Vitamin $\text{B}_{12}$ Deficiency
Diagnosis and Markers

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Vitamin B$_{12}$ Deficiency
Diagnosis and Markers

- Historical Background
- Biology and Genetic Disorders
- Deficiency states: Diagnosis and Markers
Vitamin B$_{12}$ Deficiency

Diagnosis and Markers

Historical Background
Vitamin B$_{12}$ Metabolism Timeline

1855 Addison described a remarkable form of anaemia
1926 - 1934 "eating liver cures pernicious anaemia"
1948 Vitamin B$_{12}$ isolated
1956 Vitamin B$_{12}$ crystal structure elucidated
1960s Coenzymes for mammalian B$_{12}$ enzymes identified
Vitamin $\text{B}_{12}$ crystal structure

STRUCTURE OF VITAMIN $\text{B}_{12}$

By DR. DOROTHY CROWFOOT HODGKIN, F.R.S., JENNIFER KAMPER, MAUREEN MACKAY
and JENNY PICKWORTH

Oxford

KENNETH N. TRUEBLOOD AND JOHN G. WHITE

Los Angeles Princeton

NATURE

July 14, 1956
The molecule that appears is very beautifully composed, not far from spherical in form, with all the more chemically reactive groups on the surface.
Vitamin $\text{B}_{12}$ Metabolism Timeline

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1926 - 1934 "eating liver cures pernicious anaemia"
1948 Vitamin $\text{B}_{12}$ isolated
1956 Vitamin $\text{B}_{12}$ crystal structure elucidated
1960s Coenzymes for mammalian $\text{B}_{12}$ enzymes identified
1970s Inborn Errors of Vitamin $\text{B}_{12}$ metabolism discovered
1975 – 85 Classification of cbl defects/ complementation classes
1985 – 1995 Bacterial $\text{B}_{12}$ enzymes mechanisms
1988 – 1999 cloning of genes for $\text{B}_{12}$ target enzymes
2002 – 2011 Genes for intracellular $\text{B}_{12}$ "processing"
2000 $\rightarrow$ diagnostic Markers
Structure of Vitamin B\textsubscript{12} (Cobalamin, Cbl)

In man, essential cofactor for 2 enzymes:

- Methionine Synthase
  - Methyl-Cbl
- Methylmalonyl CoA-Mutase
  - Adenosyl-Cbl
Foods rich in Vitamin B$_{12}$ (Cobalamin)

Daily requirement 3µg

Elderly ??
Vitamin B\textsubscript{12} Deficiency
Diagnosis and Markers

Biology and Genetic Disorders
Vitamin B$_{12}$ and Homocysteine Metabolism

Methionine

- S-adenosyl methionine
- S-adenosyl homocysteine

Homocysteine

- Adenosine
- Serine

Cystathionine

Cysteine

Sulphate

Methionine Synthase

Tetrahydrofolate

5-Methyl-THF

Cobalamin

MeCbl
Methylmalonic acid metabolism

Valine
Isoleucine
Methionine
Threonine
Odd-chain fatty acids
Cholesterol

Propionyl-CoA

D-Methylmalonyl-CoA ← L-Methylmalonyl-CoA ← Succinyl-CoA

Epimerase

Free Methylmalonic Acid

Mutase

Cobalamin

Succinate-CoA Ligase

Succinate-CoA

ADP

GDP

GTP

ATP

Fumarate

Isocitrate

Citrate

Oxaloacetate

Acetyl-CoA

Pyruvate

Lactate
Cbl = Cobalamin
HC = Haptocorrin-bound Cbl
deg.HC = degraded HC
IF = Intrinsic Factor
IF-Cbl = IF-bound Cbl
●-IF-Cbl = IF-Cbl attached to Cubilin (Ileal receptor)
TCII-Cbl = Transcobalamin bound Cbl.

Gastrointestinal Absorption of dietary Vit-B₁₂
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TCII-Cbl = Transcobalamin bound Cbl.
Intracellular Cobalamin Metabolism
Steps are defined by complementation classes
Intracellular Cobalamin Metabolism
Steps are defined by complementation classes

Cytoplasm

Cbl

cblF

OHcbl

TC

Lysosome

OHcbl

OHCbl

Methylmalonyl-CoA

Succinyl-CoA

Mitochondrion

Methionine

Homocysteine

Cbl

cblE

MeCbl

cblG

AdoCbl

mut

Intracellular Cobalamin Metabolism
Steps are defined by complementation classes
New Genes
The CbIC defect

Identification of the gene responsible for methylmalonic aciduria and homocystinuria, cblC type

- Discovered by homozygosity mapping
- Located on chromosome 1p
- Codes for approx. 30 kDa protein
Gene Identification for the cblD Defect of Vitamin B₁₂ Metabolism

