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Minireview

Vitamin D in Renal Transplantation—From Biological Mechanisms to Clinical Benefits

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Recent developments in our understanding of vitamin D (VitD) show that it plays a significant role in immunological health, uniquely occupying both an anti-microbial and immunoregulatory niche. VitD deficiency is widespread among renal transplant recipients (RTRs), thus providing one patho-mechanism that may influence the achievement of a successful degree of immunosuppression. It may also influence the development of the infectious, cardiovascular and neoplastic complications seen in RTRs. This review examines the biological roles of VitD in the immune system of relevance to renal transplantation and evaluates whether VitD repletion may be relevant in determining immunologically related clinical outcomes in RTRs (including graft survival, cardiovascular disease and cancer). While there are plausible biological and epidemiological reasons to undertake VitD repletion in RTRs, there are few randomized-controlled trials in this area. Based on the available literature, we cannot at present categorically make the case for routine measurement and repletion of vitamin D in clinical practice but we do suggest that this is an area in urgent need of further randomized-controlled level evidence.

Keywords: Cancer, cardiovascular disease, immune system, renal transplantation, transplant rejection, vitamin D

Abbreviations: $1,25(OH)_2D_3$, 1,25-dihydroxy-vitamin D; 25(OH)D, 25-hydroxy-vitamin D; CKD, chronic kidney disease; CVD, cardiovascular disease; DC, dendritic cell; FGF-23, fibroblast growth factor-23; IFN, interferon; PTH, parathyroid hormone; RTR, renal transplant recipient; RTx, renal transplant; Tr1 cells, type 1 regulatory T cells; Treg, regulatory T cells; VDR, vitamin D receptor; VDRA, vitamin D receptor agonist; VitD, vitamin D

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Introduction

The biology of vitamin D (VitD) is highly topical at present, with significant research being carried out in the contexts of cardiovascular, autoimmune and allergic conditions, chronic kidney disease (CKD) and cancer (1). A recent systematic review of prospective observational studies showed that VitD deficiency (definitions of VitD status are given in Table 1) is a significant determinant of all-cause mortality in patients with CKD (2). Renal transplant recipients (RTRs) have a high prevalence of VitD deficiency versus controls (3). This arises for several reasons, including the mild-to-moderate degree of renal functional impairment that characterizes most allografts (causing loss of renal tubular CYP27B1 [1-alpha-hydroxylase]), raised serum concentrations of fibroblast growth factor 23 (FGF-23) (4), immunosuppressive drugs inducing VitD catabolism (5) and medically advised sun-avoidance behavior (see below). FGF-23 actively inhibits VitD through suppression of CYP27B1, reducing 1-alpha-hydroxylation of 25-hydroxy-vitamin D (25(OH)D) and induction of CYP24A1, which enhances calcitriol and 25(OH)D degradation (6) (Figure 1). The natural history of 25(OH)D and 1,25-dihydroxy-vitamin D ($1,25(OH)_2D_3$) in incident RTRs has been reviewed elsewhere (7); while the skeletal, renal and gastro-intestinal effects of VitD on calcium and phosphate homeostasis are well known, with VitD deficiency linked to increased risk of postrenal transplantation (post-RTx) bone mineral loss and fractures (8). VitD is also recognized to exert effects on both the innate and adaptive immune systems. In so doing, VitD status in RTRs can affect immunologically driven posttransplant outcomes, notably allograft rejection, transplant function and development of *de novo* posttransplant malignancies. This minireview examines the immunological effects of VitD that are of relevance to RTx and evaluates existing clinical evidence for VitD measurement and repletion in this cohort.

Table 1: Current definitions of vitamin D status based on 25(OH)D levels

Definition	Equivalent 25(OH)D serum level (UK)	Equivalent 25(OH)D serum level (US)	Notes
Vitamin D toxic	>375 nmol/L	>150 ng/mL	(69)
Vitamin D sufficient	>75 nmol/L	>30 ng/mL	
Vitamin D insufficient	50–75 nmol/L	20–30 ng/mL	
Vitamin D deficient	<50 nmol/L	<20 ng/mL	Recent increase in threshold from <11 ng/mL has led to an estimated increase in prevalence from 2% to 14% (70)

25(OH)D, 25-hydroxy-vitamin D.

Immunological Effects of VitD Relevant to RTx (Figure 2)

The VitD receptor (VDR) is ubiquitously expressed in immune cells, including activated $CD4^+$ and $CD8^+$ T lymphocytes, and cells of the innate immune system, such as macrophages and dendritic cells (DCs). Immune cells not only express the VDR but may contain the machinery for producing biologically active $1,25(OH)_2D_3$ through inducible expression of the CYP27B1 (9). These findings, along with strong epidemiological evidence linking VitD deficiency to multiple autoimmune diseases, suggest a physiological role for VitD in immune homeostasis. Experimentally, VitD metabolites, particularly $1,25(OH)_2D_3$, have multiple effects on immune system functioning, instructing both anti-microbial and immunoregulatory functions.

