De ene vitamine D bepaling is de andere niet

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Vitamin D

Calcium en phosphorus uptake by gut

Mineralisation of bone
Vitamin D deficiency

Rickets
Deficiency and supplementation

- 30-70% of the population is vitamin D deficient
  - Cut-off
  - Seasonal influences
  - Vitamin D assay used

- Supplementation
  - Decreased risk of osteoporosis (and fractures)
Extraskeletal effects

• 3% of human genome is under control of vitamin D

• Muscle function
• Cancer
• Immune system
• Cardiovascular system
• Metabolic system
• Neuropsychiatric function
Vitamin D

Sunlight → Skin → 7-Dehydrocholesterol → Cholecalciferol (vitamin D₃) → Liver → 25-hydroxyvitamin D₃ → Kidney → 1,25-dihydroxyvitamin D₃

Dietary intake:
- Vitamin D₃ (fish, meat)
- Vitamin D₂ (supplements)

Maintains calcium balance in the body
Sources

- Sunlight (250 microgram production one day in sun)
- Fatty fish (10-25 microgram per portion)
- Supplements (recommended daily dose is 5 microgram)
Sun protection

Skin cancer versus vitamin D deficiency
Vitamin D assays are laborious. Automation is desirable.
Vitamin D binding protein (DBP)

- (25OH) Vitamin D transport
  - 85% bound to DBP
  - 15% bound to albumin
  - 0.03% free
Vitamin D assay

Extraction of vitamin D from DBP

Can automated immunoassays perform extraction well enough?
Aim

- To test the accuracy of six available routine 25(OH)D assays by comparing these assays with an ID-LC-MS/MS method, using not only plasma from healthy individuals, but also plasma from patients with various DBP concentrations.
Methods: demographic details

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Male/female</th>
<th>Age range (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy individuals</td>
<td>51</td>
<td>23/28</td>
<td>20-64</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>52</td>
<td>0/52</td>
<td>19-40</td>
</tr>
<tr>
<td>Haemodialysis patients</td>
<td>50</td>
<td>24/26</td>
<td>33-89</td>
</tr>
<tr>
<td>IC patients</td>
<td>50</td>
<td>28/22</td>
<td>18-89</td>
</tr>
</tbody>
</table>

Table 1. Demographic details of the subjects.
Methods: 25(OH)D assays

- Architect (Abbott)
- Centaur (Siemens)
- iSYS (IDS)
- Liaison (Diasorin)
- Modular (Roche)
- RIA preceded by extraction (Diasorin)
- ID-LC-MS/MS
Methods: ID-XLC-MS/MS

- 100 µl EDTA plasma
- Disruption D25 from DBP: proteolysis
- Deuterated internal standard (IS: 25(OH)D3-d6)
- Solid Phase Extraction (SPE)
- LC-MS/MS
LC-MS/MS method

- Establishing accuracy by measuring standard and control with candidate reference method

- Intra CV% <6% and inter CV% <8%; LOQ is 4 nmol/L
Methods: DBP

- ELISA (R&D systems)
- Intra CV <5%
Accuracy
Results

- ID-LC-MS/MS: 25(OH)D2 and 25(OH)D3

- Passing&Bablok
  - Immunoassay compared to ID-LC-MS/MS
    - Healthy individuals
    - Pregnant women
    - Dialysis patients
    - IC patients
Healthy individuals
Pregnant women

\[ R = 0.91 \]
\[ R = 0.92 \]
\[ R = 0.88 \]
\[ R = 0.92 \]
\[ R = 0.92 \]
\[ R = 0.96 \]
Hemodialysis patients

R = 0.92
R = 0.93
R = 0.86
R = 0.93
R = 0.97
R = 0.84
Intensive care patients

Intensive care patients not determined with iSYS

$R = 0.94$

$R = 0.81$

$R = 0.83$

$R = 0.91$

$R = 0.82$
DBP concentrations
Results: DBP concentrations
Relation between 25(OH)D deviation and DBP concentration
Discussion

- Major differences between assays
  - Standardisation problems
  - Influence of DBP (4 of 5 automated assays)
  - Other unknown interference in immunoassays
- Not all assays are suitable for measuring 25(OH)D in all patient groups
- 25(OH)D2 is not causing differences
- Clinical significance
  - Advice on supplementation
Sufficiency

- Healthy individuals
- Dialysis patients
- IC patients
- Pregnant women

- >50 nmol/L
- 30-50 nmol/L
- <30 nmol/L
Conclusion

Some of the assays used to measure 25(OH)D are not well standardized and report significantly different results from measurements performed with ID-XLC-MS/MS.

The deviations which are sometimes serious are different in various patient groups, and are dependent on the concentration of DBP and other still unknown interfering factors.

Laboratory specialists, clinicians, researchers, reviewers and authorities should carefully consider the method used when interpreting results of 25(OH)D measurements.
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