David Coelho, Ph.D., Terttu Suormala, Ph.D., Martin Stucki, M.Sc.,
               Jordan P. Lerner-Ellis, Ph.D., David S. Rosenblatt, M.D.,
               Robert F. Newbold, Ph.D., Matthias R. Baumgartner, M.D.,
               and Brian Fowler, Ph.D.
New Genes: The CbID defect

- one gene – three phenotypes
  - combined defect methylmalonic-aciduria / homocystinuria
  - isolated Hcy
  - isolated MMA

- Significant homology with bacterial genes related to ABC transporters
- Encodes a polypeptide of 296 amino acids (32.8 kDa)
- Mutations were found in all cblD patients
  - MethylMmalonic Aciduria and HomoCystinuria cblD type (MMADHC)
- Maps to chromosome 2q23.2
New Genes: the cblF defect

Identification of a putative lysosomal cobalamin exporter altered in the cblF defect of vitamin B₁₂ metabolism

Frank Rutsch¹, Susann Gailus¹, Isabelle R Miousse², Terttu Suormala³, Corinne Sagné⁴, Mohammad Reza Toliat⁵, Gudrun Nürnberg⁵, Tanja Wittkampf¹, Insa Buers⁶, Azita Sharifi⁴, Martin Stucki⁷, Christian Becker⁵, Matthias Baumgartner⁷, Horst Robenek⁶, Thorsten Marquardt¹, Wolfgang Höhne⁹, Bruno Gasnier⁴, David S Rosenblatt², Brian Fowler³ & Peter Nürnberg⁵,¹⁰

Mutations in the LMBRD1 gene (encoding LMBD1, a lysosomal membrane protein) found in cblF patients
A new Cbl complementation class mimicking cblF is caused by mutations of ABCD4

Coelho D et al. Basel, Zürich, colleagues from Montreal, Münster
Late breaking news SSIEM 2011, Geneva

Two patients with cblF phenotype but new complementation group

<table>
<thead>
<tr>
<th>N. American case</th>
<th>European case</th>
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<tbody>
<tr>
<td>– whole exome capture</td>
<td>microcell mediated chromosome transfer</td>
</tr>
<tr>
<td>→ 2 mutations of ABCD4</td>
<td>exome sequencing of chr. 14</td>
</tr>
<tr>
<td></td>
<td>→ 2 other mutations of ABCD4</td>
</tr>
</tbody>
</table>

ABCD4 presumed ABC transporter

*NEJM* 358;14  www.NEJM.ORG APRIL 3, 2008
Vitamin B\textsubscript{12} Deficiency

Diagnosis and Markers

Deficiency states

Diagnosis and Markers
Cobalamin and common diseases

• Vitamin B$_{12}$ deficiency in breastfed Newborns
  - mothers on vegan diet / B$_{12}$ absorption problems

• Mal-absorption of cobalamin especially in elderly persons
# Causes of Cobalamin Malabsorption

**Causes often leading to megaloblastic anemia**

<table>
<thead>
<tr>
<th>Autoimmune</th>
<th>- Pernicious anemia</th>
</tr>
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<tbody>
<tr>
<td>Gastric</td>
<td>- Congenital IF deficiency</td>
</tr>
<tr>
<td></td>
<td>- Gastrectomy</td>
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<tr>
<td>Intestinal</td>
<td>- Congenital transcobalamin deficiency</td>
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<tr>
<td></td>
<td>- Selective malabsorption with proteinuria (MGA1; Imerslund–Gräsbeck syndrome)</td>
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<tr>
<td></td>
<td>- Intestinal stagnant loop syndrome: jejunal diverticulosis, ileocolic fistula, anatomical blind loop, intestinal structure, etc.</td>
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<td>- Ileal resection and Crohn’s disease</td>
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<td>- Tropical sprue</td>
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<td>- Fish tapeworm</td>
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</tbody>
</table>
Causes of Cobalamin Malabsorption

Causes usually NOT leading to megaloblastic anemia

**Gastric**
- Simple atrophic gastritis (food $B_{12}$ malabsorption)
- Zollinger-Ellison syndrome
- Gastric bypass surgery
- Use of proton pump inhibitors

**Intestinal**
- Gluten-induced enteropathy
- Severe pancreatitis
- HIV infection
- Radiotherapy
- Graft versus host disease

**Nutritional**
- Deficiencies of $B_{12}$, folate, protein

**Drugs**
- Colchicine
- Neomycin
- Anticonvulsant drugs
- Phenformin
- Alcohol
- Para-aminosalicylate
- Slow-release potassium chloride
- Metformin
- Cytotoxic drugs
- Alcohol
# Vitamin B<sub>12</sub> Deficiency Diagnostics & Markers: Different Levels

