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# Vitamin D Metabolites as Clinical Markers in Autoimmune and Chronic illness

**PRESENTED BY DR GREG BLANEY  
(Canada)**

[<http://vimeo.com/1790302>]



**Session: Vitamin D Receptor (VDR) and  
Vitamin D in Autoimmune Disease**

**Chairs: T. Marshall (USA) and H. Amital (Israel)**

**"Vitamin D Metabolites as Clinical  
Markers in Autoimmune  
and Chronic illness"**

**Author: Greg Blaney (Canada)**

**Vitamin D Metabolites as Clinical  
Markers in Autoimmune and  
Chronic Disease**

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## **Co-morbidity in chronic pain patients**

Chronic fatigue  
Fibromyalgia  
Irritable Bowel Syndrome  
Interstitial cystitis  
Chronic prostatitis  
Chronic sinusitis  
Periodontal disease  
Cognitive dysfunction  
Insomnia  
Anxiety  
Osteoporosis  
Hypertension  
Renal calculi

**Dr. Trevor Marshall:** Our next speaker is Dr. Greg Blaney. After a stint teaching at Michigan State, Greg Blaney moved to Vancouver, British Columbia and pursued a clinical practice focused on chronic pain. Dr. Blaney.

**Dr. Greg Blaney:** Thank you for inviting me to come to this prestigious conference. I'm kind of a unique being because I'm a clinician, I'm not a researcher, I'm not an academic but I have been involved in clinical practice for too many years and the last 20+ has been dealing with chronic pain patients and (I'm a Mac guy so you have to give me a little pause cause I'm working with a PC).

In chronic pain patients — chronic pain manifesting most of the time in muscle-skeletal pain but also in neurological pain, discogenic pain, etcetera — in that population of patients there is a number of co-morbid conditions.

It is very common to find chronic fatigue syndrome (fibromyalgia), Irritable Bowel Syndrome, Interstitial cystitis, prostatitis, sinusitis, periodontal disease, osteoporosis, osteoarthritis, hypertension, renal calculi, colitis and various cognitive and central nervous system disorders. And they can be a challenge.

Now, in trying to find a way to support my patients in their chronic pain, there is a fairly limited number of tools. In terms of fibromyalgia and muscle-skeletal pain you know we have biomechanical factors, we have fitness factors, but other than that we are either working with muscle relaxants, NSAIDs — now we have got Neurontin, Lyrica but they have variable effectiveness — and of course often have side effects and will actually aggravate some of the co-morbid conditions.

So in my kind of endeavors to try and discover what may be going on with this patient cohort, I started looking into both infective and inflammatory potential causations.

So first thing that comes up, as you all know, is that the concept of infection presenting as a non-infectious disease. This has been discussed for many, many decades and at this moment in time, certainly recently.

Adult onset asthma now is being seen as a manifestation of a persistent infection. Interstitial cystitis is seen now more and more as being a persistent biofilm infection of the bladder. Certainly recurrent cystitis now is recognized as being a persistent infective disorder, irritable bowel, chronic sinusitis, gingivitis, periodontal

## Infection presenting as non-infectious disease

Adult onset asthma  
Interstitial cystitis  
Irritable Bowel Syndrome  
Chronic sinusitis  
Gingivitis - cardiovascular disease  
Miscarriage and premature labour  
Sarcoidosis  
Macular degeneration

## Para-Inflammation

**"This response relies mainly on tissue-resident macrophages and is intermediate between the basal homeostatic state and a classic inflammatory response. Para-inflammation is probably responsible for the chronic inflammatory conditions that are associated with modern human diseases."**

Nature 454, 428-435 (24 July 2008) Review Article  
Origin and physiological roles of inflammation  
Ruslan Medzhitov

## Persister Cells, Dormancy and Infectious Disease

Several well recognized puzzles in microbiology have remained unsolved for decades. These include latent bacterial infections, unculturable microorganisms, persister cells and biofilm multidrug tolerance. Accumulating evidence suggests that these seemingly disparate phenomena result from the ability of bacteria to enter into a dormant (non-dividing) state.