Immunoregulatory actions of VitD

VitD has clear effects on immune system functioning, characterized by inhibition of proliferation (10), IL-2 (11) and interferon (IFN)-γ production by $CD4^+$ T cells (12) and reduced cytotoxicity of $CD8^+$ T cells (13). While VitD also enhances IL-4 production by $CD4^+$ T cells, its ability to enhance regulatory T cell (Treg) differentiation is particularly important. Not only does VitD induce differentiation of suppressive $FOXP3^+$ Tregs (14), the most critical of immuno-Tregs for the prevention of autoimmune diseases in humans, but also IL-10-producing $FOXP3^-$ type 1 Tregs (Tr1 cells) (15) as well as IL-10-producing B cells (16). Although a definitive role for Tr1 cells or IL-10-producing B cells in transplant survival has not previously been described, $FOXP3^+$ Treg numbers infiltrating transplanted tissues do correlate, in general, with improved outcomes (17).

The immunomodulatory effects of VitD are mediated both through direct effects on T cells and indirectly through modification of DC function (18). DCs play a central role in the initiation, magnitude and quality of the adaptive immune response and modification of their function by VitD is clearly of relevance to transplantation as both passenger and recipient DCs are critical for induction of direct and indirect alloresponses, respectively (19). VitD inhibits the maturation and antigen-presenting capacity of DCs and induces them to behave in a ''tolerogenic'' manner preferentially stimulating naïve T cells both *in vitro* and *in vivo* (20) to mature into $FOXP3^+$ Tregs and Tr1 cells and enhancing the suppressive activity of these Tregs (21). Inhibition of DC-derived IL-12 production by VitD is also of great relevance as IL-12 is a central mediator in Th1 differentiation, a cell population intimately associated with transplant rejection.

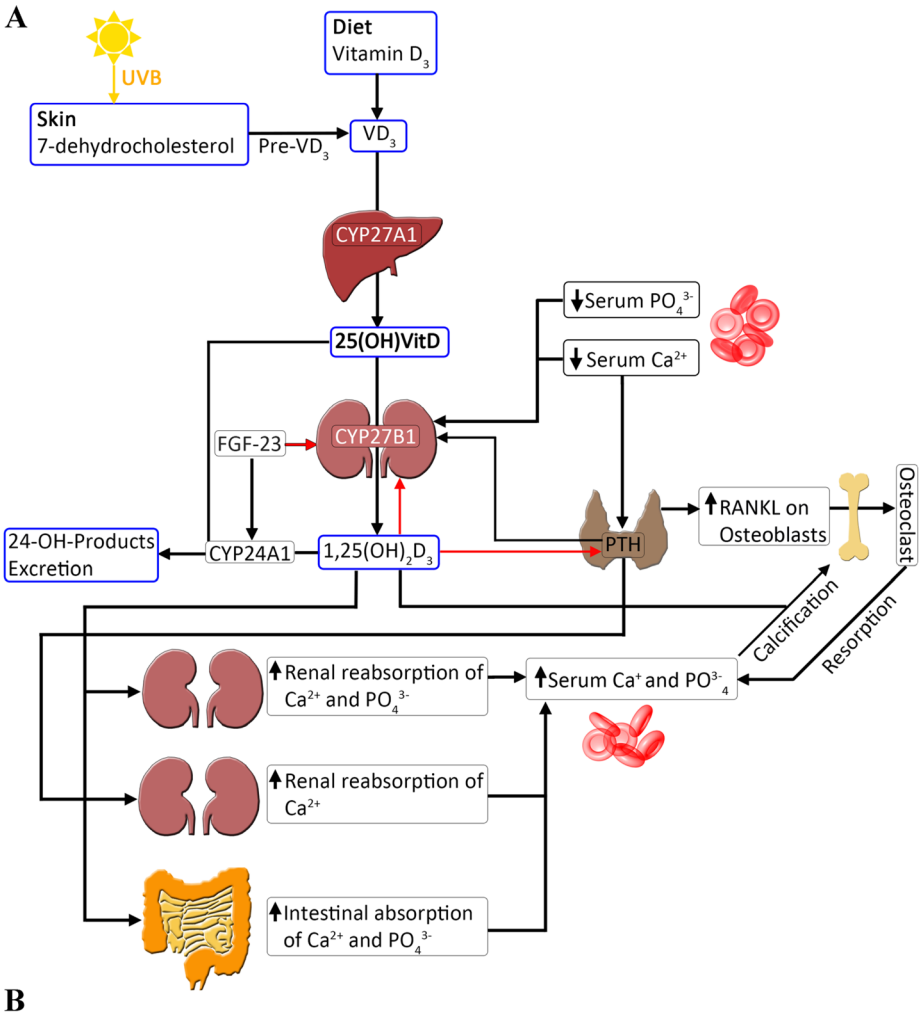
VitD also regulates chemokine-chemokine receptor interactions, key steps in migration of inflammatory cells to sites of allograft rejection (22). The CXCL10-CXCR3 axis is particularly important in transplant rejection, with levels of CXCL10 being associated with rejection in human transplant recipients (23). CXCL10 is secreted by immune cells as well as resident cells of tissues and organs (23) and recruits multiple immune cells, including T cells, natural killer cells, macrophages and DCs through engagement of CXCR3. Thus, CXCL10 plays a role in the initiation and maintenance of Th1 alloresponses (24). VitD decreases CXCL10 secretion by tubular epithelial cells, thus inhibiting immune cell infiltration of renal transplants and potentially protecting against allograft rejection (25).