<table>
<thead>
<tr>
<th>Clinical Symptoms, Haematological changes</th>
<th>- example: Child of a Veganer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutritional</td>
<td>- Dietary History</td>
</tr>
<tr>
<td></td>
<td>- Total B&lt;sub&gt;12&lt;/sub&gt; in Blood (Haptocorrin + Transcobalamin)</td>
</tr>
<tr>
<td>Active transport form</td>
<td>- Holo-transcobalamin</td>
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<tr>
<td>Metabolic Function level</td>
<td>- Methylmalonic acid (MMA), total Homocysteine</td>
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<tr>
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<td>Caveat: kidney function</td>
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<tr>
<td>Enzyme level</td>
<td>- fibroblast assays - severe genetic disorders</td>
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<tr>
<td>Genetic level</td>
<td>- mutations in severe genetic disorders</td>
</tr>
<tr>
<td></td>
<td>- polymorphisms in common conditions</td>
</tr>
</tbody>
</table>
Vitamin B₁₂ Deficiency
Total homocysteine

![Graph showing the relationship between plasma folate and vitamin B₁₂ concentrations and mean homocysteine concentration.]

Selhub et al.
JAMA
1993;270:2693-2698
Vitamin B\(_{12}\)-Deficiency in 8 Month old child of a Vegetarian (part of small intestine removed)

Clinical
- increasing tiredness over 4 weeks
- Apnea, Hypotonic, Myoclonic episodes

Lab findings

<table>
<thead>
<tr>
<th>µmol/L</th>
<th>Kind</th>
<th>Mutter</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>140</td>
<td>Child</td>
<td></td>
<td>Homocysteine</td>
</tr>
<tr>
<td>120</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td></td>
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<td>80</td>
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<td>0</td>
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</tbody>
</table>

Treatment
- B\(_{12}\) 1000 µg/d i.m. → Homocysteine normal
Markers for Vitamin B$_{12}$ disturbance in common diseases

Plasma (Urine) Methylmalonic acid

Method
- Gas Chromatography / Mass Spectrometry
- Extraction *Stable isotope dilution* using D3 - Methylmalonic acid as carrier and internal standard

cut off ca. 300 nmol/L

Accurate / sensitive but time consuming / expensive equipment
MMA Stable Isotope Dilution: B$_{12}$ deficiency

MMA: 1.1 µmol/L
Vitamin B$_{12}$ Deficiency
Plasma Methylmalonic acid

Allen et al.
FASEB J. 1993
Markers for Vitamin $B_{12}$ disturbance

Plasma Holo-transcobalamin ($B_{12}$ bound to transcobalamin)
transcobalamin
Markers for Vitamin B$_{12}$ disturbance

Plasma Holo-transcobalamin (B$_{12}$ bound to transcobalamin)

Previous assays unreliable and cumbersome

Recent methods precise and accurate (e.g. Ueland et al. 2002)
- separation of trans-cobalamin by monoclonal antibody
  $\rightarrow$ B$_{12}$ measured by radio-assay
- monoclonal antibody for HOLO-transcobalamin in chemiluminescence assay

Commercially available assays
Plasma Holo-transcobalamin as Marker for Vitamin B₁₂ disturbance

Holotranscobalamin as a Predictor of Vitamin B₁₂ Status
Anne-Metter Hvas and Ebba Nexo

Holo- transcobalamin, a marker of vitamin B-₁₂ status: analytical aspects and clinical utility
Ebba Nexo and Elke Hoffmann-Lücke
Am J Clin Nutr 2011;94:359S-365S
Cobalamin and its binding proteins in human plasma.

Nexo E, Hoffmann-Lücke E

Am J Clin Nutr 2011;94:359S
Holotranscobalamin (holoTC) and cobalamin in relation to methylmalonic acid

AUC (vs. MMA)
Total B12 ca. 80%
HoloTC ca. 90%

Nexo E, Hoffmann-Lücke E
Am J Clin Nutr 2011;94:359S
Non-radioactive Vitamin B₁₂ Absorption Test
Controls and Patients with Inherited Malabsorption of Vitamin B₁₂

Change after B₁₂ intake*
Day 1 - Day 0

* 3 x 9µg oral B₁₂ 6 hourly

Markers for Vitamin B\textsubscript{12} disturbance: Summary

Vitamin B\textsubscript{12} Handbook of Vitamins, Fourth Edition

Ralph Green and Joshua W. Miller

• Limitations of individual analyte assays indicate that no single assay is completely adequate for assessment of B\textsubscript{12} status.
• Novel or refined strategies using sequential tests in an algorithm proposed for diagnosis or detection of B\textsubscript{12} deficiency or for screening populations.
• Strategies promote panels of two or more measurements performed in sequence or simultaneously
Measure chosen parameters / e.g. haptocorrin then MMA
If still unclear contact expert
Measure chosen parameters / e.g. haptocorrin then MMA

If still unclear contact expert
Acknowledgements

- Terttu Suormala, David Coelho (Basel),
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