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"Let There Be Light": The Role of Vitamin D in the Immune Response to Vaccines

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Abstract

Vitamin D's non-skeletal actions, including immunomodulatory role, have been increasingly recognized. Of significance, many immune cells are able to synthesize a biologically active form of vitamin D from circulating 25-(OH) D with subsequent intracrine actions, and the vitamin D receptor (VDR) is broadly distributed. In this review, we discuss vitamin D's potent role in innate and adaptive immune responses and published studies evaluating the impact of serum vitamin D, vitamin D gene pathway polymorphisms or empiric vitamin D supplementation on vaccine immunogenicity. We highlight existing knowledge gaps and propose the steps needed to advance the science and answer the question of whether vitamin D may prove valuable as a vaccine adjuvant for certain vaccines against infectious diseases.

Keywords

Vitamin D; Immunization; Immunity; Innate; Immunity; Cellular; Adaptive Immunity; Vaccines

Introduction

In recent years, new research has illuminated many functions of vitamin D other than its traditionally known roles in calcium homeostasis [1]. We have a greater comprehension of the role of vitamin D in human immunity. The first speculation of vitamin D's role in human immunity was based on observations that heliotherapy used in sanatoria aided in the treatment of tuberculosis. The discoveries of the vitamin D receptor (VDR), its expression in cells of the innate and adaptive immune system, and the capability of antigen presenting cells and lymphocytes to synthesize the active form of vitamin D via local 1-α-hydroxylase, have shed further light on vitamin D's immunomodulatory actions. The goals of this review are to discuss the key immune actions of vitamin D, vitamin D receptor and signaling pathway polymorphisms, and vitamin D's role in the human immune response to vaccines

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including all relevant published clinical studies. Despite escalating knowledge in recent years, significant knowledge gaps remain that merit further study, including examining the potential role of vitamin D as an adjuvant for vaccination.

Background on Vitamin D Physiology, the Vitamin D Receptor and Signal Transduction

An understanding of vitamin D physiology and the vitamin D receptor is required in order to understand vitamin D's role in human immune responses. Vitamin D that is ingested or synthesized in the skin (via UVB spectrum 280-320 nm) is hydroxylated in the liver via microsomal and mitochondrial enzymes to become 25-hydroxyvitamin D (25-(OH) D2 or 25-(OH) D3). 25-(OH) D is the main systemically available form of vitamin D with a half-life of two to three weeks. 25-(OH) D is then hydroxylated by 25-(OH) D-1-α-hydroxylase (mitochrondrial cytochrome P450 isoenzyme CYP27B1 and microsomal CYP2R1) in the kidneys to form 1,25-dihydroxyvitamin D (1,25-$(OH)_2$D). 1,25-$(OH)_2$D, a steroid hormone, is the most active form of vitamin D that is transported to target tissues [1,2]. The main vitamin D transport proteins are the vitamin D-binding protein (DBP) and albumin (with lower affinity) [3]. 1,25-$(OH)_2$D serves as the ligand for the nuclear vitamin D receptor (VDR) with subsequent transcription related effects,[1,2] as is illustrated in Figure 1 [1,4].

VDR is extensively distributed across many tissues and works in conjunction with other transcription factors. VDR forms a heterodimer with nuclear retinoid X receptor alpha (RXRA) upon binding with 1,25-$(OH)_2$D, which then mediates intra-cellular signaling and its nuclear actions. RXRA plays a key regulatory role in pathways connected to vitamin D binding to VDR and downstream signaling. VDR also regulates gene expression for genes that contain promotors with particular DNA sequences, called vitamin D response elements (VDRE) [1,3]. Due to its direct transcriptional activity, in addition to gene expression regulation, VDR affects key physiological processes involving bone health, bone mineralization, detoxification, cell life processes (including proliferation, differentiation, migration and death), immune actions, as well as cell metabolism [1,4].

The important extra-skeletal actions of vitamin D have now attracted tremendous interest [5]. There were two initial observations that supported the potent immunomodulatory role of vitamin D. Firstly, many cells from the innate and adaptive immune system express VDR. In addition, antigen presenting cells (macrophages and dendritic cells) and T and B lymphocytes express CYP27B1 (1-α -hydroxylase), which leads to local production of the active 1,25-$(OH)_2$D3 from serum 25-(OH) D, thereby leading to tissue-specific intracrine and paracrine actions. These immune cells express CYP27B1 only when activated by an invading organism. This facilitates enhanced generation of the antimicrobial peptides cathelicidin and beta-defensin 2, autophagy and other cytokine mediated effects [4]. Activation of toll-like receptors has also been shown to upregulate expression of 1-α-hydroxylase enzyme and VDR in monocytes and macrophages, likely resulting in increased transcriptional activity of vitamin D [6].

CYP27B1 is present in many non-renal sites in addition to immune cells, specifically epithelial cells of the gut, skin, prostate, breast, lung, bone and parathyroid glands.

Regulation of non-renal CYP27B1 appears to be different than that in the kidney. PTH does not influence CYP27B1 activity in these cells, nor in immune cells [4]. The vitamin D intracrine system is dependent on sufficient 25-(OH) D substrate to generate local 1,25-$(OH)_2$D3 for many tissue specific actions [1]. In turn, 1,25-$(OH)_2$D3 and FGF23 (fibroblast growth factor) inhibits CYP27B1 gene transcription via a negative feedback loop [4].

The action of vitamin D in tissues is dependent upon the local synthesis of 1,25-$(OH)_2$D3 from 25-(OH) D, transport of adequate concentrations of 1,25-$(OH)_2$D3, expression of sufficient VDR and RXR co-receptor proteins, and regulation of transcription of select genes [1]. Serum 25-(OH) D levels serve as a reliable indication of vitamin D status. 1,25-$(OH)_2$D levels are finely maintained regardless of vitamin D stores due to compensatory secondary hyperparathyroidism; therefore, they are inaccurate for determining vitamin D deficiency, especially in the early stages. Maintaining adequate or high-circulating 25-(OH) D levels is necessary for these extra-skeletal actions of vitamin D. However, the specific serum 25-(OH) D level that would be relevant to vitamin D's immune actions has not yet been defined. There are also inter-individual variations in production of vitamin D, dietary intake, VDR and other pathway genetic polymorphisms that might make a universal recommendation for an ideal "target" 25-(OH) D level difficult given our relative lack of knowledge.