Anti-microbial actions

Monocyte activation with IFN-γ or lipopolysaccharide results in up-regulation of both CYP27B1 as well as the VDR (26). Autocrine engagement of the VDR results in production of natural anti-microbial peptides, such as cathelicidin and β-defensin 4 (27), enhancing innate immune clearance of pathogen. Production of cathelicidin is further increased by the presence of pro-inflammatory IL-17, synergizing to remove inciting pathogens. Likewise, active ($1,25(OH)_2D_3$) VitD can be stimulatory to other innate immune cells, such as monocytes and macrophages, promoting proliferation and secretion of highly inflammatory IL-1 (11).

How can these immunological functions impact on transplant outcomes?

The balance between regulatory and inflammatory immune components is a key determinant of graft outcomes, resolution of chronic infections and responsiveness to neoantigens such as cancerous cells. From an immunological



B

Enhancing CYP27B1	Inhibiting CYP27B1
Hypocalcemia	FGF-23
Hypophosphatemia	$1,25(OH)_2D_3$
PTH	Dietary calcium intake*
Dietary calcium intake*	

Figure 1: Effects of vitamin D on mineral biology. (A) Schematic showing biogenesis of vitamin D. Vitamin D_3 derived from either the diet or UVB irradiation in the skin is metabolized to 25-hydroxy-vitamin D (25(OH)D) in the liver through an enzymatic reaction catalyzed by CYP27A1. 25(OH)D is subsequently metabolized to the active form 1,25-dihydroxy-vitamin D ($1,25(OH)_2D_3$) in the kidneys by CYP27B1. Both 25(OH)D and $1,25(OH)_2D_3$ are converted by CYP24A1 to 24 hydroxylated products and excreted. CYP27B1 is tightly regulated: a drop in serum calcium levels is detected by the parathyroid gland and results in secretion of parathyroid hormone (PTH). Both PTH and reduced serum calcium and phosphate concentration directly stimulate CYP27B1 activity, and thus increased $1,25(OH)_2D_3$ production. $1,25(OH)_2D_3$, in a negative feedback loop, down-regulates its own production through inhibiting CYP27B1 activity as well as PTH production. $1,25(OH)_2D_3$ has multiple systemic effects that ultimately result in restoration of serum calcium levels, as well as re-calcification of bones. Fibroblast growth factor-23 is produced by osteocytes and decreases circulating concentrations of $1,25(OH)_2D_3$, through induction of CYP24A1 and suppression of CYP27B1. In the schematic, black arrows represent induction and red arrows represent inhibition. (B) Factors controlling CYP27B1 activity. *A low-calcium diet reduces extra-renal CYP27B1, particularly in the colon, and enhances renal CYP27B1.

perspective, the dual functions of VitD (anti-microbial vs. immunoregulatory) appear counterintuitive; however, these functions are context- and time-dependent and carefully regulated, with the balance between the two in any given situation, dictating outcome. By modulating adaptive immune responses and down-regulating DC proliferation, maturation and antigen presentation capacity, VitD can ameliorate the risk of transplant rejection. Additional mechanisms, including regulation of chemokines responsible for leukocyte infiltration and down-regulating renal TGF-β1 production (which has pro-fibrotic activity), may also inhibit the evolution of rejection in RTx (28). The ability of VitD to inhibit cell growth, promote apoptosis, alter cell adhesion and inhibit metastasis and angiogenesis is of

