Vitamin D and Tuberculosis

Dr. David Connell
Academic Clinical Fellow in Respiratory Medicine
Tuberculosis Service
Imperial College Healthcare NHS Trust

Professor Ajit Lalvani
Tuberculosis Research Unit
Department of Respiratory Medicine
Imperial College London
A Global Epidemic

• TB infects approximately 1/3 of the world’s population
• Estimated 9.2 million new cases in 2006
• Estimated 4.1 million smear positive cases in 2006
• 1.7 million deaths from TB in 2006
• 7.7% HIV positive
• New threat: MDR and XDR disease
Figure 1
Tuberculosis rate, London and England and Wales, 1982 - 2003

From: Statutory Notifications (NOIDs) and London 2000-2003: Enhanced Tuberculosis Surveillance
Therapies for TB

- Streptomycin: 1944
- PAS: 1949
- Pyrazinamide: 1954
- Ethambutol: 1962
- Isoniazid: 1952
- Cycloserine: 1955
- Rifampicin: 1963
- Moxifloxacin: 2000s

MDR-TB
XDR-TB
A Need for “Novel” Therapies

The aspects of things that are most important to us are hidden because of their simplicity and familiarity

-Ludwig Wittgenstein
Back to the Future...Ancient Remedies

• Galen (130-200AD) suggested ass’ milk as a treatment for TB

• Diets rich in eggs and milk were recommended prior to the introduction of modern chemotherapy

• Cod Liver Oil as treatment was popular in the 19th century: 1 500 gallons were used per year at the Royal Brompton Hospital, London
Fatty Acids of Cod-liver Oil in the Treatment of Tuberculosis

Sir,—Professor Cummins and Dr. Weatherall's article on the inhibitory action of "alepol" on the growth of the tubercle bacillus, in the Journal of January 14th (p. 48), is of very special interest to me, as for the last eight years I have been investigating the action of the fatty acids of cod-liver oil on the tubercle bacillus.

My main plan was to treat cases suffering from tuberculous infection—the potential consumptive—rather than those suffering from definite tuberculous disease, as I have long held the view that these cases are not considered seriously enough or treated as they might be. No one can prognosticate in whom the infection will pass on to definite disease—the regulating factors are so many and varied. My cases were therefore mainly confined to those in whom tuberculous infection was, on clinical grounds, definitely established, but I also treated a few cases of old-standing pulmonary tuberculosis. I instinctively avoided early and acute pulmonary invasion, but I see no reason why the same treatment should not be applied to such cases after the quiescence has been established for a reasonably long period. The results in cases of tuberculous infection were uniformly good, the improvement was, without exception, excellent, and was freely acknowledged as such by the patients. The old-standing cases of pulmonary tuberculosis also showed
Phototherapy for TB 1903: Nobel Prize
Switzerland, Sanatoria and Sun

Thomas Mann
Der Zauberberg
Roman

S. Fischer

Tuberculosis Research Unit
Linking sunshine and oily fish?

Mellanby, T. The part played by an "accessory factor" in the production of experimental rickets. A further demonstration of the part played by accessory food factors in the aetiology of rickets. J Physiology 1918; 52:11.

Goldblatt, H, Soames, KM. A Study of Rats on a Normal Diet Irradiated daily by the Mercury Vapour Quartz Lamp or kept in Darkness. Biochem J 1923; 17:294

Vitamin D: “The Sunshine Vitamin”

25OHD-1α hydroxylase (CYP27B)

Diet/supplements

Liver

Calcitriol (1,25-dihydroxy Vitamin D)

Kidney

Calcidiol (25-hydroxy Vitamin D)

Cholecalciferol (Vitamin D3)

Ergocalciferol (Vitamin D2)

↓ Intestinal absorption of calcium

↑ PTH-mediated bone resorption

↓ Renal Ca++ and phosphate excretion

24,25-dihydroxyvitamin D
Vitamin D and TB: a link?

Vitamin D Deficiency

Environmental factors

Eggs
Milk

Oily Fish

Tuberculosis Research Unit
1961: Widespread rickets and Vitamin D deficiency found in Pakistani immigrants to Glasgow

TB also much more common in this population compared with the white population

Could a link be possible?
1985: Davies et al demonstrated lower levels of 25-OHD Vitamin D in patients with TB when compared with matched controls.
Scientific Causality: proving a link

Austin Bradford-Hill: “Father of the RCT” and TB sufferer

1. **Strength**: stronger associations are more likely to be real
2. **Biological gradient**: higher exposures lead to more disease
3. **Consistency**: the association is found in different places and people over time
4. **Plausibility and coherence aka Experimental evidence *in vitro***: a biological model exists to explain the association
5. **Experimental evidence *in vivo***: interventions to modify the exposure alters disease rates: RCTs
6. **Temporality**: exposure precedes disease
7. **Analogy**: have similar associations been found with other exposures or diseases
8. **Genetic basis for disease**: not considered at time

Strength and Gradient of Association

- 25-OHD deficiency was significantly associated with active TB disease
- Undetectable serum 25-OHD carried the highest risk of TB

