Review

Vitamin D levels and potential impact in systemic sclerosis

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ABSTRACT

Vitamin D is essential not only for calcium and bone metabolism, but it also may exert other biological activities, including immunomodulation through the expression of vitamin D receptor in antigen-presenting cells and activated T cells. Evidence from animal models and human prospective studies of rheumatoid arthritis, multiple sclerosis, type I diabetes, and systemic lupus erythematosus, indeed suggested an important role for vitamin D as a modifiable environmental factor in autoimmune diseases. In systemic sclerosis (SSc), this role has not been completely dissected, although recent studies clearly evidenced a high prevalence of vitamin D deficiency. Moreover, some degree of association between vitamin D deficiency and disease activity or phenotype characteristics has also been observed. Vitamin D deficiency in SSc may be related to several factors: insufficient sun exposure due to disability and skin fibrosis, insufficient intake because of gut involvement and malabsorption. Although it is advisable to regularly check vitamin D status in these patients, there is no consensus about which vitamin D supplementation regimen might be sufficient to modulate immunological homeostasis, and possibly reduce disease activity or severity, thus further prospective studies are needed. Moreover, novel vitamin D analogues with more pronounced immune modulatory effect and lower activity on calcium metabolism are in the pipeline, and might represent a great innovative opportunity for the treatment of vitamin D deficiency in such autoimmune disorders.

Vitamin D physiology

Vitamin D is essential for calcium homeostasis and prevention of bone diseases but it is now suggested that vitamin D also plays a role in a large number of physiologic processes and, as such, adequate levels are necessary for optimal health. However, deficiency of vitamin D seems to be pandemic with more than half of the world's population currently at risk (1, 2).

There are two main forms of vitamin D: vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol) (3). The former derives from 7-dehydrocholesterol of the cell membranes of the skin after ultraviolet B radiation exposure, the latter enters circulation through the diet. Similar to vitamin D2, vitamin D3 is also available from animal sources and some foods are fortified with vitamin D (2).

After vitamin D is ingested from the diet or synthesised in the skin, it is metabolised in the liver to a biologically inactive 25-hydroxyvitamin D (25-(OH)D), which is the major circulating form of vitamin D and the parameter used to determine an individual's vitamin D status.

Subsequently, 25-(OH)D is converted in the kidneys by the enzyme 1α-hydroxylase into its active form, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). This enzyme is also present in a variety of extrarenal sites, including osteoclasts, skin, colon, brain, and macrophages, which may be the cause of its broad-ranging effects. In addition, the vitamin D receptor (VDR) is present in most tissues and cells, allowing $1,25(OH)D_2$ to be one of the most potent regulators of cellular growth in both normal and cancer cells (4-7). $1,25(OH)_2D$ inhibits renin production in the kidney and has an immunomodulatory activity on monocytes and activated T and B lymphocytes (8-11).

Vitamin D deficiency

As stated previously, vitamin D status is determined by measuring 25-(OH)D. The cut-off value for deficiency remains controversial (2); this probably relates

Table I. *In vitro* vitamin D actions in the immune system.

Inhibits T-cell proliferation, expression of interleukin-2 (IL-2), interferon-γ (IFNγ) mRNA and protein in T-cells, and CD8 T-cells mediated cytotoxicity (24-29).
Enhances T-cell suppressor activity (29).
Blocks the induction of T-helper-1 (Th1)-cells cytokines, particularly IFNγ, and promote Th-2 response, through the indirect action of IFNγ and the direct intervention of IL-4 and IL-5 (29).
On antigen-presenting dendritic cells (DCs), suppresses the synthesis of IL-12, a cytokine that promotes Th1-cell responses (29).
Inhibits indirectly Th 17-cell responses, through inhibition of IL-6 and IL-23 expression (30).
Enhances the release of IL-10 (31).
Induces the reciprocal differentiation of regulatory T cells (Tregs) (30-32).
Decreases B-cell proliferation, plasma cell differentiation and Ig secretion (24,33-35).
Acts on cells of innate immune system, through the inhibition of the differentiation, maturation, and immunostimolatory capacity of DCs by decreasing the expression of MHC class II molecules and of CD 40, CD 80, and CD 86 (36).
Stimulates human monocyte proliferation *in vitro* and increase the production of IL-1 and the antimicrobial peptide cathelicidin by monocytes and macrophages (1, 26).