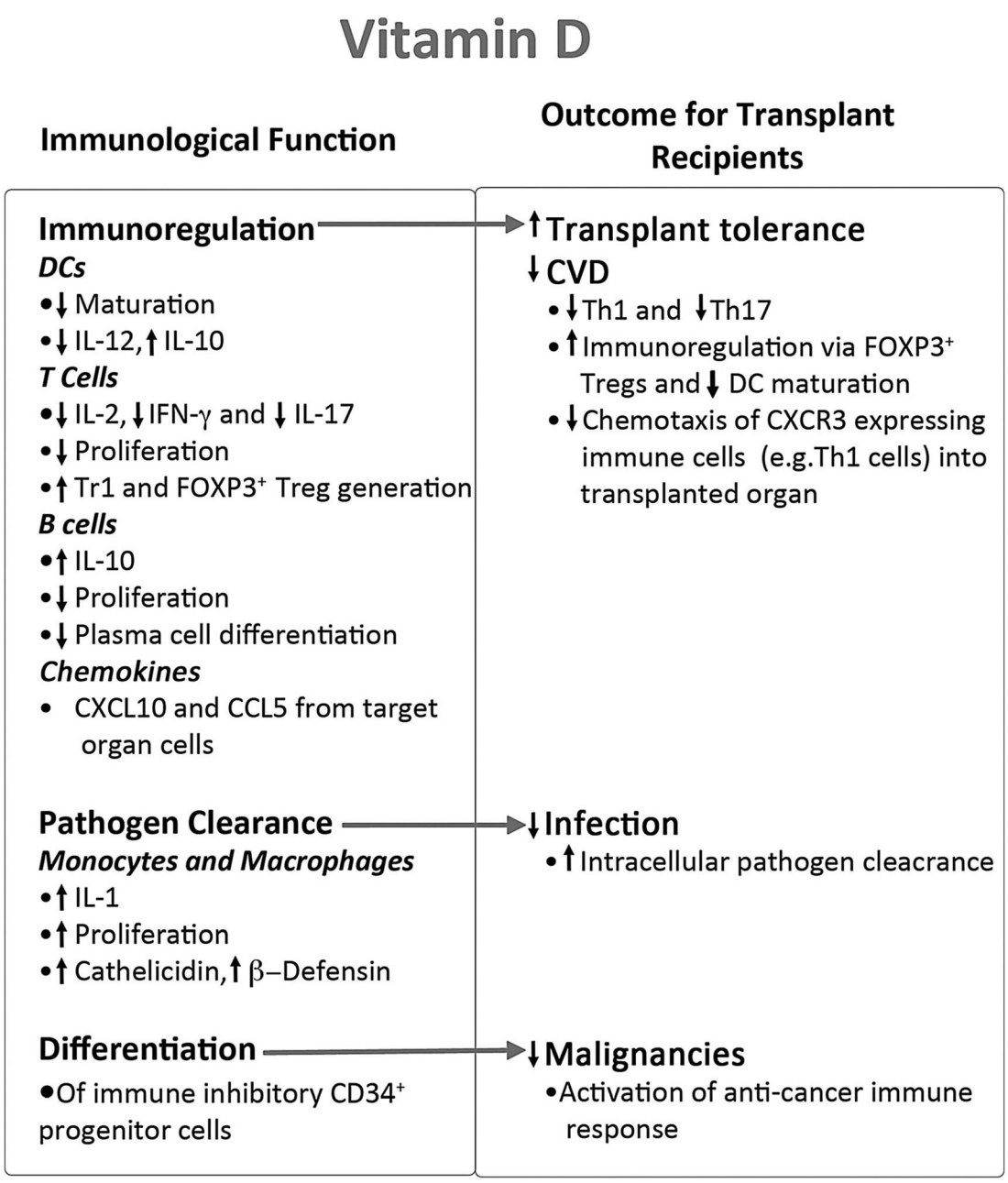


Figure 2: Biological functions of vitamin D in the immune system and their potential relevance to transplantation. The biological impact of vitamin D on different immune parameters are shown on the left and the mechanisms by which these effects may impact on renal transplantation is indicated on the right.

great relevance to the risk of cancer development in RTRs (see below), as is the ability of VitD to induce differentiation of immune inhibitory $CD34^+$ progenitor cells (observed in higher amounts in some cancers) (29). These potential protective roles of VitD are supported by multiple empirical observations.

Experimental evidence from animal models shows that survival of allografts of bone marrow, heart, kidney, liver, pancreatic islets, skin and small intestine is significantly prolonged by administration of VitD and its analogues (30), with increased resistance to opportunistic infections (31), supporting the assertion that immunomodulation by VitD is a determining factor of outcomes. Additionally a small (nine donors and nine transplant recipients) prospective study in which donors received calcitriol therapy, which was then continued in the recipients, showed an expansion of $CD4^+CD25^+$ Tregs in the calcitriol-treated group (32).

Another small prospective study treating 24 transplant recipients with calcitriol observed decreased costimulatory molecule expression (HLA-DR, CD28, CD86 and CD40) on white blood cells. Together these studies provide evidence of the immunomodulatory properties of VitD receptor agonists (VDRAs—active VitD compounds, such as calcitriol and paricalcitol) after transplantation. VDRAs could thus be used as potentially immunomodulatory agents in RTx. Calcitriol analogues, such as paricalcitol, which could exert immunomodulatory activity with a lower risk of causing hypercalcemia, have been developed for clinical use for secondary hyperparathyroidism (33,34).