Tuberculosis Research Unit

Consistency across populations

Nnoaham and Clarke (2008) examined the association in a meta-analysis. 7 papers met their eligibility criteria from 151 originally selected. They represented a global geographical mix of research and confirmed the significant association between Vitamin D deficiency and TB disease.

\[ \text{Int J Epidemiol. 2008 Feb;37(1):113-9} \]

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Cases ( N )</th>
<th>Control ( N )</th>
<th>Effect Size (SE)</th>
<th>Weight</th>
<th>Effect Size (random)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Davies, UK</td>
<td>40</td>
<td>40</td>
<td>0.8600 (0.2300)</td>
<td>16.69</td>
<td>0.86 [0.41, 1.31]</td>
<td></td>
</tr>
<tr>
<td>Grange, Indonesia</td>
<td>40</td>
<td>38</td>
<td>0.1800 (0.2300)</td>
<td>16.69</td>
<td>0.18 [-0.27, 0.63]</td>
<td></td>
</tr>
<tr>
<td>Davies, Kenya</td>
<td>15</td>
<td>15</td>
<td>1.0400 (0.3900)</td>
<td>8.34</td>
<td>1.04 [0.28, 1.80]</td>
<td></td>
</tr>
<tr>
<td>Davies, Thailand</td>
<td>51</td>
<td>51</td>
<td>0.9600 (0.2100)</td>
<td>18.31</td>
<td>0.96 [0.55, 1.37]</td>
<td></td>
</tr>
<tr>
<td>Chan, Hong Kong</td>
<td>24</td>
<td>24</td>
<td>0.3300 (0.3000)</td>
<td>12.14</td>
<td>0.33 [-0.26, 0.92]</td>
<td></td>
</tr>
<tr>
<td>Milkinson, UK</td>
<td>103</td>
<td>42</td>
<td>0.8900 (0.2300)</td>
<td>16.69</td>
<td>0.59 [0.14, 1.04]</td>
<td></td>
</tr>
<tr>
<td>Sasidharan, India</td>
<td>35</td>
<td>15</td>
<td>0.9400 (0.3200)</td>
<td>11.13</td>
<td>0.94 [0.31, 1.57]</td>
<td></td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td><strong>308</strong></td>
<td><strong>226</strong></td>
<td><strong>0.68 (0.43, 0.93)</strong></td>
<td><strong>100.00</strong></td>
<td><strong>Effect Size (random)</strong></td>
<td></td>
</tr>
</tbody>
</table>

Test for heterogeneity: \( \chi^2 = 10.44, df = 6 (p = 0.042), I^2 = 40.8\%

Test for overall effect: \( Z = 5.19 (p < 0.00001) \)

Extra-renal production of Vitamin D

- Expression of CYP27B by macrophages
- Expression of intracellular VDR in a variety of cell types outside Ca^{2+} homeostasis

Hypercalcemia in an anephric patient with sarcoidosis: evidence for extrarenal generation of 1,25-dihydroxyvitamin D
G. L. Barbour and Others
Biological Plausibility: Vitamin D and cells of the immune system

- Rook et al (1986) demonstrated that the addition of IFN-γ to monocytes allowed detection of 25OHD-1α hydroxylase (CYP27B) activity.
- Incubation of monocytes with three cholecalciferol metabolites induced anti-tuberculosis activity.

*Immunology* 1986 57 159-163
Tuberculosis Research Unit

25-OH Vit D

M. tuberculosis

CYP27b

Macrophage

Phagosomal killing

?
Biological Plausibility: *in vitro* experimental evidence

- How might Vitamin D interact with M.tb?
- Toll-like-Receptors (TLRs) interact with M.tb surface antigens as part of the innate immune response
- Liu *et al* characterised changes in gene expression following ligation of Macrophage TLRs by M.tb antigens
  - Upregulation of CYP27B and VDR gene products
M. tuberculosis

25-OH Vit D

TLR

CYP27b

VDR

Macrophage
• Liu *et al* also showed the production of mRNA for an antimicrobial peptide - cathelicidin - in response to Vitamin D in the same cells
• The gene for this molecule - *hCAP18* - had previously been shown to have a Vitamin D response element
• The nuclear VDR mediates *in vitro* Vitamin D killing of M.tb

*Science*. 2006 Mar 24;311(5768):1770-3
Tuberculosis Research Unit

M. tuberculosis

25-OH Vit D

TLR

CYP27b

VDR

25-OH Vit D

1, 25-OH Vit D

Cathelicidin

Phagosome killing

Macrophage
Biological Plausibility: *in vitro* experimental evidence

- Cathelicidin knockdown with specific small interfering RNA
  - Ablation of cathelicidin release
  - Ablation of the anti-mycobacterial effect of Vitamin D in human monocytes

• If Vitamin D deficiency is associated with development of TB disease, and Vitamin D has an important role in the innate immune response to M.tb....

Could Vitamin D supplementation alter the natural history of TB infection?
10 prospective case series and 3 RCTs of low quality were identified from 1947-2006

No effect on disease course seen

Some of these comment on paradoxial reactions during Vitamin D treatment
Martineau et al. used a BCG-lux assay as a correlate of bacillary metabolic activity in a double-blind RCT.