Vitamin D Receptor and Vitamin D Signaling Pathway Gene Variants

VDR and *RXR* are highly polymorphic genes. *VDR* gene alterations may have functional consequences, specifically regarding gene activation, and/or alterationsin VDR protein structure. The commonly studied polymorphisms are *Fok*I, *Bsm*I, *Apa*I, *and Taq*I. The latter three polymorphisms are apparently "silent" polymorphisms; however, they could have a regulatory role in gene expression, or may be linked to other functional sequences that have not yet been discovered [6-8].

The *Fok*I is a candidate functional polymorphism and is not in linkage disequilibrium (LD) with the aforementioned polymorphisms. It occurs at the 5' end of the *VDR* gene in exon II, with a resultant change in the structure of coded protein. The T-> C change in the *F* allele produces a VDR protein that is three amino acids shorter than the protein encoded by the *f* allele. The *F* allele derived protein has greater activity than that of the *f* allele [6,7,9,10].

VDR gene polymorphisms, including those noted above, have been associated with greater infection susceptibility and perturbations in the natural course of some infections, such as HIV-1, hepatitis B, RSV, human T-cell lymphotropic virus type-1 (HTLV-1), leprosy, tuberculosis and dengue [9,11-15]. A majority of the evidence showing associations between *VDR* gene polymorphisms and infectious diseases arises from clinical case-control studies in certain populations with many other confounding variables. Such findings need further replication before they can be taken as definitive. In addition, the functional consequences of various *VDR* gene polymorphisms and mechanistic pathways that influence the acquisition and natural course of these infections have not been defined.

Vitamin D and Impact on the Immune System

Several *in vitro* studies have elucidated vitamin D's actions (both 1,25- $(OH)_2$ D and substrate 25-(OH) D) on innate and adaptive immune cells. These studies were not performed in subjects following vaccination, but they do provide evidence for the physiological role of vitamin D in immunity, as well as in response to certain pathogens. In addition, there are a few animal studies demonstrating the impact of immunization with the use of vitamin D as an adjuvant [16-18].

Vitamin D and the Innate Immune System

Support for vitamin D's role in the innate immune system, specifically macrophages, first came from observations related to *Mycobacterium tuberculosis* (MTB). Rook *et al*. showed 1,25-$(OH)_2$ D could inhibit the growth of MTB [19]. 1,25-$(OH)_2$ D has been demonstrated to augment the fusion of phagosome and lysosome in macrophages infected with MTB, independently of IFN-γ dependent macrophage activation [6].

Liu *et al*. also noted that killing of MTB only occurred for cells that were cultured in serum that had sufficient 25-(OH) D levels. Human monocytes and macrophages with higher VDR expression of and CYP27B1 had greater activation of TLR2/1 when exposed to a lipoprotein from MTB. There was also induction of production of antimicrobial peptide cathelicidin [20,21]. Cathelicidin has direct bactericidal effects in humans, is toxic for MTB, and also acts as a chemo-attractant for various immune cells (monocytes, T cells, neutrophils) [6,21]. The expression of cathelicidin is induced by 1,25-$(OH)_2$D3 in both myeloid and epithelial cells [6,20-22]. Recent research suggests vitamin D may impact the formation of reactive oxygen species and boost IL-1β expression, which in turn impacts antimicrobial peptide production.[23,24] The majority of supportive evidence for vitamin D's action on the innate immune system is based on studies involving mycobacterial and granulomatous diseases. Although there is burgeoning literature on the association of vitamin D's role in the course of respiratory viral infections and influenza, there still remains a notable knowledge gap with respect to vitamin D's actions on the innate immune system inresponse to other bacterial and viral pathogens. [5,25-34] . It appears cathelicidin has an effect on disease severity and course for certain viral and bacterial infections, and this is an area of active research. [23,35,36]

Vitamin D and the Adaptive Immune System

Vitamin D has a notable role in the adaptive immune system, which is partly illustrated in Figure 1. Intracrine synthesis of 1,25-$(OH)_2$D by dendritic cells (DCs) decreases their maturation, with resultant suppressed antigen presentation and decreased T cell proliferation. This intracrine expression is also linked to generating tolerogenic regulatory T cells (Tregs) and suppressing Th17 cells.[2] 1,25-$(OH)_2$D3 inhibits differentiation of DCs from monocytes or murine bone marrow-derived precursors. There is also inhibition of surface expression of co-stimulatory molecules, including IL-12 production. 1,25-$(OH)_2$D3 increases IL-10 production, which opposes IL-12 induced Th1 responses. 1,25-$(OH)_2$D3 inhibits IFN-γ and IL-2 producing Th1 cells, and IL-17 and IL-22 producing Th17 cells. There is a shift to a Th2 phenotype with increased production of IL-4, IL-5, and IL-10.

CD4+/CD25+ Treg cells are increased with increased IL-10 production, which may be the mechanism for blocking of Th1 and Th17 development [4,37,38]. The other mechanism for increased CD4+/CD25+ Treg cells may be via increased FoxP3 expression that is induced when DCs are treated with 1,25-$(OH)_2$D3. FoxP3 expression is essential for Treg generation and IL-10 production, which is important in immune tolerance [20]. In summary, the main effects of vitamin D on cellular immunity are the bias toward a Th2 phenotype, compared to Th1 and Th17, as well as enhanced Treg cells.

The influence of vitamin D on humoral immunity, specifically B cells and antibody production, is less clear. 1,25-$(OH)_2$ D3's role in modulating human B cell differentiation were examined in an *in vitro* study involving healthy subjects. 1,25-$(OH)_2$D3 was found to inhibit activated B cell proliferation while promoting the apoptosis of these cells, and similarly inhibit the production of plasma cells and memory B cells. 1,25-$(OH)_2$D3 has been found to regulate B cell mRNA expression for 1α-hydroxylase, 24-hydroxylase and VDR, which are all key in the action of vitamin D. These results suggest B cells can directly respond to 1,25-$(OH)_2$D3. 25-(OH) D had comparable effects on purified B cells, although this was observed at higher concentrations compared with the active form [37,38]. The overall impact of vitamin D's immune actions may be in establishing immune homeostasis and playing an immunomodulatory role [5,25,27,39].

Impact of Vitamin D on Immune Response to Vaccines

With the various aforementioned actions of vitamin D on the immune system, it is intriguing to consider the impact of vitamin D and/or the VDR and vitamin D pathway gene polymorphisms on the adaptive immune response to vaccines. The impact of such understanding could be in the design of better vaccines using vitamin D or vitamin D-like analogs as an adjuvant.