VitD Repletion Studies in RTx

Given plausible biological links between VitD and the pathophysiology of diseases endemic in the RTR population, the clinical evidence for VitD repletion in RTRs is reviewed here, excluding those predominantly focusing on skeletal outcomes, which are reviewed elsewhere (35). It should be noted that important clinical safety data for VitD repletion can be found in three separate comprehensive Cochrane reviews of bone disease in nondialysis, dialysis and RTx (8,36) where adverse effects of VitD repletion were described only in the minority of studies (4/16 studies in CKD, 8/60 studies of dialysis and 0/23 studies in RTx) suggesting it is generally a well-tolerated and safe therapy. However, higher repletion doses than those used in these studies are needed to bring serum levels significantly above 30 ng/mL (75 nmol/L).

VitD and allograft outcomes (Table 2)

Given the immunomodulatory effects of VitD, it has been hypothesized that reduced serum 25(OH)D concentrations are associated with poorer graft outcomes. Reduced serum 25(OH)D concentrations in RTRs is commonplace (37). Three out of four observational studies published to date draw a direct link between VitD levels and allograft outcomes (summarized in Table 2). Notably, in an observational study of 90 Polish RTRs, 25(OH)D deficiency at time of transplantation was significantly associated with delayed graft functioning and an increased risk of acute rejection episodes over a 2-year follow-up period (38). This would be clinically highly significant as both of these are known risk factors for graft fibrosis and impaired allograft function. The other two observational studies showed an association between 25(OH)D levels at time of transplantation and renal function over a 2- to 4-year follow-up period (39,40). The more recent study of 634 patients (40), demonstrated an association between low serum 25(OH)D at 3 months posttransplantation and increased risk of interstitial fibrosis/tubular atrophy on 12-month transplant biopsies at, but not with mortality. The fourth observational study is not directly comparable to the first three as it was carried out in a pediatric cohort with stable graft function some time (mean $\pm$ SD 4.9 ± 0.5 years) after transplantation (41). Given the low event rate (only 6 patients out of 64 had a decrease in GFR of $\geq$50% and there were only 14 acute rejection episodes), this was an underpowered study to determine the effects of VitD on long-term transplant function.

Interventional studies of VitD supplementation in the context of RTx have also yielded conflicting data, most likely attributable to difference in patient selection, control group selection, time since transplantation, VitD repletion regimen and formulation of VitD. These caveats mean that it is difficult to directly compare study cohorts and formulate an ideal repletion strategy. While supplementation posttransplant with calcitriol was associated in three studies with either reduced numbers of acute rejection episodes (42,43), better transplant function (44) and improved graft survival (43) a smaller interventional study, using cholecalciferol in the first year posttransplantation, gave conflicting results (45). There are significant difficulties in conducting clinical VitD research, which are elaborated below, but these trials can be individually critiqued. The data set of Tanaci et al (42) is a retrospective small series with baseline imbalances between osteoporotic and non-osteoporotic cohorts; the study of Özdemir et al (44) does not disclose the calcitriol dosing regime and has a surprisingly high late rejection rate in the control group while Courbebaisse et al (45) was not a randomized prospective study and the repletion strategy only achieved a mean 25(OH)D concentration of 31.8 ± 7.1 ng/mL, arguably below the nephroprotective threshold. Some of the discrepancy between studies may also be explained by the lack of a contemporary control population in the latter study.

In conclusion, there is an association between serum VitD concentrations and allograft outcomes; however, the evidence for causality has yet to be tested in an RCT.

VitD and cancer (Table 3)

RTRs are at a three- to fivefold increased risk of developing malignancies compared to the general population and an inverse correlation between general population serum 25(OH)D concentrations and the risk of solid organ malignancies (especially breast and colorectal cancer) is observed epidemiologically (46).

Limited observational epidemiological data exist analyzing VitD status and *de novo* malignancies in RTRs (47,48). The shorter of the two studies (47), with a 3-year follow-up period, describes a significant increase in malignancy risk with VitD deficiency, with a hazard ratio of 1.12 for every 1 ng/mL decline in 25(OH)D_3. However, a longer follow-up study with the same number of patients found no association over a 10-year follow-up period between VitD levels and risk of *de novo* malignancy (48). Further work is needed to establish whether these results can be explained by risk segregation with cancer type, particularly viral-related cancers. A single interventional

Table 2: Clinical studies of the correlation between vitamin D and allograft function