Over 99% of all species that are present in the environment fail to grow on laboratory media.

Nature Review Microbiology Jan. 2008

## Hypothesis

**Could a mechanism for bacterial persistence be the production of ligand(s) by intracellular pathogens which act as an VDR antagonist resulting in VDR hyporesponsiveness and reflected by elevated levels of 125OH Vitamin D as proposed by Marshall, et al.**

disease. Recently there has been a finding of increased bacterial load present in women who either miscarried or had premature labour. Sarcoidosis has been implicated as being caused by a bacterial infection. And Macular degeneration with Chlamydia trachomatis as being a potential causative agent.

Now, we also find that there is a persistent inflammatory condition going on in my chronic pain patients. This is a recent quote from Nature, 2008, which basically describes this type of inflammation as a para-inflammation, which is expressed, arising from resident macrophage dysfunction.

Now, the other thing that has been coming up again — this is like medicine, things go through cycles and the cycle now is this re-awakening of an interest in persistent and chronic infections. And this is again a recent publication where it states the various aspects of bacteria, both in their nature as well as their ability to evade the immune system, as becoming more and more recognized as a problem.

In terms of bacteria, we are now looking at and recognizing that they have a colony type of pattern and they also have quorum consciousness. And so that, you can almost think of them as like a beehive. You have got the worker bees, we have got the active bacteria, we have got bacteria in that same colony that are designated persister cells, and we also have part of the bacterial load that are in dormant or in very low metabolic rate.

And the other thing that is interesting is that it is being speculated that over 99 percent of all the species that are present in the environment fail to grow on laboratory medium. They also grow very slow, like Tuberculosis bacteria. And so, if you are looking for a pathogen in a urine, in a bladder infection — a chronic recurring urine bladder infection — and you do 24 to 48 hour culture, you may not pick up any significant colony growth and you will have a report coming back that you have no infection. Also, because the adaptive immune system is not functioning very well — it is more an inflammatory condition — that same urinalysis will come back negative for leukocytes.

Now, this is really following up in terms of Dr. Marshall's presentation. So, there I am. I am a clinician, I have a significant number of people who are not functioning very well, who are dependent on medications that are not very effective and have significant side effects and adverse effects. And in terms of some of the more recent drugs, the biologicals, have a significant economic load on their finances.

So, in researching this out, this hypothesis that Dr. Marshall has stated which I have learned about, about five years ago, and that hypothesis was: 'Was there a possibility that one of the ways that bacteria persist in the body, is that its able to sabotage the innate immune response?'

And because, interestingly enough, there seems to be a pathological calcification component to a lot of these conditions



## Physician Initiated Study

100 community based patients with diagnosed Autoimmune and associated chronic diseases, such as CFS, fibromyalgia, type 2 diabetes, metabolic syndrome were measured for serum levels of 25OH Vit D, 125OH Vit D, CRP, CK and ferritin.

## Findings

125OH Vit. D (>110 pmol/L)  
- 85 out of 100 patients showing levels from 110 to 350 pmol/L.  
25OH Vit. D deficiency (<50 nmol/L) -  
- 26 patients out of 100 patients with a range of 20 to 49 nmol/L

## Findings

Elevated CRP (>5.0 mg/L) was positive in 17/100 patients  
Elevated CK (>235 U/L, males, >190 U/L, females) was positive in 12/100 patients  
Elevated ferritin (male > 350 ug/L, <49 y. female > 156 ug/L, >49 y. > 204 ug/L) in 4/100 patients.

## Interpretation

Elevated levels, >110 pmol/L of 125OH Vitamin D was found in the majority of patients exhibiting autoimmune and associated diseases. This finding supports the presence of VDR hyporesponsiveness

and relates to Vitamin D metabolism. And now as we know the nuclear VDR is very important in terms of innate immune response, that this VDR could become hyper-responsive through some mechanism that the bacteria have learned how to do.