Several *in vivo* studies involving adult mice vaccinated subcutaneously or intramuscularly with inactivated vaccine co-administered with 1,25-$(OH)_2$D3 demonstrated production of antigen-specific mucosal immunity (IgA and IgG antibodies), as well as enhanced systemic immune responses [16-18]. The studies involved inactivated polio vaccine (IPV) [16], *Haemophilus influenzae* type b oligosaccharide conjugated to diphtheria toxoid vaccine [17], and hepatitis B surface antigen (HBsAg) [18]. The observation of induction of mucosal immunity is significant, as the traditional paradigm suggests this requires direct antigen presentation at the mucosal surface [40].

Influenza Vaccine

There has been great interest in the role of vitamin D deficiency in viral respiratory illnesses and influenza in children and adults, as well as its influence on immune response to influenza vaccine. Table 1 summarizes the studies evaluating the effect of vitamin D status and/or vitamin D supplementation on the immune response to TIV (trivalent inactivated influenza vaccine). The largest prospective observational cohort study involved 1,103 adult volunteers aged > 50 years old in Marshfield, WI, and Nashville, TN, spanning two influenza seasons (2008-2009 and 2009-2010) [41]. Mean vitamin D levels were 31+/- 11 ng/mL in year 1, with similar levels in year 2 of the study, and 25% of the population was

defined as vitamin D deficient (vitamin D < 25 ng/ml). The end point was a hemagglutination-inhibition (HAI) titer with evaluation for rate of seroprotection (HAI titer≥40) and seroconversion (≥4 fold rise in HAI post-vaccination). Vitamin D serostatus had no association with the achieving seroprotection or seroconversion in either season in unadjusted, as well as adjusted (logistic regression), analyses. Cell-mediated immune outcomes were not measured in this study [41].

Another large prospective cohort study of 221 children in Hutterite communities in Canada evaluated the impact of baseline serum vitamin D level on HAI response post-vaccination with TIV and found no effect of baseline vitamin D levels on antibody response to TIV. Post-vaccination HAI titers were measured at a median time of 11 weeks following the initial HAI titer (IQR 6.9, 21.3). The median serum 25-(OH) D level was 24.4 ng/ml (IQR 20, 28.4 ng/ml). No association found between baseline vitamin D level and seroprotection or seroconversion (i.e., HAI titer ≥40 if pre-vaccination titer < 10, or four-fold rise in titer if pre-vaccination titer ≥10) against any of the vaccine strains. There was a suggestion of decreased odds of seroprotection with baseline vitamin D levels below 20 ng/ml, but this was not statistically significant. One study limitation is baseline serum vitamin D levels in this cohort were relatively high. The authors acknowledge the study's sample size had limited power to detect a meaningful effect [42].

Other studies investigating the effect of vitamin D on influenza vaccine response were not necessarily methodologically geared to address the basic premise in terms of generalizability and external validity. These studies involved select specific populations, specifically HIV-positive patients [43] and prostate cancer patients [44]. Higher HAI response was seen in vitamin D-replete subjects in the study involving prostate cancer patients [44].

Three studies have examined the influence of vitamin D supplementation on influenza vaccine response. A placebo-blinded trial involving young adult volunteers who had received an intramuscular injection of calcitriol (or placebo) at the time of influenza vaccination did not find any significant difference in post-vaccination HAI response between the treatment and placebo groups. However, neither baseline nor post-calcitriol serum vitamin D levels were measured in this study [45]. The second study was conducted in a cohort of 116 children (mean age 3.0 +/- 1.0 y) who had not previously received influenza vaccination. Fifty-nine children received vitamin D supplementation for four months, and 57 received placebo. The baseline vitamin D levels at initiation of the study were similar in both groups. The immune response to TIV was measured at four months, and there was no correlation between vitamin D level and seroprotection. This study revealed no significant difference in post-vaccination immune response between the treatment and placebo groups [46]. Finally, Antonen *et al.* evaluated the HAI response in patients on dialysis who had received TIV [47]. The enrolled subjects included 42 hemodialysis (HD) patients, 15 continuous ambulatory peritoneal dialysis (CAPD) patients, 20 patients with renal impairment but who were not on dialysis, and 31 cardiac patients with normal renal function. Twenty of the HD patients were treated with calcitriol (14 with oral formulation, and six with intravenous) due to secondary hyperparathyroidism. Serum 1,25-$(OH)_2$D3 levels were measured and, as expected, there was a dose-response relationship noted; the lowest levels were seen in the group that was not on calcitriol, with higher levels observed in

the group on oral calcitriol, and finally, the highest levels were found in the group on intravenous calcitriol. HAI titer was measured at baseline and week five. HD patients were least likely to have a protective titer (defined as postvaccination titer log≥1.6 for at least two of the antigens). Although numbers were small in this subset analyses, there appeared to be a dose-response relationship of calcitriol on HAI response in the HD patients. Multiple regression analyses revealed calcitriol treatment as the most significant variable (p=0.06); however, none of the other variables were statistically significant. The effect of serum 1,25-$(OH)_2D_3$ level, which was available for both HD and CAPD patients, was not analyzed in univariate or multivariate analyses [47].

There are conflicting results and limitations to these studies, as discussed in Table 1. All the aforementioned studies only evaluated HAI response without any measurement of cell-mediated immune outcomes. Notably, these studies did not have sufficient numbers of patients with vitamin D deficiency and likely had limited statistical power to detect differences. Although the exact threshold for vitamin D deficiency with respect to non-skeletal, and specifically immunomodulatory, actions has not yet been defined, it is still imperative to study patient cohorts with an adequate range of serum vitamin D levels (from <25 ng/ml, or even 10 ng/ml, to > 50 ng/ml). Additional studies that evaluate the role of vitamin D receptor and signaling pathway polymorphisms in influenza vaccine immune responses are also needed.