Study	Design	Study population and use of vitamin D	Outcome and notes
Observational studies			
Falkiewicz et al 2009 (38)	Prospective study of adult transplant recipients (n = 90) with measured $1,25(OH)_2D_3$ on day 3, months 1, 6, 12, 18 and 24 posttransplant	Patients were followed up for 24 months The effect of $1,25(OH)_2D_3$ levels on outcomes (incidence of acute rejection, graft function, *de novo* malignancy and cardiovascular events) was analyzed	All patients had received alfalcalcidol as part of routine care pretransplant. Despite this, severe $1,25(OH)_2D_3$ deficiency was present in 83% on day 3. In only 50% the concentration rose to normal levels during follow-up The incidence of delayed graft function was higher in those with $1,25(OH)_2D_3$ deficiency. There was a negative correlation between initial and 1 month $1,25(OH)_2D_3$ levels and graft function during follow-up. Those with $1,25(OH)_2D_3$ deficiency had poorer outcomes (death from cardiovascular events, acute rejection episodes, graft loss and cancer)
Wesseling-Perry et al 2011 (41)	Prospective analysis of pediatric transplant recipients with stable transplant function at recruitment (n = 68)	Associative study analyzing link between mineral ion abnormalities and GFR/acute rejection over a 2-year follow-up period. Measurement of 25(OH)D, $1,25(OH)_2D_3$ and FGF-23 was made at mean ± SD 4.9 ± 0.5 years posttransplant and correlated with transplant outcomes over the next 2 years	Four patients were lost to follow-up, so only 64 were included in the analysis VitD levels do not, but FGF-23 levels do, correlate with number of episodes of acute rejection and decline in eGFR over 2-year follow-up
Kim et al 2012 (39)	Observational study of adult transplant recipients (n = 106) with known VitD levels prior to transplantation	Measurement of 25(OH)D pre- and posttransplantation with exclusion of osteoporotic patients. Patients were followed up every 6 months for 36 months	Pretransplant VitD deficiency was identified in multiple logistic regression analysis as a significant independent risk factor for decline in eGFR over 36 months posttransplantation
Bienaimé et al 2013 (40)	Prospective cohort study of adult transplant recipients (n = 634) with measured 25(OH)D levels at 3 months posttransplant	Measured 25(OH)D levels at 3 months posttransplantation were correlated with clinical variables over a median follow-up of 48.6 months	19 patients were lost to follow-up and 30 had lost their graft; 28 had died with a functioning graft There was no association between 3-month VitD levels and either graft loss or death during the follow-up period 25(OH)D level at 3 months was an independent predictor of mGFR and progression of IF/TA at 12 months

(*Continued*)

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Interventional studies			
Tanaci et al 2003 (42)	Retrospective cohort analysis of adult patients (n = 92) treated, or not, with VitD	Outcomes of 43 transplant recipients in whom VitD was prescribed for clinically	Eight patients in the treatment arm were excluded from analysis due to noncompliance

(*Continued*)

Table 3: Clinical studies of the correlation between vitamin D and malignancies

Study	Design	Use of vitamin D	Results
Observational studies			
Ducloux et al 2008 (47)	Retrospective cohort analysis of adult kidney transplant recipients (n = 363) with known pretransplant 25(OH)D levels	Pretransplant 25(OH)D levels were correlated with risk of development of posttransplant cancers, with respect for other known risk factors, over a 3-year follow-up period	32 cancers were observed, more frequently in those with VitD deficiency and insufficiency Low VitD level was identified as an independent risk factor for development of posttransplant cancer over 3 years of follow-up (hazard ratio 1.12, for each 1 ng/mL decline in 25(OH)D)
Marcén et al 2012 (48)	Observational prospective study of adult kidney transplant recipients recruited posttransplantation (n = 389)	25(OH)D levels measured at 3, 6 and 12 months posttransplant were correlated with cardiovascular events and new malignancies	331 patients were analyzed as those that had lost their grafts within the first 12 months posttransplantation were excluded Over a 10-year follow-up, no difference was observed between cumulative incidence of malignancy in patients with normal VitD level, VitD insufficiency or VitD deficiency (21.3% vs. 22.7% vs. 16.7% cumulative incidence, respectively)
Interventional studies			
Obi et al 2012 (49)	Prospective cohort analysis of adult Japanese kidney transplant recipients recruited 1 year posttransplantation (n = 218), with 25(OH)D levels measured at recruitment	Patient exposure to VDRAs (calcitriol and alfacalcidol) and baseline 25(OH)D was correlated with development of malignancies	92 patients had received AVDs at recruitment During median follow-up of 2.9 years, 5 AVD (2.1 per 100 patient years) users and 11 non-AVD users (3.5 per 100 patient years) developed malignancies. Although there was no correlation between 25(OH)D level and risk of malignancy, AVD users were at lower risk of developing malignancy by Cox proportional hazard regression (hazard ratio 0.21; 95% CI 0.07–0.65)

25(OH)D, 25-hydroxy-vitamin D; VitD, vitamin D; VDRAs, vitamin D receptor agonists; CI, confidence interval.

repletion study exists in the literature (49) describing a decreased posttransplantation malignancy risk associated with VDRA supplementation (calcitriol and alfacalcidol). This study needs to be assessed with the caveat that the overall ''event rate'' was exceedingly small (2.1 and 3.5 *de novo* malignancies per 100 patient years in VitD-treated and -untreated subjects, respectively).