So I did my own unfunded study, which is: I took a hundred of my patients and the breakdown was basically, out of that hundred, 3 of them were documented autoimmune diseases, 42 of them were chronic fatigue syndrome (fibromyalgia) and the rest were some metabolic syndrome and there was some, it was called post treatment lyme disease syndrome — patients, basically people who have had some event where they were infected with a zoonotic infection that was treated or not treated and had persistent symptoms afterwards.

And what I did is, I basically measured their serum levels for 25-dihydroxyvitamin D, 1,25-dihydroxyvitamin D, CRP which is a phase 1 protein, CK which I have found in my interest in muscle skeletal medicine was often elevated, and ferritin.

**And what I found was, is that the most sensitive or the most consistent finding was a finding of an elevation of the 1,25-hydroxyvitamin D.**

I used the cut-off point of 110 pmol per liter. If you want to convert that into American terminology feel free to divide by 2.4. And I used that level because that is kind of the consensus as the high normal for 1,25-D. Some researchers have put it at 130 which is when you will start seeing hypercalcemia but in my experience there is not a lot out there in terms of normal values of 1,25-dihydroxy D but that was the best that I came up with. And the ranges were anywhere from 110 up to 350 pmols per liter, which is exceptionally high, with no hypercalcemia, interestingly enough.

The 25-hydroxyvitamin-D I put as deficiency at less than 50 nmols, which I think is a pretty well and an agreed-upon determination of deficiency state. Only 26 of those 100 patients had that finding, with a range between 20 and 49 nmols per liter.

CRP was positive in 17 out of the 100 patients. Elevated CK was positive in about 12, and elevated ferritin was positive in only 4 of that 100 patients.

So, interpretation: Well, interpretation was that elevated levels of 1,25 dihydroxyvitamin D above 110 was a pretty sensitive and consistent indicator — in this small cohort of patients — with autoimmune disease and autoimmune-associated diseases.

At the time I did this, chronic fatigue syndrome was not seen as an autoimmune-type disease, while there was certainly evidence for suggesting that there was altered immunity, but it was not put into that category. It now is.

And this does suggest the fact that there is a presence of the Vitamin D receptor hyper-responsiveness. And again, I am a clinician. The one that stands out for me is type2 diabetes where there is an increased resistance to insulin and one of the

## Protocol & Rationale

Olmesartan - dosing more frequent than normally used for BP control - 40 mg q6-8 h - to reduce levels of angiotensin 11 induced pro-inflammatory cytokines.

Olmesartan also appears to have VDR agonistic properties

## Protocol & Rationale

Bacteriostatic antibiotics - sulfa-trimeth, minocycline, clindamycin & azithromycin - used at very low dose (sub inhibitory) and in a pulsed manner - q2d to q10d

Reduces bacterial virulence factors  
Prevents development of mutant resistant strains

No apparent toxicity

## Therapeutic Trial

Olmesartan 40 mg q6-8h  
Minocycline 25 - 100 mg q2d  
Clindamycin 37.5 - 150 mg q2d  
Azithromycin 25 - 125 mg q10d

manifestations of that is an elevation in insulin levels in the early phases of type2 diabetes and it is only later — even if it does occur — where the insulin levels start dropping, which interestingly enough, is what we find in some of our chronic patients with chronic disease that we are talking about.

So, again, "clinician." I went, "well if this is the case maybe we should try and do something about their condition and use a treatment."

And so, using the information developed by Dr. Marshall and also doing the research into angiotensin receptor blockers and how they are now being increasingly used for non-antihypertensive conditions such as rheumatoid arthritis — where they are using 10mg per kilogram levels of Valsartan in one study — and also being used in research in terms of metastatic cancer — again in 10mg per kilogram dosages — with no toxic effects.

So we used Olmesartan, which, because of molecular modeling information as well as patient response, we used Olmesartan even though you can use some of the other ARB's and we used them in dosages of 40mg every 6 to 8 hours. And with the main intention of reducing angiotensin-II pro-inflammatory cytokines, which are implicated in a lot of these conditions, such as atherosclerosis and many of those co-morbid conditions.

