Chronobiology of Vitamin D: 

...implications for human cancer

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...vitamin D: where it came from

- in 1919/20, Sir Edward Mellanby (1884-1955),

  ...working with growing dogs raised exclusively indoors, who developed bone disease „rickets“, devised a diet that allowed him to unequivocally establish that this disease was caused by a deficiency of a trace component present in the diet.
...in 1921 he wrote:

- “...The action of fats in rickets is due to a vitamin or accessory food factor, which they contain, probably identical with the fat-soluble vitamin...”

- ...later on, he established that **cod liver oil** was an excellent antirachitic agent.
Biosynthesis

...in the presence of sunlight

7-Dehydrocholesterol $\xrightarrow{\text{Ultra violet light}}$ Cholecalciferol (Vitamin D₃)

Hydroxylation of carbon 25 by liver 25-hydroxylase

Hydroxylation of carbon 1 by kidney 1-hydroxylase

Active vitamin 1,25-Dihydroxycholecalciferol (25-Hydroxyvitamin D₃)

25-Hydroxycholecalciferol (25-Hydroxyvitamin D₃)
Seasonality in oncology: lessons from history

- **1941** Apperly FL
  - cancer mortality connected to sunlight

- **1981** Abe et al
  - vitamin D role in differentiation of CML cells and a growth inhibition of malignant melanoma cells

- **2005-2007** animal models
  - prodifferentiation effect of vitamin D on hyperproliferative processes

- **2000-2007** epidemiological studies
  - publishing an inverse relationship between vitamin D intake and colorectal cancer

- **2010** Jenab et al
  - metaanalysis of 35 prospective studies- inverse relationship between 25-OHD plasma levels and CRC risk
...blood levels of 25-OHD and CRC

• Metaanalysis by Gorham, ED, et al 2007
  • 25-OHD levels > 82 nmol/l
    – ...resulted in 50 % lower incidence of CRC than levels of vitamin D < 30 nmol/l.

• Dana-Farber Cancer Ng,K et al 2008 n=304
  – ...patients with higher „prediagnostic“ levels of 25-OHD resulted in about 50 % reduction of death
...OUR EPIDEMIOLOGICAL RESEARCH
...a research question: *is there seasonality pattern in incidence of cancer?*  
... = yes, it is.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Observed incidence pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal cancer (C18-C20)</td>
<td>Significantly increased incidence in spring and decreased in autumn</td>
</tr>
<tr>
<td>Pancreatic cancer (C25)</td>
<td>No observed seasonal pattern</td>
</tr>
<tr>
<td>Breast cancer (C50)</td>
<td>Significantly increased incidence in spring and decreased in October</td>
</tr>
<tr>
<td>Ovarian cancer (C56)</td>
<td>Increased incidence in March</td>
</tr>
<tr>
<td>Prostate cancer (C61)</td>
<td>Significantly increased incidence in spring and decreased in September</td>
</tr>
<tr>
<td>Kidney cancer (C64)</td>
<td>Increased incidence in spring</td>
</tr>
<tr>
<td>Malignant melanoma (C43)</td>
<td>Highly significantly increased incidence in spring and summer, decreased in autumn and winter</td>
</tr>
</tbody>
</table>
methodological approach to analyze seasonal patterns

• 1. Cumulative incidence mass was calculated over 20 years
  - (i.e. all Januaries through all Decembers across 20 yrs)
  - => this integrated approach is robust in analysis of seasonal differences, since annual fluctuations partially mask seasonal patterns

• 2. Age-specific and stage-specific profiles of incidence were obtained
  - „spring“ was defined as March+April+May, i.e. not astronomical spring
  - (reporting is obviously by months)
Cumulative incidence profile: colorectal cancer
Colorectal cancer; 1992-2011; stage 1
Age-specific incidence

Colorectal cancer; 1992-2011; stage 2
Age-specific incidence

Colorectal cancer; 1992-2011; stage 3
Age-specific incidence

Colorectal cancer; 1992-2011; stage 4
Age-specific incidence

March
April
May
September
October
...we observed most significant differences ($p < 0.001$) for CRC and RCC.
Malignant melanoma – …peaking in summer (p < 0.001)
Cases per 100,000 inhabitants

Annual incidence and mortality in selected tumours – recent data (2011-2012)

- Prostate cancer
- Breast cancer
- Colorectal cancer
- Kidney cancer
- Pancreatic cancer
- Ovarian cancer
- Malignant melanoma
...OUR EPIDEMIOLOGICAL AND LABORATORY RESEARCH
...circannual pattern of blood 25-OHD levels, does it exist?

- spring cohort, **437 observations** from March+April:
  - mean 42.7 nmol/l (95% confidence interval 21.9–90.1 nmol/l)

- autumn cohort, **508 observations** from Sept.+Oct.:
  - mean 70.8 nmol/l (95% CI 37.0-137.5 nmol/l)

-ARCHITECT i2000sr (Abbott)

=> interpretation: yes, levels vary with cca 90% amplitude and cca 6 months frequency interval
...then, how to validate it?

validation question: what is the variability of vitD-regulated biomolecule?