Due to the increased risk of skin malignancies with immunosuppression (particularly squamous cell carcinomas), there has been long-standing advice to RTRs to avoid solar UV exposure. In RTRs, regular application of SPF-50 sunscreen is associated with fewer skin lesions over a 2-year period, but also a lower mean concentration of 25(OH)D levels (mean value 53 ng/mL vs. 60 ng/mL) (50). Higher levels of VitD are similarly associated with an increased risk of cancer, explained by greater UV exposure conferring increased disease risk (51). These data demonstrate the difficulties of drawing conclusions using only epidemiological studies.

Other Key Effects of VitD in RTRs

VitD status contributes significantly to skeletal health. A Cochrane review (8) in 2007 concluded that from 24 trials (1299 patients) no individual intervention (bisphosphonates, VitD sterol or calcitonin) was associated with reduced fracture risk in RTRs compared with placebo, but by combining results for all active interventions against placebo it could be demonstrated that any treatment of bone disease was associated with reduced risk of fracture (relative risk 0.51, 95% confidence interval 0.27–0.99). Bisphosphonates (any route), VitD sterol and calcitonin all increased lumbar spine bone mineral density. Bisphosphonates and VitD also had a beneficial effect on the bone mineral density at the femoral neck. This represents the ''classical'' VitD therapeutic paradigm and is reviewed in depth elsewhere (35).

Cardiovascular disease (CVD) is the most common cause of death in RTRs, with chronic inflammation a key etiological factor. As well as epidemiological data showing a link between low serum VitD concentrations and predisposition to cardiovascular events, meta-analyses have shown that oral VitD treatment contributes to improved all-cause mortality through an associated reduction of deaths from cardiovascular events (52). However, a recent systematic analysis showed that the quality of current trial data is inadequate to draw conclusions about the relationship between VitD status and mortality from CVD in the general population (53). Further discussion of the role of VitD in CVD is beyond the scope of this review but has been reviewed elsewhere (54).

Issues in VitD Research

There are several caveats that cloud the interpretation of clinical VitD research data. First, reliably assessing VitD status and activity is itself a challenge (55). Measurement of serum 25(OH)D concentration is widely used because this species has a 3–4 week half-life, whereas the biologically most active VitD species—$1,25(OH)_2D_3$—has a life-life of only hours. 25(OH)D is an indirect test as it does not measure the most active VitD species and does not accurately predict VitD concentrations in tissues. The biological function of VitD can also be modulated by polymorphisms in VitD binding protein and the VDR, which are not accounted for in currently available trials. This is relevant because up to 3% of the human genome can be influenced by VitD (9), including steroid sensitivity (56). Additionally there remains controversy over the accuracy of different VitD assays. Standardization of assays has recently been improved but not resolved (57). Second, as there is no consensus on what should constitute repletion in interventional trials, seasonal (UVB-driven) effects on study cohorts' serum VitD concentrations are important and relevant to patients with CKD, on dialysis or after RTx (58).

Third, the species and route of administration of VitD treatment used in interventional studies are confounding. There are six to eight different possible forms of ViD, including ergocalciferol, cholecalciferol, calcidiol, calcitriol, 1-alfacalcidol and paricalcitol, with almost no head-to-head studies comparing them in RTRs. These have different affinities for the VDR, potencies, biological activities and side-effect profiles—for a detailed discussion see (59). VitD can raise serum creatinine, due to either an effect on the renin–angiotensin–aldosterone system or direct alteration in tubular handling of creatinine (60). Further variables include the route (oral, intramuscular and intravenous—the latter confers greater bioavailability) and frequency of administration, whether daily, weekly or monthly (61).

Fourth, although there is a high prevalence of VitD insufficiency in transplantation, there is no consensus dosing strategy for VitD repletion. One study showed that 100 000 IU of cholecalciferol fortnightly for 2 months (equivalent to 6600 IU/day) corrected 25(OH)D insufficiency in RTRs and significantly decreased serum parathyroid hormone (PTH) concentrations without side-effects. This study also highlighted that 100 000 IU of cholecalciferol every other month from months 6 to 12 posttransplant (the ''maintenance period'') was insufficient to maintain serum 25(OH)D levels above 30 ng/mL in about half of the patients studied, consistent with a previous report (62). The authors pharmacokinetically simulated an optimal dosing regimen to maintain 25(OH)D concentrations between 30 and 80 ng/mL (100 000 IU six times fortnightly, then 100 000 IU monthly until the end of the first year) (63), but this proposal remains to be tested prospectively.