It also, as indicated by modeling, has a VDR-agonistic effect and this is confirmed clinically because that when you use Olmesartan in a significant number of patients they show symptoms of immune activation which we call immunopathology and sometimes will even express to the point where it will be very similar to IRIS, so-called Immune Reconstitution Inflammatory Syndrome that is seen in AIDS-patients with high dose HAART.

Then we went, at the infective component, we used bacteriostatic antibiotics of a variety of type. We initiate with one of them initially and then, after we built up to a certain level of dosage with that, we add a second. Clindamycin in particular is interesting because it has an amplification effect of other antibiotics when given in concert with other antibiotics. We start with fairly significant low dosages: 25mg minocycline typically every 48 hours. Azithromycin we start with 25mg every 10 days. The reason for that is, as you know, azithromycin has a very long half life -60 hours- and has very good tissue penetration and stays in the tissues for a long time.

The intention of using these low dose sub inhibitory levels of antibiotics is to reduce the bacterial virulence factors. Again this is something that has been shown in the research, that if you use low dose antibiotics you don't kill the bacteria and therefore you don't induce resistance or dormancy or persister cells but you do reduce the ability of them to avoid the immune system response and to persist. It also, we've used it in a pulsed manner because in that way there is not a consistent environment of antibiotic concentrations, which again will have a tendency or have



### Case History #1 - MS

DS, 52 y.o., M - MS Dx 1995, wheel chair bound  
April 2005  
25OHD - 79 125OHD - 120  
IgG (IFA) 1:320 Bartonella henselae  
June 2005, initiated Benicar  
July 2005, initiated minocycline  
Feb, 2006, initiated clindamycin  
Dec 2006, full dose Minocycline & clindamycin  
25OHD - 51 125OHD - 90 CK - 48 CRP - 0.91  
IgG (IFA) 1:64 Bartonella henselae

### Case History #1 - MS

Jan 2007 Initiated azithromycin 25 mg q10d, Nov 2007, full dose  
25OHD- 19.9 CK- 66 CRP- 1.0  
Improved muscle strength, decreased tremor, fatigue  
  
Dec 2007  
25OHD- 40 125OHD- 100  
Plateau, some increased muscle weakness  
July 2008  
25OHD- 38.6 125OHD- 84 CRP- 1.7 PTH- 37 ng/ml  
Testosterone (bioavailable) - 5.1 nmol/L

### Case History #2

JZ, 30 y.o., F - CFS x 6 years, positive ANA, +ve IgM, WB Borrelia  
  
Sept 2006  
25OHD - 94 125OHD - 160 CK - 65 CRP - 0.52  
IgG (IFA) Rickettsia rickettsii- 1:32  
  
Nov 2005, initiated olmesartan  
Dec 2005 added 25 mg minocycline q2d  
  
Mar 2006, 100 mg minocycline q2d  
25OHD - 62 125OHD - 100 CK - 121

### Case History #2

May 2006, initiated azithromycin 25 mg q10d  
July 2006, 100 mg azithromycin, Sept 125 mg  
Oct 2006, initiated 37.5 mg clindamycin q2d  
  
April 2008, clindamycin 112.5 mg  
25OHD - 43 125OHD - 200 CK - 60 CRP - 0.1  
IgG (IFA) Rickettsia rickettsii: 1:32 titre  
ANA negative  
  
Significant improvement in CFS symptoms

effectively reduced the development of mutant resistant strains and at dosages that obviously are very low or no toxicity.

I'm going to go real quick in a couple of case histories. This is a 52 year old male with multiple sclerosis diagnosed 1995, wheel chair bound. Initial assessment: his 25-D was 79. His 1,25-D was 120. Interestingly enough he had an IgG, antibody level of 1 to 320 for bartonella henselae and we initiated the therapy so that roughly a year and a half later he was on full dose of minocycline-clindamycin and his 25-D was now into 50 and his reduction of his 1,25. And his bartonella levels went to 64.