Measles Vaccine

To our knowledge, no studies have examined the role of vitamin D levels on the outcome of measles vaccine immune responses. Ovsyannikova and Poland *et al.* used a candidate gene approach to study the impact of genetic variations in vitamin D (*VDR*/ *RXRA*) and vitamin A receptor genes (*RARA, RARB, RARG*) on the adaptive immune response to measles vaccine. 745 healthy children who had received two documented doses of measles, mumps, rubella (MMR) vaccine were enrolled in this study. Their DNA was genotyped using the Illumina platform. There were notable associations found between intronic polymorphisms in *RXRA* (rs6537944, p=0.0010), *VDR* genes (rs2239181, p=0.08), and allele-dose related IFN-γ ELISPOT responses [48]. *VDR* and *RXRA* gene polymorphisms were associated with measles-virus induced cytokine responses. The *RXRA* genotype GG (SNPs rs6537944 and rs3118571) was associated with greater IFN-γ ELISPOT and secreted IFNλ-1 responses in Caucasians. There was a three-fold difference in IFN-γ response between the homozygous minor allele genotype of *RXRA* rs6537944 when contrasted with the homozygous major allele genotype, with the former having a higher response. This study showed that allelic variants in the *VDR* and *RXRA* genes were associated with inter-individual measles vaccine responses [48]. Vitamin D levels were not assessed in this study.

The *RXRA* gene is a moderator of vitamin D signaling pathway and is involved in both B cell antibody production [49] and cytokine responses to measles and rubella vaccine [48,50]. Furthermore, an association with a specific intronic SNP, rs1805352, in the *RXRA* gene and with antibody response to smallpox vaccine was discovered [51]. This suggests that RXR pathways play a role in vaccine immune response.

Rubella Vaccine

In another study, 714 healthy 11-19 year-old children who had received two doses of rubella-containing vaccine were assessed for rubella-vaccine induced cytokine immune response .. [50] These subjects were genotyped using a candidate gene approach that included select genes involved in innate immune response, including the vitamin D receptor (*VDR*), *RXRA*, and select genes in the vitamin A receptor family. The goal was to determine the impact of polymorphisms in these selected genes on the cytokine immune response to rubella vaccine. There were significant associations found between polymorphisms in promoter and intronic regions of *VDR* and *RXRA* genes with rubella-specific cytokine responses. The minor alleles for SNPs rs7970314 and rs11568820 in the VDR gene were associated with an allele dose-related reduction in TNF-α levels in response to rubella vaccine [50]. The rs11568820 SNP is located in the *VDR*'s promotor region and is a functional polymorphism. It can affect *VDR* transcription, where the G allele is associated with diminished transcription compared to the A allele [52,53]. Similarly, for SNP rs3118536 in the *RXRA* gene, the minor allele was associated with an allele dose-related decrease in rubella-specific IL-10 levels. To our knowledge, no studies have evaluated the role of vitamin D levels in rubella vaccine immune responses.

Hepatitis B vaccine

The strongest support for a relationship between vitamin D and vaccine response may be for the hepatitis B vaccine response in chronic kidney disease (CKD) patients. One retrospective study noted a clear association has been demonstrated between a lack of hepatitis B vaccine seroconversion (post-vaccination anti-HBs titer of ≥ 10 IU/L) and vitamin D-deficient patients with CKD and very low vitamin D levels (<10 ng/ml). This study evaluated antibody response to three doses of 40 μg recombinant hepatitis B vaccine among 200 patients with CKD stages 3-5. Thirty-five percent of patients in this retrospective cohort of 200 patients had vitamin D levels < 10 ng/mL. Only 57% of patients mounted an antibody response ≥10 IU/L [54]. The seroconversion rate for patients with vitamin D levels < 10 ng/mL was lower compared to those who had levels ≥ 10 ng/mL (45 % vs 64%, p =0.011). The vitamin-D deficient group also had lower median anti-HBs antibody titers. Non-responders had lower 25-(OH) D levels compared to responders (12.9+/-6.5 ng/mL vs 15.1+/- 7.4 ng/mL; p=0.034). In a multivariate analysis (logistic regression) model, vitamin D deficiency was one of the independent significant negative predictors of seroconversion. [54] This finding may partially explain why, on average, only 50-60% of ESRD (end-stage renal disease) patients seroconvert following hepatitis B vaccination compared to more than 90% of the general population [55]. The impact of very low serum vitamin D levels on hepatitis B vaccine response likely varies based on the specific stage of kidney disease and whether the patient is on hemodialysis, since advanced kidney disease and HD is associated with its own immune-related changes [56].

Grezegorzewska *et al.* studied associations between polymorphisms in vitamin D binding protein (GC), *VDR* and *RXRA* genes with hepatitis B vaccine response in patients on hemodialysis [57]. A total of 915 subjects were included: 692 were hepatitis B vaccine "responders" and 223 were "non-responders." "Responders" were subjects who had post-vaccination anti-HBs titers of ≥ 10 IU/L following primary vaccination with hepatitis B

(four doses of 40 ug vaccine), or after any additional vaccine doses following the primary vaccination. “Non-responders” were subjects who failed to develop anti-HBs titers of ≥ 10 IU/L following three or more vaccine doses after the primary vaccination series. 25-(OH) D levels were measured in a randomly selected cohort of 184 patients (135 responders, 49 non-responders). 25-(OH) D level was not associated with vaccine response, although, as mentioned, levels were only measured in a subset of these patients. Men had higher 25-(OH) D levels compared to women (median 15.3, range 5.1-41.6 ng/ml compared to median 11.8, range 4.5-50.0 ng/ml; p=0.00002) [57]. A number of variables were significantly different between responders and non-responders. Younger age, longer time since initiation of hemodialysis, higher PTH levels, male gender, and chronic glomerulonephritis as a cause of renal disease were factors that were positively associated with seroconversion to hepatitis B vaccine. These variables were used for adjustment in association analyses for genetic polymorphisms. The AA genotype of *VDR* rs1544410 polymorphism was associated with a higher odds of non-response (adjusted OR 1.50; 95% CI 1.17-1.94; p=0.002) compared to the GG genotype [57]. The functional significance of this polymorphism is not entirely clear. In certain tissues, there is some evidence that the A allele of the *VDR* rs1544410 polymorphism is associated with lower levels of VDR mRNA,[58] but this difference was not seen in PBMCs [59]. Additional studies are needed to replicate this finding.

The potential association between vitamin D deficiency (particularly with very low vitamin D levels <10 ng/ml) and lack of hepatitis B vaccine response in patients with CKD merits additional study among different stages of chronic kidney disease with larger cohorts in order to determine if the findings can be replicated. Close attention to vitamin D and other biochemical markers important in bone metabolism is standard clinical practice in the care of patients with CKD. Studying the role of vitamin D (and effectiveness of supplementation) in hepatitis B vaccine immune response in this population is highly relevant due to the high incidence of deficiency. It is also important to determine if vitamin D levels are associated with sub-optimal hepatitis B vaccine response in other populations.