Fifth, and most importantly, the optimum marker denoting biological VitD repletion has yet to be determined. Although biochemical markers (principally PTH and alkaline phosphatase) have traditionally been used to monitor repletion, the reliability and clinical relevance of PTH levels to infer changes in 25(OH)D levels in RTRs have been called into question. In a cohort study of 419 RTRs, 25(OH)D, estimated GFR and serum phosphate combined only accounted for 19% of the variance in PTH levels, indicating that VitD supplementation alone is likely to have only a limited effect on PTH levels (64). Bone mineral density, graft and patient survival are all relevant, additional, parameters/biomarkers for consideration.

Future Directions—Upcoming Trials

Although tentative associations have been made between VitD repletion and improvement of clinical outcomes in RTRs, this review highlights several deficiencies in our current knowledge that need to be addressed. Table 4 lists three actively recruiting VitD repletion trials, evaluating a range of primary end points. Encouragingly, there is focus

Table 4: Trials currently recruiting for vitamin D in RTRs

Trial, location	Design	Primary end points
VITA-D, Vienna (65)	Phase 3 placebo-controlled trial. 200 kidney transplant recipients with 25(OH)D <50 nmol/L will be randomized 5 days posttransplant to either placebo or VitD (6800 IU daily for 1 year)	1-year MDRD eGFR, number of infections, CRP, number of acute rejection episodes, bone mineral density (DEXA scans within the first 4 weeks, then at 5 and 12 months posttransplant)
VITALE, Paris (66)	Phase 4 placebo-controlled trial, comparing high (100 000 IU fortnightly then monthly) versus low (12 000 IU fortnightly then monthly) dose VitD over 2-year follow-up to patients 12–48 months posttransplant, with stable renal function over the previous 3 months, and VitD insufficiency (25 (OH)D <30 ng/mL) at recruitment. n = 320 patients in each group	*De novo* development of diabetes, cardiovascular complications, *de novo* cancer, patient death
CANDLE-KIT, Osaka (67)	Phase 4 open-label trial VitD supplementation and anemia correction (with Mircera®) over 2-year follow-up. 246 patients will be recruited who are at least 12 months posttransplant, with eGFR ranging from 15 to 60 mL/min. Inclusion criteria will not include VitD levels but patients must have Hb <10.5 g/dL without iron deficiency. They will be randomized to low Hb (≥9.5 and <10.5 g/dL) with no VitD, low Hb (≥9.5 and <10.5 g/dL) with VitD (1000 IU/day), high Hb (≥12.5 and <13.5 g/dL) without VitD or high Hb (≥12.5 and <13.5 g/dL) with VitD (1000 IU/day). Outcomes will be followed up for 2 years	Change in MDRD eGFR over 2 years of follow-up

All three trials currently recruiting will use cholecalciferol as the vitamin D (VitD) formulation. RTRs, renal transplant recipients; IU, international units; 25(OH)D, 25-hydroxy-vitamin D; Hb, hemoglobin; eGFR, estimated GFR; CRP, C-reactive protein.

on allograft function, cardiovascular outcomes and *de novo* malignancy.

The VITA-D trial (65) is a randomized, placebo-controlled double-blind study of 200 transplant recipients with follow-up duration of 1 year, with entry criteria being 25(OH)D serum concentration of <50 nmol/L. Incidence of acute rejection episodes, number and severity of infections (as measured by C-reactive protein) and GFR will be monitored. VITA-D is primarily aimed at evaluating short-term outcomes as only newly transplanted patients are being recruited and will be the first trial to report on VitD supplementation in *de novo* RTRs. The VITALE trial (66) will evaluate the differential effect of low- and high-dose cholecalciferol supplementation. Six hundred forty patients ranging from 12 to 48 months posttransplantation will be recruited to capture medium-term outcomes, particularly the development of new cancers and CVD. Although better powered than VITA-D, follow-up is still short at 24 months, in comparison with epidemiological literature in general. CANDLE-KIT (67) will recruit 246 RTRs, of at least 1 year posttransplantation, and randomize them to receive no additional treatment or combinations of cholecalciferol and an erythropoiesis-stimulating agent. Transplant function over a 2-year follow-up period will be the primary outcome measure of this trial. Interestingly, entry criteria for this trial do not include baseline VitD insufficiency/deficiency.

Conclusion

Research concerning the benefits of VitD supplementation in RTRs is clearly still evolving. While there is consistent epidemiological evidence suggesting an association between replete VitD status and improved clinical outcomes in RTRs, particularly skeletal outcomes (bone mineral density and fractures), we lack compelling evidence at the moment that measurement and repletion of VitD are mandatory for RTRs. The KDIGO guidelines recommend the use of VitD in RTRs for the prevention and treatment of transplant bone disease (68), but as yet a hard case for VitD repletion to optimize immunomodulation in RTRs has not been made. Given recent developments in our understanding of its molecular properties, VitD probably has a multifaceted role, which cannot be fully appreciated by examining hard clinical end points such as mortality alone. Future work is urgently needed to translate molecular biology into clinical outcomes.

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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