We then continued the protocol of that period of time. He had a significant reduced 25-D at that time but at the same time clinically was better. And then we checked him again in 2007, he was starting to go to more normal pattern of 25-D and 1,25-D which is persistent.

Second case is a 30 year old female chronic fatigue for six years, positive ANA at the Mayo clinic. She was also positive for IgM, Western Blot Borellia. Again you'll see that her 25-D was not below 50. She was 94. She had a 160 1,25-D. She also had an evidence of being exposed to rickettsia with no past history of rickettsial infection. She then started to see the same pattern of the 25-D going towards a certain 50 to 60 pattern and the 1,25 dropping below 110.

What is interesting, in 2008, she was continuing to improve symptom-wise but her 1,25 all of a sudden shut up to 200 and this is an expression of this delayed increased immune response that we can characterize as similar to an IRIS pattern, but she became ANA negative.

Then the last one is chronic fatigue syndrome. This one I just want to identify in terms of the questioning 25-D levels as being assumption of Vitamin D storage. We assessed her in August of 2007, her 25-D level was 138 nmols per liter. She had elevated CRP, she had an elevated ferritin and she had an elevated IgG for Anaplasma. Then in December 2007, no therapy initiated, she was down to 96/130 still elevated therapy, then we initiated Olmesartan only and there her 25-D was now down to 65.

So, basically this is what we have observed: With Olmesartan alone we had pretty reliable reduction of 1,25 levels, we have improved symptomatology with 50 percent of the patients we had increased with 10 percent. Then when we add the antibiotics we get aggravation of symptoms through immunopathological response and then later increased inflammatory response. But as treatments progressed the Vitamin D metabolites would tend to normalize down to 25-D between 50 and 70 and 1,25 between 50 and 90.

So, summary: To say 1,25 Vitamin D is a sensitive clinical marker of an autoimmune in chronic disease and reflects para-inflammation and VDR hypo-responsiveness. This para-

### Case History #3

VK, 60 y.o., F - FM, CFS, metabolic syndrome  
Aug 2007  
25OHD- 138  
125OHD- 100  
CRP- 6.49  
Ferritin- 842  
IgG (IFA) Anaplasma 1:128

inflammation could be due to persistent bacterial infection both because of what I have found serologically and also what we have found in response to anti-bacterial medication.

Pro-inflammatory cytokines can be safely reduced with ARB's. Sub inhibitory levels of bacteriostatic antibiotics are effective in reducing bacterial burden.

And finally, resolution of the inflammatory condition is reflected in the normalization of Vitamin D metabolites.

Thank you.

### Case History #3

Dec 2007  
25OHD- 96  
125OHD- 130  
CRP- 7.6  
Ferritin- 814  
Jan 2008, started olmesartan  
Mar 2008  
25OH- 65  
125OHD- 110  
CK- 60  
CRP- 7.7

### Therapeutic Response

Olmesartan alone  
- resulted in reduced levels of 125OHD  
- reduced symptoms in > 50% of patients  
- Increased symptoms in 10% of patients  
Antibiotic dosage increase and addition of synergistic antibiotics resulted in aggravation of symptoms (Immunopathology) and changes in laboratory finding (I.R.I.S)  
As treatment progressed, Vitamin D metabolites normalized - 25OHD - 50 - 70 nmol/L and 125OHD - 50 - 90 pmol/L

### Summary

Elevated 125OH Vitamin D is a sensitive clinical marker in autoimmune and chronic disease and reflects para-inflammation  
Para-inflammation could be due to persistent bacterial infection  
Pro-inflammatory cytokines can be safely reduced with ARBs used more frequently than with anti-hypertensive dosing  
Sub inhibitory levels of bacteriostatic antibiotics slowly and progressively reduce bacterial burden  
Resolution of inflammatory condition is reflected with normalization of Vitamin D metabolites and improved patient-reported outcomes