BCG Vaccine

There has been a large variability in reported BCG vaccine efficacy. A meta-analysis that included more than ten prospective trials and a dozen case-control studies attempted to address efficacy of BCG vaccine in preventing disease from tuberculosis. The overall protective efficacy was found to be 51%. Higher rates of protection were seen against disseminated TB, TB meningitis, and death due to TB. lAbout forty per cent of the between-study variability of BCG vaccine efficacy among 13 prospective studies was explained by latitude, as shown by analysis from a two-covariate, random-effects regression model. BCG vaccine efficacy was higher in places further from the equator. There was no hypothesis, or proposed explanation provided for this observed association [60].

Geographic latitude has a known influence on temperature, humidity, UV radiation and skin pigmentation. Other factors could be modified with latitude: host-microbe interactions, population genetic susceptibility to TB, and transmission factors could all impact an assessment of BCG vaccine efficacy [61]. The relationship of sunlight to vitamin D status is likely not a linear one; other factors such as ethnicity, skin pigmentation, population

genetics, dietary intake, etc., play a role. Nonetheless, the above observation, as well as several studies describing the protective effect of vitamin D on *M. tuberculosis* disease, [10,62] raises the question of any possible impact of vitamin D status on BCG vaccine response. The published studies in this field have yielded disparate results.

A study involving 79 BCG-vaccinated infants in the UK examined 25-(OH) D levels at three months (n=47), and 1 year post-BCG vaccination (n=37), with IFN-γ response to the MTB purified protein derivative (PPD) test. IFN-γ response was assessed by testing supernatants from diluted whole blood stimulated with MTB PPD for six days. There were 32 and 28 age-matched unvaccinated controls at the respective time-points. BCG-vaccinated infants with greater 25-(OH) D levels (> 30 ng/ml) possessed significantly lower IFN-γ responses to MTB PPD compared to infants with lower vitamin D levels at three months post-BCG vaccination.. A similar, but weaker inverse association was observed at 1 year post-vaccination. . The authors suggested vitamin D may play an immunomodulatory role in the immune response to BCG vaccine [63]. As discussed earlier, vitamin D inhibits IFN-γ producing Th1 cells. It enhances phagosome and lysosome fusion in TB infected macrophages via a mechanism that is independent of IFN-γ driven macrophage activation.

A recent study conducted in China enrolled 597 infants who did not have a PPD response (i.e., < 0.5 cm) following routine BCG vaccination at birth, and divided them into an intervention group and a control group. All of these infants were revaccinated with the intervention group receiving oral vitamin A (1,500 IU) and vitamin D (500 IU) supplementation for three months, and the control group was revaccinated but did not receive supplementation. PPD response was measured again after three months. The rate of PPD response was higher in the supplementation group (96.1% compared to 89.7% in the control group, $p<0.05$; prevalence ratio 1.07, 95% CI 1.02-1.12). The authors also found a positive correlation between BCG scar formation and PPD response ($r=0.17$, $p<0.05$). The results from this study suggest there is a positive impact of vitamin A and D supplementation on response to BCG vaccine, but there are a number of limitations. There was no objective lab assay to assess immunological response, vitamin D serum levels were not measured, and there may have been dietary and nutritional factors (breastfeeding versus non-breastfeeding) that could influence the results [64]. Furthermore, it is important to note that tuberculin skin test reactivity after BCG vaccination does not correlate with protective immunity [61]. In order to evaluate the relationship between vitamin D levels, vitamin D receptor polymorphisms, and immunogenicity with BCG vaccine or future tuberculosis vaccines, the correlates of protection for *Mycobacterium tuberculosis* will need to be defined [65].

Pneumococcal, Meningococcal, *Haemophilus influenzae* serotype b (Hib) Vaccine

Eighty-five asplenic patients who had been vaccinated against *S. pneumoniae* using the 7-valent pneumococcal conjugate vaccine (PCV7) (n=40), MenC (n=77) and/or Hib (n=61) had serum samples collected prior to vaccination and 21 days after vaccination for IgG antibody detection. There was overlap among subjects between the three vaccinations. Patients were classified as responders to the pneumococcal vaccine where post-vaccination antibody titers were ≥1.0 ug/mL for ≥5/7 pneumococcal serotypes. Response to MenC

vaccine was defined as antibody titer ≥ 2.0 ug/mL after vaccination. Response to HiB vaccination was defined as antibody titer ≥1.0 ug/mL after vaccination.

Serum 25-(OH) D levels were measured prior to vaccination. The median vitamin D level in this cohort was 66 ng/ml (IQR 39-74 ng/ml). The serum 25-(OH) D levels were similar in responders and hypo-responders to the pneumococcal vaccine. No association was found between serum 25-(OH) D level and specific antibody titer to any single pneumococcal serotype. There was also no association observed between vitamin D level and vaccination response to *Neisseria meningitidis* type C vaccine. Differences could not be assessed in the HiB-vaccinated group, as there were only two hypo-responders. There was a weak negative correlation between anti-Hib IgG titers and serum 25-(OH) D (r=-0.343, p=0.008).

There are limitations to this study. All the vaccines used were protein-conjugated vaccines, where T-cell dependent B-cell responses can occur in secondary lymphoid tissue, and antibody response assessment alone may not be adequate. Some patients had received vaccination 3-5 years prior to inclusion in the present study, and in those patients the vaccine given in this study might have been a booster vaccination, thus confounding possible associations. Most patients were also vitamin D sufficient [39].

In a separate study, baseline vitamin D was evaluated in 25 children with idiopathic nephrotic syndrome who had received the 23-valent pneumococcal vaccination. Pneumococcal antibody levels were measured at baseline, and at intervals 1 month, 3 months, and 6 months after vaccination. Antibody response after vaccination ranged from a 2- to 50-fold increase. There was no association between baseline levels of 1,25-$(OH)_2$D3 or 25-(OH) D with antibody response at any time-point. Antibody response was also not explained by patient age, plasma albumin or immunosuppression [66]. To our knowledge, there have not been any published studies in other patient populations evaluating the role of vitamin D in the response to polysaccharide or conjugate pneumococcal vaccination.