to the various assay methodology and vitamin D geographic variability (12). Most agree that a 25-(OH)D concentration <50 nmol/L or <20 ng/mL, is an indicator of vitamin D deficiency and a more severe deficiency is indicated by concentrations <25 nmol/L or <10 ng/ml, while a 25-(OH)D concentration of 51-74 nmol/L or 21-29 ng/mL indicate insufficiency, and concentrations >75 nmol/L or >30 ng/mL are considered to be sufficient (1, 13-17). This is based on the observation that in post-menopausal women, intestinal calcium absorption is maximised for values above 80 nmol/L or 32 ng/mL (18) and that parathyroid hormone (PTH) concentrations decline and reach their nadir at 75-30 nmol/L or 30-40 ng/mL (13, 16, 17, 19).

Immunoregulatory effects of vitamin D

Cellular data

Most of the biological effects of $1,25(OH)_2D3$ are mediated trough the VDR, a member of the superfamily of nuclear hormone receptors. The discovery that the VDR is constitutively expressed by antigen-presenting cells (APCs) such as macrophages and dendritic cells (DCs), and inducibly expressed by lymphocytes following activation, suggests a role for $1,25(OH)_2D3$ in the immune system (Table I) (20-36).

Animal models

Data from animal models of autoimmune diseases support a regulatory *in vivo* role for vitamin D in preventing and treating such diseases. Administration of vitamin D *in vivo* to rats and mice prevents the onset of experimental autoimmune encephalitis (EAE), which is a model of multiple sclerosis (MS) (37, 38), and slows the progression of established disease. With 1,25-dihydroxyvitamin D3 treatment in the early phase, collagen-induced arthritis (CIA) was preventable (39). With the administration of vitamin D, the progression of arthritis decreased compared with the untreated control animals (40). Vitamin D has also been studied in the animal model of systemic lupus erythematosus (SLE), MRL/lpr mice, where the administration of vitamin D resulted in a loss of dermatologic manifestations, without effects upon renal disease in one study (41), in contrast improved renal disease and survival were observed in another study (42). In a third study on MRL/lpr mice, the effect of vitamin D was similar to high dose of steroids with a significant prevention of the disease (43). Conversely, in NZB/W mice, vitamin D worsened the disease (44). A therapeutic effect for vitamin D has also been described for the non-obese diabetic (NOD) mouse (45), as well as for multiple animal models of transplantation (23).

Human data

Both experimental and observational studies seem to provide the evidence that vitamin D is an environmental factor affecting the prevalence of certain autoimmune diseases beside the geographical, climate and ethnic background (26). There is an association with living at higher latitudes and increased risk of many autoimmune diseases such as type I diabetes, multiple sclerosis (MS), and Crohn's disease (1, 6, 46). Vitamin D deficiency has been associated with increased risk of developing multiple sclerosis, and women who took more than 400 IU of vitamin D supplementation had a 42% reduced risk of developing MS (47) and a 41% reduced risk of developing rheumatoid arthritis (RA) (48). In Finland, more than 10.000 children who received 2000 IU of vitamin D were followed from 1960s for subsequent 31 years and they were found to have a 78% reduced risk of developing type I diabetes compared to children free from the supplementation (49).

Vitamin D and systemic sclerosis

Vitamin D and pathogenesis

Systemic sclerosis (SSc) is a connective tissue disorder characterised by early widespread microangiopathy that culminates in systemic fibrosis. Although its pathogenesis is not completely understood, it is suggested that both genetic and environmental factors are implicated in disease susceptibility and in clinical expression or progression (50, 51). Different association studies have been recently reported in SSc. Similar to SLE, different variants of interferon regulatory factor 5 (IRF5), which plays a role in Toll-like receptor signalling, have been found to be associated with susceptibility to SSc. STAT4 is involved in signalling through the IL-12 and IL-23 receptors and may also be activated in response to type I IFN receptors. STAT4 directs helper T cells toward the proinflammatory Th1 cell development and Th17 lineages, and data have convincingly identified that STAT4 rs7574865 variant is a genetic factor conferring susceptibility to SSc (51). Considering these data, the above mentioned *in vitro* inhibitory effects of vitamin D on adaptive immune cells might have some influence on SSc