=> ...annual p_Ca variability (median):

Red: C50, n=1040
Gold: healthy, n=31982
Blue: C18,19,20, n=763
...and how to validate it further? Staff levels of 25-OHD in spring

Reference cohort 437 obs.

March-April

- Sufficient: 7%
- Insufficient: 27%
- Difficient: 60%
- Severe deficient: 6%

March-April

- Sufficient: 1 (3%)
- Insufficient: 8 (27%)
- Difficient: 16 (53%)
- Severe deficient: 5 (17%)
...And in autumn

Reference cohort 503 obs.

- Sufficient
- Insufficient
- Deficient
- Sev. deficient

September-October

11 (37%)
14 (47%)
5 (17%)

September-October
...interim summary I

- on the basis of epidemiological and laboratory data one can indeed conclude that **circannual variations of vitamin D blood levels** along with vitamin D-regulated bioactive molecules were
  - i) **robustly established and validated** thereof and
  - ii) **consistently projected in the seasonal cancer incidence curves** being mostly apparent for
    - iii) CRC and RCC
    - whilst inversely apparent for melanoma
...OUR EPIDEMIOLOGICAL, LABORATORY AND CLINICAL RESEARCH
...how vitamin D exerts its biological effects

• ...genomic and nongenomic effects

• nongenomic = through membrane bound receptor-type molecules, yet poorly characterized
  » ...rapid insulin release
  » ...rapid calcium absorption in the intestine

• genomic = nuclear receptor-mediated response:
  » ...through VDR, a „new“ member of steroid hormone receptor superfamily
…brief vitamin D physiology overview
VDR expression: breast cancer

breast cancer, 400x, heterogenous epithelial and stromal staining

breast cancer, 100x, strong homogenous staining of epithelia and stroma, both are ER positive
VDR in normal breast, more staining in luminal cells (different antibody used).

mCRC timeline

Vitamin D and prospective trials
...so what can we learn from?

...2007: Patients who follow a **low-fat diet and exercise** regularly are found to have a **lower risk of colon cancer recurrence** after surgery for early-stage disease demonstrating that lifestyle factors can have a significant impact on cancer recurrence. The results provide patients with new tools for reducing the risk that their cancer will return...

...a questions is then:

......*Individuals with higher blood 25-OHD levels have a lower risk of developing colorectal cancer, but the influence of 25-OHD on mortality after CRC diagnosis is unknown.*
Time-course patterns of blood 25-hydroxycholecalciferol are significant predictors of survival outcome in metastatic colorectal cancer: clinical practice-based, proof-of-principle study, Valik et al, Neoplasma, accepted

- We studied blood levels of 25-hydroxycholecalciferol in relation to other clinical and laboratory variables in metastatic colorectal cancer patients to ascertain whether their variations may be a prognostic or predictive parameters of survival outcomes
Patient characteristic

- We included 84 patients treated with first-line oxaliplatin-based chemotherapy with or without bevacizumab.

- The patients were enrolled on the intent-to-treat basis considering their performance status, comorbidities and laboratory parameters to be medically apt for intensive chemotherapy.
  - The study was made possible due to having a research BBMRI-affiliated biobank at MMCI storing besides tissues also periodical aliquots of patient sera from CEA determinations.
Time-course typology patterns of 25-OHD blood levels during therapy of metastatic CRC

<table>
<thead>
<tr>
<th>Number of samples in given time</th>
<th>T01</th>
<th>T02</th>
<th>T03</th>
<th>T04</th>
<th>T05</th>
</tr>
</thead>
<tbody>
<tr>
<td>At least 1 25-OHD value 40 or above</td>
<td>40</td>
<td>40</td>
<td>32</td>
<td>24</td>
<td>8</td>
</tr>
<tr>
<td>All 25-OHD &lt; 40 - fluctuating</td>
<td>14</td>
<td>14</td>
<td>12</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>All 25-OHD &lt; 40 - stable</td>
<td>30</td>
<td>30</td>
<td>15</td>
<td>11</td>
<td>7</td>
</tr>
</tbody>
</table>

Consecutive measurements
## Potential predictors of OS in Cox proportional hazard regression model