Tetanus Toxoid

Thirty-two healthy adults who had last received tetanus/diphtheria immunization at least five years prior, and were due to receive a booster immunization, were enrolled in a double-blind intervention study in Germany [67]. Twenty subjects were randomized to receive oral vitamin D supplementation (daily 2000 IU vitamin D3) for a period of nine weeks, and the rest received placebo (neutral oil) for the same duration. 25-(OH) D levels were assessed before and after the supplementation period. All subjects received tetanus/diphtheria toxoid booster immunization at the end of supplementation period. The two groups did not differ based on demographics or vitamin D levels at baseline. The median 25-(OH) D level increased significantly in the supplementation group (80.3 nmol/L from 28.4 nmol/L), and decreased in the placebo group. TT-specific IgG increased in both groups following booster immunization, and this was slightly higher in the vitamin D group (p=0.04). There was also a positive correlation between an increase in 25-(OH) D level following the supplementation period and change in anti-tetanus-IgG following vaccination (r=0.351, p=0.042) [67]. The immunogenicity and efficacy of tetanus toxoid vaccine in preventing tetanus is high across most age groups; however, adults with chronic renal failure have been noted to have lower antitoxin levels after tetanus toxoid vaccination than healthy controls [68-70]. These

populations would benefit from additional studies to determine if vitamin D deficiency is related to the decreased tetanus toxoid vaccine responses.

Expert Commentary

The published literature regarding the role vitamin D plays in the human immune response to vaccines demonstrates disparate findings. Clearly, not all vaccines elicit the same immune response phenotype regardless of vitamin D status. If vitamin D does indeed play a role in the immune response to vaccines, it is likely to differ for live attenuated viral or bacterial vaccines, toxoid vaccines, inactivated viral, inactivated bacterial polysaccharide or conjugate vaccines. Most of the studies in this review had small sample sizes, were retrospective, and were not adequately powered to specifically evaluate the role of vitamin D in the outcome of vaccine response.

Strategies for examining the potential role of vitamin D in immune response to vaccines should consider several issues. First, studies assessing the role of vitamin D in vaccine response must include subjects who have a large range of vitamin D levels (including sufficient numbers of subjects who have low vitamin D levels). Such studies should then proceed to boost baseline serum vitamin D levels to a threshold conducive to observing its immunomodulatory actions (not yet defined). It is important to highlight that the vitamin D level used to define “deficiency” is based on its effects on bone health and calcium homeostasis, which cannot be necessarily extrapolated to its immune relevant actions. Heaney elegantly discusses considerations for clinical studies involving response to nutrient effects and highlights how the model of deficiency being equal to one specific disease may not be all encompassing. [71,72] Importantly, the nutrient dose-response curve may be sigmoid-shaped instead of linear. Consideration of the nutrient dose-response curve should inform the recruitment of subjects with a certain nutrient basal status and ensure the change in intake spans the ascending limb of the curve to investigate effects and benefit. An illustrative example is the inverse association of vitamin D with insulin resistance. [73] The strongest association with insulin resistance was seen for 25-(OH) D levels in range 16-36 ng/ml, and there was no clear benefit for levels higher than that range. [73] Similarly, the optimal threshold/range for vitamin D’s immune relevant actions needs to be defined in the context of local populations. A recently completed population based study did not find any evidence of acute clinical toxicity (hypercalcemia) with increased incidence of 25-(OH) D levels above 50 ng/ml. [74]

A second strategy for study design would be to administer exogenous calcitriol to increase levels quickly and in a short time frame prior (days to weeks) to vaccination in select groups at high risk of vitamin D deficiency. A third strategy could be co-formulating certain vaccines with vitamin D or vitamin-D like analogs to examine if vitamin D can function as a vaccine adjuvant. Animal studies have had success when co-administering vitamin D at the time of vaccination (for inactivated polio, hepatitis B, *Hemophilus influenza*e conjugated to diphtheria toxoid) vaccines [16-18]. However, the exact mechanism behind vitamin D’s association with enhanced antibody production and mucosal immunity was not entirely defined. Mechanistic studies looking at differences in gene expression, cytokine response, and other systems-level interactions will be important to further understand this.

Additional studies are also needed to evaluate the immune response to vaccines in a more comprehensive manner (e.g., assess innate, humoral and cellular immune responses) and include sufficient number of vaccine non-responders to detect meaningful differences and correlations. The overwhelming majority of the studies we reviewed did not meet these criteria. It is also important to note that the role of systemic 25-(OH) D status by itself may be too small to stand out separately. It may exert a larger influence in partnership with a host of other factors. We still do not understand the functional consequences of several *VDR* gene and other vitamin D pathway gene polymorphisms, which will certainly impact any associations observed. For this reason, we again emphasize the need for systems biology-level studies in order to better understand and detect such interactions.

There are also certain populations with a higher incidence of vitamin D deficiency that are of public health importance with respect to the immunological response to vaccination and, thus, disease prevention. It may be that vitamin D may play a different role in vaccine response among patients who are vitamin D deficient and have CKD versus those who do not have any medical comorbidity; other relevant groups include the elderly, the obese, and patients with CKD. Patients with CKD have sub-optimal hepatitis B vaccine response, especially at more advanced stages,[56] and vitamin D did appear to have a role in immunogenicity [54]. Further studies involving the role of vitamin D in immune response to other vaccines in CKD are also needed.

As part of the aging process, the skin becomes less efficient in making vitamin D (for the same amount of UVB exposure), and the kidneys are less able to make the active metabolite [2]. The elderly also face immunosenescence with observed reduced vaccine efficacy [75,76]. Ensuring a vitamin D-replete state in this population may be a potentially modifiable factor that could favorably impact vaccine response.. Large cohort studies have demonstrated that increased body mass index (BMI) and body fat are strongly and inversely related to serum 25-(OH) D concentrations [3]. It has been increasingly recognized, specifically with pandemic influenza A/H1N1,[77] that the obese population has a more severe clinical course with certain infectious diseases. Therefore, the obese population represents a vulnerablegroup for whom better designed vaccines could have a potentially significant preventive impact.

It is noteworthy that UV radiation has been shown to diminish resistance to certain local and systemic infections in experimental animal models. The evidence in humans is much more limited, but UV light has been shown to trigger the reactivation of latent infections such as HSV (Herpes Simplex Virus), VZV (Varicella Zoster Virus), and HPV (Human Papilloma Virus). [78] There are a few studies investigating the effect of UV light or season at time of immunization on the immune response to vaccination in humans. [79,80] UV-induced DNA damage and certain mediators are thought to be involved, and individuals with certain cytokine polymorphisms (IL-1β) are more susceptible to immune effects of UV exposure. [78] There are numerous unanswered questions including mechanisms of UV induced immune effects, and certain variables are key (such as nature of UV light; i.e., natural vs artificial, spectrum (UVA or UVB), dose, duration, inter-individual differences, etc.) in future studies. Findings from animal models cannot be directly extrapolated to humans. The role of vitamin D in this aspect is controversial and unclear. In fact, 1,25-$(OH)_2$ D has been

shown to be photoprotective and reduce UV induced DNA damage. [81] Studies aiming to investigate the effect of homeostatic 25-(OH) D levels and pharmacological supplementation on immune response to vaccination should also attempt to factor in UV exposure/season of study in addition to other variables discussed.