pathogenesis, especially through its action on T-cell proliferation, expression of IFNγ and Th 17-cells responses. Moreover, in murine studies, vitamin D seemed to have a direct anti-fibrotic effect through a decreased expression of TGFβ1, collagen I and collagen III, besides it enhanced expression of some anti-fibrotic factors (52).

Vitamin D and clinical studies

Few studies reported low vitamin D levels in patients affected by SSc (Table II summarises the main features of these studies) (53-61).

A small uncontrolled study failed to demonstrate low levels of 25-OH and 1,25-OH vitamin D in 20 patients with SSc, although significant lower concentration of 1,25-OH vitamin D were found in 7 patients with sub-cutaneous calcinosis compared to 13 patients without (53). Similar results were found in a controlled study performed in 19 patients with SSc and their corresponding matched healthy controls, in which 25-OH vitamin D levels (28±3 *vs*. 29±3 ng/mL) did not differ in the two groups (54).

One retrospective study (56) reported a high prevalence of vitamin D hypovitaminosis (25-OH vitamin D values <12 ng/mL) in 26/40 SSc patients (46%), and in 13 patients (22%) who had lower 25-OH vitamin D levels (9.4±1.8 ng/mL) parathyroid hormone (PTH) was found elevated. Low levels of vitamin D (7.3±2.2 ng/mL) were found in half of the patients with acroosteolysis (AO), with or without calcinosis, in comparison to only 28% in those without AO (8.2±1.8 ng/mL), and PTH was elevated (80.4±22.8 ng/L) in 13/42 (31%) patients with AO. A statistically significant association was observed between AO and PTH (p= 0.01) but not between AO and vitamin D. No correlation was observed between PTH or vitamin D and disease duration or severity.

The exact mechanism which leads to AO in SSc patients is not completely understood, but vascular injury plays a key role (62). Besides, the hyperparathyroidism secondary to low vitamin D might contribute to the development of AO in some patients, in fact low vitamin D levels are associated with low calcium absorption rates, hyperparathyroidism, and increased bone turnover (6, 18). PTH increases the expression of RANKL on osteoblast: this is the signal for pre-osteoclast through its RANK interaction with RANKL to stimulate the formation of multinucleated osteoclasts, which release enzymes and hydrochloric acid to dissolve the collagen matrix (33). It remains to be evaluated in larger prospective studies whether vitamin D supplementation might lower the risk of AO in SSc.

A further controlled study evaluated 60 SSc patients and 60 matched controls. 25-OH vitamin D levels were lower in patients than in controls [median 23 ng/mL(range 3-92) and 39 ng/mL (range 14-138), respectively, p<0.001]. In detail, 38 patients had 25-OH vitamin D levels below 30 ng/mL, 4 had values <10 ng/mL, 17 had values included in the range ≥10 and <20 ng/mL, and 17 within the range ≥20 and <30 ng/mL. No association with disease characteristics or activity has been found, instead vitamin D tended to correlate with physical performance score assessed by the Medical Study Short Form-36 (SF-36) questionnaire scores (57).

Recently, we observed low 25-OH vitamin D values (mean values 19±11 ng/mL) in 156 consecutive patients, 90 coming from northern France and 66 from southern Italy (58). According to current recommendations, 25-OH vitamin D concentrations <30 ng/mL were considered as insufficiency, while values <10 ng/mL were classified as deficiency. We found 131/156 SSc patients (84%) with insufficiency and 44/156 (28%) with deficiency. Vitamin D deficiency was not associated with any markers that could be evocative of malabsorption syndrome such as haemoglobin, ferritinemia, albuminemia, vitamin B12, folates, nor with auto-immune serological markers such as anti-nuclear antibodies prevalence and levels. However, a modest univariate association was observed with the subgroup of SSc patients positive for anti-centromere antibodies (p=0.04). A significant negative correlation was found between low vitamin D levels and European Disease Activity Score, and even more with acute-phase reactants. Vitamin D deficiency was associated with systolic pulmonary artery pressure (sPAP), pulmonary fibrosis and ESR. In multivariate analysis vitamin D deficiency was associated with sPAP. Moreover, our study showed that usual vitamin D supplementation with 800 IU of cholecalciferol did not completely prevent the deficiency.