<table>
<thead>
<tr>
<th>Endpoint: overall survival</th>
<th>Univariate estimates</th>
<th>Multivariate-adjusted estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td>Any surgical procedure</td>
<td>0.239 (0.098; 0.583)</td>
<td>0.002</td>
</tr>
<tr>
<td>Radical resection of metastases</td>
<td>0.101 (0.014; 0.747)</td>
<td>0.025</td>
</tr>
<tr>
<td>25-OHD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-OHD (max) &lt; 40</td>
<td>2.238 (1.029; 4.868)</td>
<td>0.042</td>
</tr>
<tr>
<td>25-OHD (max) &lt; 50</td>
<td>3.178 (1.107; 9.068)</td>
<td>0.032</td>
</tr>
<tr>
<td>All 25-OHD in time series &lt; 50</td>
<td>3.344 (1.168; 9.576)</td>
<td>0.024</td>
</tr>
<tr>
<td>All 25-OHD in time series &lt; 40</td>
<td>2.466 (1.133; 5.371)</td>
<td>0.023</td>
</tr>
<tr>
<td>Typology of 25-OHD time series (binary code)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All values &lt;40 - stable, without any elevation</td>
<td>2.710 (1.333; 5.510)</td>
<td>0.006</td>
</tr>
<tr>
<td>CEA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEA (first) &gt; 11</td>
<td>4.610 (2.115; 10.051)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Survival of patients according to time-course typology patterns of 25-OHD blood levels (nmol/l)

--- NORMAL (N) ---

LOW (L): At least one 25-OHD value 40 or above (N=40)

LOW (L): All 25-OHD < 40 (N=44)

<table>
<thead>
<tr>
<th>Time</th>
<th>No at risk</th>
<th>0</th>
<th>12</th>
<th>24</th>
<th>36</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>40</td>
<td>28</td>
<td>11</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>44</td>
<td>21</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td></td>
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<tbody>
<tr>
<td>N</td>
<td>40</td>
<td>36</td>
<td>24</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>44</td>
<td>35</td>
<td>18</td>
<td>8</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

\[ p \text{ (log rank test)} = 0.015 \]

\[ p \text{ (log rank test)} = 0.019 \]
Vitamin D is a pleiotropic bioactive molecule that exerts influence on the course of active disease. Its plasmatic levels assessed as trend patterns, but not single samplings, possess robust predictive power towards mCRC outcome.

Although it is tempting to speculate that pharmacological administration of cholecalciferol may have favorable effect on the disease clinical course, this assumption needs to be proven under clinical trial conditions.

From the point of oral administration, clinical fear of overdosing is probably very unsubstantiated, cholecalciferol preparation are probably safe to up to 10000 IU/day.
Annual incidence and mortality in selected tumours – recent data (2011-2012)

- **Prostate cancer**
- **Breast cancer**
- **Colorectal cancer**
- **Kidney cancer**
- **Pancreatic cancer**
- **Ovarian cancer**
- **Malignant melanoma**
...vast number of diseases and all-cause mortality linked to vitamin D deficiency

- respiratory infections
- muscular weakness
- psoriasis
- **diabetes** – studies from Finland: 10,366 children got 2000 IU of D3 during first day after birth, 31 years followup, risk of diabetes decreased by 80% !!
- **asthma** – studies from Japan: 1200 IU / day = less asthmatic attacks
- parodontic diseases
- **cardiovascular disease** – deficiency linked to high risk for hypertension
- schizophrenia and depression
- **cancer** – vitamin D decreases incidence and improves prognosis

*J Clin Endocrinol Metab, 2012, 97(8):2792–2798*
- Africa – blacks, i.e. low synthesis but outside, India – undressed outside, Arabs – dressed, Florida – in airconditioned houses and cars, Czech Republic – summer vacations in Greece and Croatia...??
...but how to cover requirements for vitamin D

How many minutes of mid-day summer sun needed for adequate vitamin D?

<table>
<thead>
<tr>
<th>Location</th>
<th>Age</th>
<th>Position</th>
<th>Time Needed</th>
</tr>
</thead>
<tbody>
<tr>
<td>San Diego</td>
<td>Youth</td>
<td>Lying down</td>
<td>42 min, 19 min, 11 min, 7 min</td>
</tr>
<tr>
<td>Seattle</td>
<td>Youth</td>
<td>Standing</td>
<td>168 min, 76 min, 44 min, 28 min</td>
</tr>
<tr>
<td>Seattle</td>
<td>Senior</td>
<td>Standing</td>
<td>504 min, 228 min, 132 min, 84 min</td>
</tr>
</tbody>
</table>

Adequate = 40 nanograms/ml  
Does not include Obese, Dark Skin

Details: http://www.is.gd/timeinsun
...overall summary

- **Vitamin D is a significant biological modulator of incidence of CRC and other human cancers as well**
  - and its blood levels may be an informative prognostic factor in the course of active disease

- **Current recommendation on sun exposure may be perhaps too restrictive**
  - mostly being postulated on skin cancer incidence only.

- **Due to significant societal impact of CRC, lay public may be educated towards moderate sun exposure** whilst remaining **alert on suspect skin lesions** that need to be periodically checked
  - ...this may also mean better education of primary care physicians, but such recommendations need to be first adopted by professional societies
thank you for your attention

- Lenka Zdrazilova Dubska
- Kristina Greplova
- Iveta Selingerova
- Vlad Popovici
- Radek Pilny
- Regina Demlova
- Ladislav Dusek
- Vit Dusek
- Radka Obermannova
- Jiri Jarkovsky
- Rostislav Vyzula
- Rudolf Nenutil