Five-Year View

Determining whether vitamin D will prove to be a promising adjuvant for vaccination will require further study. Vitamin D's role in innate, humoral, and cellular immune responses to different classes of vaccines (bacterial, viral, polysaccharide, protein, etc.) remains unclear and yet is amenable to scientific inquiry. Understanding the functional and mechanistic relationships of vitamin D on vaccine response via well designed studies, which take into account geographic, ethnic, population genetic and dietary variations, will be important. The first steps involve conducting well designed studies across an adequate sample size of subjects with a sufficient range of vitamin D levels and immune outcomes to be able to detect whether vitamin D does indeed play a role in vaccine responses. Studying vitamin D signaling pathway polymorphisms and other high throughput assays is also important. Systems biology-level studies adequate to examine multiple factors simultaneously (and better reflecting the human *in vivo* milieu) are imperative.

There are a number of active studies registered on Clinicaltrials.gov investigating the impact of vitamin D on vaccine immunogenicity. A randomized clinical trial (NCT01561989) is planning to enroll healthy volunteers in two arms of vitamin D supplementation (high dose 4000 IU cholecalciferol and lower dose 400 IU) who will receive influenza vaccination with assessment of impact of 25-(OH) D levels on humoral immune outcomes at week four [82]. Another supplementation study (NCT01262300) plans to examine impact of vitamin D on VZV-specific CMI and humoral immune outcomes in older individuals receiving Zostavax® [83]. A phase 2 trial (NCT01893385) with vitamin D supplementation in elderly subjects (with 25-(OH) D < 30 ng/ml) is looking at variation in plasma levels of cathelicidin and impact on influenza vaccine response [84]. A randomized trial with vitamin D supplementation and assessment of immune response to tetanus vaccine (NCT01399151) has been completed in April 2014, but results are not yet available [85]. Since vitamin D is also being studied with great interest with respect to its role in auto-immune diseases and multiple other chronic diseases, it is possible that concerted interventions will prove beneficial in furthering the science, while positively impacting public health and prevention of infectious diseases.

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* Of interest

** Of considerable interest

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Key Issues

- Vitamin D has various immunomodulatory actions, including potent actions on the innate immune system, enhancing production of antimicrobial peptide, and biasing toward a Th2 skewed phenotype
- The vitamin D level/threshold that is relevant to immune actions has not been defined, as current definitions of deficiency are based on effects on bone health.
- Vitamin D's role has been examined in the immune response to vaccines in studies looking at vitamin D levels as well as vitamin D signaling pathway polymorphisms (influenza, hepatitis B, measles, rubella, BCG vaccine, pneumococcal, meningococcal, etc.), but the results have been variable, and such studies remain un-replicated to date.
- Higher HAI response to influenza vaccine was seen in vitamin D replete patients in a small study involving prostate cancer patients. There was suggestion of dose-response relationship of improved HAI response in HD patients who were receiving calcitriol in a separate study. Similarly, vitamin D deficiency was an independent negative predictor of seroconversion to hepatitis B vaccine in patients with CKD stages 3-5. Anti-tetanus specific IgG responses were noted to be higher in patients who received vitamin D supplementation compared to placebo, and this group had higher 25-(OH) D levels.
- Certain *VDR* and *RXRA* gene polymorphisms were associated with measles and rubella vaccine induced adaptive immune responses in two separate studies. A single study found an association with a particular *VDR* gene polymorphism with higher odds of non-response to hepatitis B vaccine.
- Animal studies have shown superior immunogenicity with vitamin D co-administered with inactivated polio vaccine, hepatitis B, and *Hemophilus influenzae* vaccines
- Elderly, obese and CKD patients have a higher incidence of vitamin D deficiency, and often have suboptimal vaccine responses, hence they remain important patient populations to study
- Future studies need to include patients with a wide range of vitamin D levels and vitamin D gene polymorphisms
- Mechanistic and systems biology-level studies are also needed, examining strategies of either boosting homeostatic levels, or co-administering vitamin D with vaccine.

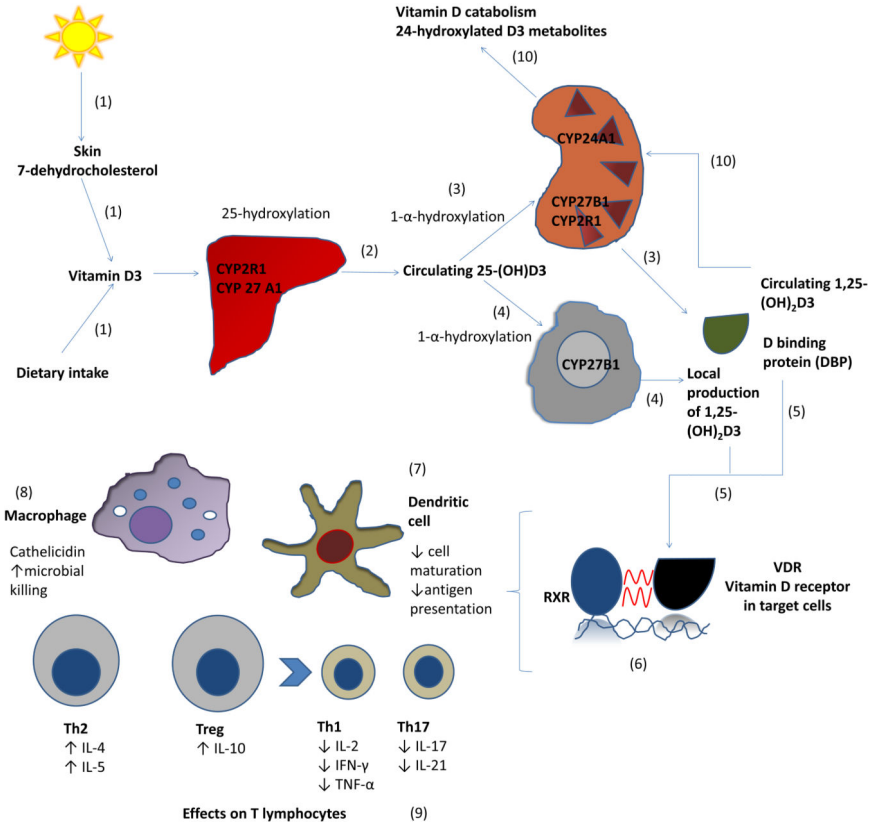

Figure 1.