Table II. Summary of the studies evaluating vitamin D levels and association with disease characteristics in Systemic Sclerosis.

Reference	No patients	Controlled group	Mean value vitamin D (ng/mL)	Vitamin D insufficiency %	Vitamin D deficiency %	Associations with disease characteristics
Matsuoka LY *et al*. (54)	19	yes	28 ± 3	0	0	Acroosteolysis
Braun-Moscovici Y *et al*. (56)	40	no	NA	46	22	none
Calzolari G *et al*. (57)	60	yes	23	63	0.6	none
Vacca A *et al*. (58)	156	no	19 ± 11	84	28	Disease activity, sPAP, pulmonary fibrosis, ESR, CRP, ACA
Rios Fernandez *et al*. (60)	48	no	NA	81	9.5	none
Caramaschi P *et al*. (59)	65	no	15.8 ± 9	66	29	Disease duration, DLCO, sPAP, CRP, ESR, late nailfold VCP
Belloli L *et al*. (61)	43	yes	18.1 ± 15	51	35	none

sPAP: sistolic pulmonary arterial hypertension; ESR: erytrocyte sedimentation rate; CRP: C- reactive protein; ACA: anti-centromere antibodies; DLCO: diffusing lung capacity for carbon monoxide; VCP: videocapillaroscopic pattern.

Although our data do not support any differences between the two main cutaneous subtypes (*i.e.* limited *vs.* diffuse), the modulation of ultraviolet radiation on vitamin D metabolism should be investigated, keeping in mind that the role of skin synthesis as a determinant of serum vitamin D levels has not been completely defined because of the absence of a direct measure of personal sunlight exposure. Interestingly, although the different UV exposure in the two cohorts, we found a similar prevalence of vitamin D deficiency, and probably other factors that might be related to the disease itself or to lifestyle should be considered.

In our patients, differently from the study by Braun-Moscovici *et al*. (56), we did not find any correlation between vitamin D deficiency and the occurrence of acroosteolysis and calcinosis, thus other factors might be implicated in the development of such complications.

Vitamin D seems to have a role in modulating disease activity in other inflammatory rheumatic conditions such as RA and SLE (63-67). We can postulate that also in SSc patients vitamin D deficiency might influence the development of a more active disease suggested by the associations observed in this sample but more work is needed. Among the factors that could contribute to vitamin D deficiency we have to consider that because of disability, these patients are less exposed to UV, may have insufficient intake of dietary vitamin D, and a potential malabsorption due to intestinal involvement should be considered. Nevertheless no association in our hands with other biochemical markers of malabsorption are available.

Interestingly, the observation that in our patients standard vitamin D supplementation does not completely correct the deficiency, suggested that higher dosages should be used to modulate disease activity and/or severity. In fact, if increasing 25(OH)D to >80 nmol/L may reduce secondary hyperparathyroidism to bone loss, it is still unclear what level is needed to influence immune homeostasis (14).

This result was also confirmed by another group which enrolled 48 SSc patients and found, respectively, 81% and 9.5% of vitamin D insufficiency and deficiency, despite of conventional supplementation; moreover no correlation with a lower bone mineral density (BMD) was observed (60).

A more recent study in 65 Italian SSc patients further confirmed a high prevalence of vitamin D insufficiency and deficiency (respectively 66% and 29%) with mean level of 15.8±9.1 ng/mL. Conversely to our previous study, the authors did not considered any patients under vitamin D supplementation. They found that vitamin D deficiency was associated to longer disease duration, lower diffusing lung capacity for carbon monoxide, higher estimated pulmonary artery pressure, higher values of CRP and ESR. Moreover, they also observed in these patients a more frequent late nailfold videocapillaroscopic pattern, while they failed to reach statistical significance for disease activity. Multiple linear regression analysis showed a correlation between vitamin D level and DLCO value (p=0.021) and disease duration from Raynaud's phenomenon onset (p=0.041). In accordance with our findings, overt malabsorption syndrome was not implicated in determining hypovitaminosis D but they rather considered other factors such as skin hyperpigmentation and reduced sun exposition (59).