Single dose 2.5mg oral Vitamin D significantly enhanced whole blood’s ability to restrict *ex vivo* mycobacterial growth 6 weeks post-supplementation (p=0.03).

Am J Respir Crit Care Med. 2007 Jul 15;176(2):208-13
Experimental evidence *in vivo*

- What is the systemic effect of replacement and how much should be given?
- Vitamin D deficiency in patients with TB can be corrected at 1 week but not 8 weeks with 2.5mg Vitamin D
- Optimal dosing still unclear

## Experimental evidence *in vivo*

<table>
<thead>
<tr>
<th>Paper</th>
<th>Site</th>
<th>Type</th>
<th>Intervention</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nursyam et al. (2006)</td>
<td>Indonesia</td>
<td>Double Blind RCT</td>
<td>0.25mg Vitamin D per day during 6th week of TB treatment in 67 patients with TB</td>
<td>Improved percentage sputum conversion and improved radiological features</td>
</tr>
<tr>
<td>Wejse et al. (2009)</td>
<td>Guinea-Bissau</td>
<td>Double Blind RCT</td>
<td>100 000 IU cholecalciferol at 0, 5, 8 months in 281 patients with TB</td>
<td>No influence on sputum conversion rate or a TB severity score</td>
</tr>
</tbody>
</table>
Further trials looking at the effect of Vitamin D on TB are now ongoing:

- Sputum and culture conversion rates in active TB (London, UK)
- Cathelicidin levels in sputum and blood, and sputum conversion rates in active TB (Atlanta, USA)
- Culture conversion and clinical improvement in active TB (PNG, Australia)
- Sputum conversion in active TB (India)
Temporality: Can TB infection influence Vit D?

- What is the direction of effect?
- What happens during successful TB treatment and reduction of mycobacterial load?
- 6 months of anti-TB therapy in a cohort of patients with TB disease did not produce any significant change in Vitamin D.
- However, Vitamin D concentrations fell significantly in the Indian Sub-continent sub-group.

*Connell et al, Thorax 2008;63(Suppl VII)*
Abstract at British Thoracic Society, December 2008
Temporality: Can TB infection influence Vit D?

• Why could this be?
  1. Intrinsic 25-(OH)D-24-hydroxylase activity in some Asian Indians may be higher compared to non-Asian controls
  2. Possible effect of rifampicin/isoniazid on Vitamin D metabolism, exacerbating the above effect
  3. Possible Paradoxical reactions: more severe disease leading to paradoxical depletion of Vitamin D metabolism during therapy

The Genetics of Vitamin D and TB

• Specific polymorphisms in the Vitamin D receptor (VDR) have been looked at in a number of studies
  – Babb et al (2007): “AA” genotypes of Apa1 associated with faster sputum and culture conversion in South Africans
• Gene-Environment also key: “ff” genotype of Fok1 conferred higher risk of TB in conjunction with Vitamin D deficiency in Gujurati Indians

J Infect Dis. 2004 Nov 1;190(9):1631-41.
## Bradford-Hill re-visited

<table>
<thead>
<tr>
<th>Evidence</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strength</strong></td>
<td>SIGNIFICANT ASSOCIATIONS BETWEEN VITAMIN D DEFICIENCY AND TB REPEATEDLY FOUND</td>
</tr>
<tr>
<td>Biological gradient</td>
<td>HIGHER RATES OF TB IN THOSE WITH LOWER VITAMIN D</td>
</tr>
<tr>
<td>Consistency</td>
<td>STRONG ASSOCIATIONS ACROSS POPULATIONS</td>
</tr>
<tr>
<td>Plausibility and coherence: in vitro experimental evidence</td>
<td>LARGE BODY OF CELL BIOLOGY WORK DEMONSTRATING A BIOLOGICAL PATHWAY</td>
</tr>
<tr>
<td>In vivo experimental evidence</td>
<td>CONVINCING EX VIVO WORK IN SUPPLEMENTATION; IN VIVO RCTs UNDERWAY</td>
</tr>
<tr>
<td>Temporality</td>
<td>SEEMS PROBABLE...NEEDS A PROSPECTIVE STUDY</td>
</tr>
<tr>
<td>Analogy</td>
<td>DIVERSE WORK ON VITAMIN D AND OTHER CONDITIONS</td>
</tr>
</tbody>
</table>
The Future

- Clinical Trials: can Vitamin D therapy alter the natural history of disease in the age of XDR-TB?
- A definitively proven temporal relationship between Vitamin D and TB should be established if possible
- Reverse effects of TB on cellular Vitamin D metabolism could also be further examined
- A window to understanding innate host-responses to infection and identify other therapeutic targets
TB Immunology Group
Imperial College London

Kerry Millington
Suzie Hingley-Wilson
Damien Montamat-Sicotte
Muhunthan Thillai
Rosalyn Casey
Joanne McDonald
Katrina Pollock
Joanne McDonald
Katrina Pollock
Davinder Dosanjh
Tim Hinks
Valerie Guyot-Revol
David Connell
Hansa Varia
Sarah Hackforth
Murphy Magtoto
Samuel Bremang
Manuela Rehr