Overview of vitamin D biosynthesis, metabolism, transport to target sites with binding to VDR and transcription related effects, including effects on various immune cells. (1) Sources of vitamin D3 in the body include dietary intake, and biosynthesis in the skin from 7-dehydrocholesterol upon exposure to UVB light (2) Vitamin D3 is transported to the liver where it hydroxylation to 25-(OH)D3 by 25-hydroxylase in the liver via microsomal and mitochondrial enzymes (i.e. CYP2R1, CYP27A1). (3) Circulating 25-(OH) D3 in the kidney then undergoes further hydroxylation by 1-alpha-hydroxylase (CYP27B1) to form the biologically active form of vitamin D, 1,25-$(OH)_2$D3. (4) Several target cells, including immune cells such as human T lymphocytes, dendritic cells and macrophages, have the capacity for local production of 1,25-$(OH)_2$ D3 from circulating 25-(OH) D substrate, as these cells also express 1-α-hydroxylase (CYP27B1) when activated by an invading organism. (5) Circulating 1,25-$(OH)_2$D3 is transported to target cells while bound to D binding protein. Locally produced 1,25-$(OH)_2$ D3 as well as transported circulating 1,25-$(OH)_2$D3 then binds to nuclear Vitamin D receptor in target cells. (6) The vitamin D receptor forms a heterodimer with the RXR receptor. VDR regulates gene expression for genes which contain vitamin D response elements, with downstream transcriptional changes. (7) Vitamin D's effects on dendritic cells includes decreased cell maturation and decreased antigen presentation. (8) There is induction of anti-microbial peptide, cathelicidin production when macrophages are exposed to the active form of vitamin D with increase microbial killing. This is seen specifically in MTB. (9) Vitamin D promotes a shift to a more Th2 phenotype and Treg profile, compared to Th1 and Th17. There is increased production

of Th2 mediated cytokines, and reduced IL-2, IFN-γ, TNF-α. This effect on T lymphocytes may either be a direct effect of Vitamin D or indirect via its effects on antigen-presenting cells. (10) Circulating 1,25-$(OH)_2$ D3 undergoes catabolism to 24-hydroxylated D3 metabolites in the kidney, mediated by CYP24A1.

Table 1

Vitamin D and response to Influenza Vaccine

Author/Journal	Population	Vaccine response/End point	Findings/ Conclusion	Comments
Sundaram M. *et al.*[41]	1,103 healthy adults Age > 50 yo 2008-9 (591 subjects), 2009-10 (512 subjects)	Pre and 21-28 day post-vaccination HAI titers	25% vitamin D deficient (< 25 ng/ml) No association found between baseline vitamin D level and odds of seroprotection.	Increased sero-protection for seasonal A/H1N1 not others season 1. Low prevalence of vit D deficiency (28%). No cell-mediated immune response (CMI) measured.
Science M. *et al.* [42]	391 children 3-15 years of age (221 had post-vaccination titers) Hutterite communities, Canada Single-dose 2008-09 TIV	Pre and post-vaccination HAI titers. Post-vaccination titers performed at median time of 11 weeks following initial HAI titer (IQR 6.9, 21.3).	Median serum 25-(OH) D level was 61 nmol/L(IQR 50.0, 71.0). No association found between baseline vitamin D level and seroprotection or seroconversion	Suggestion of decreased odds of seroprotection with baseline vitamin D < 50 nmol/L, but not statistically significant. Relatively high baseline vitamin D levels. Variability in timing of post-vaccination HAI titer. No CMI measured.
Cooper C. *et al.*[43]	298 HIV infected adults (18-60 yo) on antiretroviral therapy. Median CD4 T cell count 470 cells/mm^3, 76% were virologically suppressed.	3 vaccine groups: one group received 2 standard doses 28 days apart, second group had 2 double doses, and last group received a single standard dose. HAI titer week 4, 8, 20	Looked at vitamin D supplementation (28-37% of patients were on supplemental vitamin D). No correlation between vitamin D use and sero-protection.	Vitamin D levels not measured. Vitamin D doses/ formulations varied. Subjects had high baseline HAI titer.
Chadha M. *et al.*[44]	35 prostate cancer patients Median 25- (OH) D 45 (9-72 ng/ml)	HAI > 1:40 titer ratio or 4X rise at 3 months	Higher response in vitamin D replete (p=0.046)	19 were on vitamin D supplementation. HAI response measured at 3 mo. HAI response was only measured for 1 strain influenza A/H3N2.
Kriesel J. *et al.*[45]	175 healthy volunteers Age 32+/- 8 yo 1.0 mL (1.0 μg) of intramuscular calcitriol administered at time of vaccination	HAI titer at 0, 30 and 90 days	No significant difference in HAI titer.	Vitamin D levels not measured. Calcitriol was administered at the same time as the vaccine.
Principi N. *et al.*[46]	116 children with history of recurrent acute otitis media Age 3.0 +/- 1.0 yo	2 doses TIV (trivalent inactivated vaccine). Vitamin D 1000 IU/daily or placebo for 4 mo HAI titer measured at 4 months.	No difference in HAI titer measured at 4 mo between the two groups.	HAI titer measured at 4 mo. Vitamin D analyzed as categorical variable. No CMI measured.
Antonen J. *et al.* [47]	42 HD patients, 15 CAPD patients, 20 patients with renal impairment but not on dialysis, and 31 cardiac patients with normal renal function. 20/42 HD patients were treated with calcitriol (14 with oral formulation, and 6 with intravenous).	Single vaccination standard dose TIV. HAI titer measured at baseline and week five.	Subset analyses (small numbers): appearance of dose-response relationship of calcitriol on HAI response in HD patients. Multiple regression analysis showed calcitriol as almost statistically significant variable (p=0.06) influencing HAI response.	$1,25\text{-}(OH)_2D3$ levels were available for both HD and CAPD patients, but not analyzed in univariate or multivariate analyses. No CMI measured.