By contrast, another italian study did not observed neither a significant difference in the prevalence of vitamin D insufficiency and deficiency compared to a control group affected by osteoarthritis nor associations with diseases characteristics (61).

Only one study demonstrated a remarkable high prevalence of vitamin D insufficiency also in juvenile onset systemic sclerosis compared to controls (100% *vs.* 40%); besides, the authors found a correlation between bone mineral apparent density in femoral neck and total femur and 25-OH vitamin D levels (68).

Vitamin D and therapeutic trials

Skin involvement in SSc presents clinical signs similar to that of localised scleroderma (LS), and the two conditions are histopathologically identical, thus treatment approaches for LS could also be applicable to sclerotic skin in SSc.

Oral calcitriol and calcipotriol, because of their anti-proliferative effect on fibroblasts, has been investigated as therapy in LS and SSc in open studies with promising results with respect to skin extent and joint mobility (69-75). Calcitriol can inhibit the growth of normal human dermal fibroblast and is involved in their differentiation (76). Moreover, it has been shown that it has an immunoregolatory effect on T-helper proliferation, along with the production of cytokines by monocytes and macrophages (77). Calcitriol inhibits the synthesis of collagen and proliferation of cultured fibroblast from patients with scleroderma and from persons with a normal skin (78, 79).

Only one study evaluated the efficacy of calcitriol in SSc. This is a double-blind, placebo controlled study performed in 20 patients with morphea and 7 patients with SSc (5 with the diffuse cutaneous form and 2 with the limited type). Patients were assigned to receive either 0.75 mcg/day calcitriol for 6 months and 1.25 mcg/day for an additional 3 months or placebo for 9 months. Calcitriol or placebo was administered as a single oral dose just before bedtime, and dietary calcium intake was moderated. The efficacy parameters included the skin score, and for SSc patients also monthly oral aperture measurements, lung function studies, and esophagus motility. In patients with morphea, the skin score showed no significant differences between the placebo and calcitriol group (mean percentage reduction [SD] in skin score -29.3 [57.9] *vs.* -19.4 [46.6]). The small group of SSc patients was inadequate to draw any conclusions regarding efficacy (75).

A better understanding of the molecular mechanism of excessive fibrosis in the skin has led to novel approaches with topical vitamin D analogues (80). Calcipotriene is a synthetic analogue of vitamin D, with binding affinity for VDR and effects on keratinocyte proliferation and differentiation similar to calcitriol. In contrast, calcipotriene is 100-200 times less potent than calcitriol in causing *in vivo* calcium absorption and mobilisation. The mechanism of action

of calcipotriene in LS is unknown, but it probably suppress the function of activated T-cells. In psoriatic skin calcipotriol downregulates the expression of intercellular adhesion molecule-1, endothelial cell leukocytes adhesion molecule-1, and leukocyte function-associated antigen-1 (81).

The only open-label study (80), which evaluated the efficacy of topical calcipotriene in LS, was performed on 12 patients with active disease, who failed to respond to potent topical steroids or systemic medications. The patients applied a thin layer of calcipotriene ointment 0.005% to the affected areas twice daily, with occlusion at night, for 3 months. Dyspigmentation, induration, erythema, and teleangiectasia were rated on a scale of 0 (none), 1 (mild), 2 (moderate), and 3 (severe). The sclerodermatous lesions of all 12 patients improved during the 3 month trial. Erythema, teleangiectasia, and dyspigmentation significantly decreased by 1 month, while induration significantly reduced after 3 months. All the patients felt that the texture and colour of the lesions were improved, and 11/12 requested to continue calcipotriene therapy. In conclusion, this study demonstrated that topical vitamin D analogues may have a potential beneficial effect on LS, however double-blind placebo-controlled trials are required to establish its real efficacy.

Vitamin D and other connective tissue diseases

Systemic lupus erythematosus

Initial evidences regarding the prevalence of vitamin D deficiency in SLE came from studies on bone health (82-84).

A cross-sectional study reported statistically lower levels of 25-OH-D (mean 13 ng/ml) in 21 SLE patients, compared with 29 patients with RA (mean 24 ng/ml), 12 patients with osteoarthritis (mean 32 ng/ml), and 24 controls (mean 27 ng/ml) (85).

A second cross-sectional study of 25 Caucasian SLE patients showed a prevalence of vitamin D deficiency of about 50% (using a cut-off <50 nmol/l or <20 ng/ml). However, this not significatively differed from a control group of patients with fibromyalgia (SLE mean 46.5 nmol/l; fibromyalgia mean 51.5 nmol/l) (86).

In a recent study performed in 123 SLE patients with short disease duration and 240 controls, there was a trend in both African Americans and Caucasians toward lower 25-OH vitamin D levels in cases compared to controls, controlling for age, sex, season, and smoking (p=0.15). The cases had a mean 25-OH vitamin D levels of 21.6±12.9 ng/ml compared to 27.4±15.7 ng/ml for controls. The overall prevalence of vitamin D deficiency (cut-off <30 ng/ml) was 67%; the mean 25-OH vitamin D levels were significantly lower among African-Americans (n=138, 15.9±9.4 ng/ml) than among Caucasians (n=255, 31.3±4.9 ng/ml). Severe deficiency (cut-off <10 ng/ml) was noted in about 18% of patients and was associated with renal disease and photosensitivity (66).

Many studies, but not all, have observed an association between higher disease activity and low levels of vitamin D (55, 87-89). In one study 25-OH vitamin D levels have been evaluated in two populations of SLE patients coming from northern Europe (Estonia) and from southern Europe (Italy). All SLE patients had significantly lower values of vitamin D when compared with their controls, although serum levels did not differ between the two populations. A significant negative correlation was found between 25-OH vitamin D levels in all SLE patients and Systemic Lupus Erythematosus Disease activity Index (SLEDAI) and European Consensus Lupus Activity Measurement (ECLAM) scores (87).

A recent cross-sectional study in 92 SLE patients from Spain reported a vitamin D insufficiency (<30 ng/ml) in 69 patients (75%) and a deficiency (<10 ng/ml) in 14 patients (15%). Female sex, treatment with hydroxychloroquine (HCQ), and treatment with calcium and vitamin D predicted higher levels of 25-OH vitamin D. Higher age and HCQ use protected against vitamin D deficiency. Patients with vitamin D deficiency had a higher degree of fatigue, while no relationship was seen between vitamin D insufficiency or deficiency and disease duration, SLEDAI or Systemic Lupus International Collaborative Clinics (SLICC) scores (88). Observational two years study of this series showed a beneficial effect on fatigue coming from increasing vitamin D levels with standard regimens, while no effect has been observed in respect of disease severity and activity (89).

Patients with SLE have multiple risk factors to develop vitamin D deficiency: avoidance of sunlight exposure or use of sunscreen, chronic use of steroids that may alter the metabolism of vitamin D, renal involvement with consequences on 1-hydroxylation to make 25-OH vitamin D active (66), and finally, the possible presence of anti-vitamin D antibodies (90).

Undifferentiated Connective Tissue Diseases (UCTD)

In a recent study (91), the prevalence of vitamin D insufficiency (25-OH-D levels <30 ng/ml) among 161 UCTD patients was 42% during the summer and 54% during the winter, while the prevalence in the control group was 19% (11/59 subjects) in both seasons. Vitamin D deficiency (<10 ng/ml) was found only in 5 of the UCTD cases. Regarding the correlations with clinical and laboratory parameters, the presence of dermatological symptoms and pleuritis was associated with low levels of vitamin D (<30 mg/dl). During the average follow-up period of 2.3 years, about 22% (35/161) of UCTD patients further developed into well-established connective tissue disease (CTD). Patients who progressed into CTD had the lowest vitamin D levels (14.7±6.45 ng/ml *vs*. 33±13.4 ng/ml, p=0.0001).

In conclusion, these results showed a high prevalence of hypovitaminosis D also in UCTD patients, and that vitamin D may could play a role in the subsequent progression into well-defined CTDs .

Mixed Connective Tissue Disease (MCTD)

The prevalence of low vitamin D levels is high in patients with MCTD (59%) and they are associated with the inflammatory process and traditional risk factors which may favour cardiovascular events in MCTD (92).

Behçet's disease (BD)

A very recent study showed that vitamin D insufficiency in active BD patients is common and low levels of vitamin D correlated with several inflammatory and immunological markers, suggesting also in this disease a potential immunomodulatory effect (93).

Perspectives

The recent findings on vitamin D hypovitaminosis in patients with SSc and possible links with the disease characteristic, prompt to further observational studies to confirm these findings and establish the mechanism underlying these observations. Moreover, randomised clinical trials will be needed to determine whether vitamin D supplementation could have any potential benefit in reducing disease severity or activity or could influence the disease onset. In this view, vitamin D supplementation represents an attractive strategy to ensure suitable levels for adequate immune function, giving the opportunity to eliminate one of the proposed environmental risk factors involved in autoimmunity.

Patients with SSc have a high prevalence of vitamin D deficiency, and comparable to other autoimmune diseases, some degree of association between the deficiency and disease activity has been observed. Vitamin D deficiency in SSc may be related to several risk factors: insufficient sun exposure due to disability and skin fibrosis; insufficient intake and malabsorption. The potential influence of the therapies commonly used in SSc on vitamin D metabolism still remains to be clarified. Differently from RA and SLE, steroids and HCQ are less used and the effects of other drugs prescribed in SSc has not been yet evaluated.

The physiologic and clinical significances of vitamin D deficiency are not completely known as well as there is no consensus about the correct vitamin D supplementation regimen necessary to modulate immunologic homeostasis and whether this is different from the dosage required to maintain bone health. Since contrasting data are available on the correlation between disease activity and vitamin D deficiency, we need to consider the "tissue-specific vitamin D deficiency", as levels for optimal health may vary between organ system (94).

There are several ways to improve vitamin D status including fortification of food, annual vitamin D administration, and supplementation with tablets containing vitamin D and calcium, and clinicians should be aware of the issue surrounding interpretation of vitamin D levels (95). Another research challenge would be to develop calcitriol analogues with low or absent activity on calcium homeostasis, to avoid the major adverse effects of VDR agonists, hypercalcemia and hypercalciuria. Presently, calcemic side effects remains a great challenge, but in future, thanks to biotechnological development, novel vitamin D analogues could be designed to have more pronounced immunemodulatory capacity rather than impact on calcium homeostasis (96).

Several studies suggested that vitamin D deficiency increases the risk of colon (97) and possibly other cancers (98), increases the risk of hypertension (99), myocardial infarction (100), cardiovascular (101) and overall mortality (102), infections (103) and diabetes (104). Thereby, low vitamin D levels could be interpreted as a marker of poor general health.

Recent recommendations of an *ad hoc* expert panel, suggested that in patients with autoimmune disorders, 25(OH)D level should be above 30 ng/mL, and 100 ng/mL should be considered as a safety limit but not as an upper limit to target in clinical practice. In patients with values below 30 ng/mL, a large correcting dose can be proposed initially, followed by a maintenance treatment of 800 IU/day (or equivalent with intermitting dosing), which can be increased if levels remain insufficient during monitoring. Monitoring of serum 25(OH)D is recommended after at least 3 months (105).

Conclusion

Measurement of vitamin D levels in SSc might be included among the parameters monitored in this disease, although randomised clinical trials will be needed to determine whether vitamin D supplementation at higher dosages could have any potential benefit in influencing the disease outcome.

The role of vitamin D deficiency in the pathogenesis of SSc and other autoimmune disease remains still unclear thus the advice to widely prescribe vitamin D integration to prevent the development of such diseases needs further prospective studies, while it is advisable to regularly check vitamin D levels in these patients.

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