Vitamin D Deficiency and Insufficiency in 2 Independent Cohorts of Patients with Systemic Sclerosis

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ABSTRACT. *Objective.* To investigate 25-OH vitamin D concentrations in 2 independent systemic sclerosis (SSc) populations from France and Italy.

Methods. We studied 156 consecutive SSc patients comparable for demographic characteristics: 90 from Northern France and 66 from Southern Italy. 25-OH vitamin D, intact parathyroid hormone, and serum total calcium and phosphorus were measured in all patients. Vitamin D concentrations < 30 ng/ml were considered insufficiency, while values < 10 ng/ml were classified as deficiency.

Results. Vitamin D insufficiency and deficiency rates were very high and comparable between the 2 populations: 74/90 (82%) versus 57/66 (86%) for insufficiency and 29/90 (32%) versus 15/66 (23%) for deficiency, respectively, in the French and Italian patients. They were not influenced by vitamin D supplementation, which was not statistically different in the 2 groups. In the combined populations, a significant negative correlation was found between low vitamin D levels and European Disease Activity Score (p = 0.04, r = –0.17) and an even more significant correlation was found with acute-phase reactants (p = 0.004, r = –0.23 for erythrocyte sedimentation rate), and low levels of vitamin D were associated with the systolic pulmonary artery pressure (sPAP) estimated by echocardiography (p = 0.004). In multivariate analysis, vitamin D deficiency was associated with sPAP (p = 0.02).

Conclusion. Vitamin D deficiency was very common in the 2 SSc populations, independent of geographic origin and vitamin D supplementation. This suggests that common vitamin D supplementation does not correct the deficiency in SSc patients, and that a higher dose is probably needed, especially in those with high inflammatory activity or severe disease. (First Release Aug 1 2009; J Rheumatol 2009;36:1924–9; doi:10.3899/jrheum.081287)

Key Indexing Terms:
VITAMIN D   SYSTEMIC SCLEROSIS   DISEASE ACTIVITY
INFLAMMATION   PULMONARY ARTERIAL HYPERTENSION

Vitamin D is a steroid hormone that regulates calcium metabolism and bone homeostasis[1,2]. It is widely recognized that vitamin D exerts important effects on many other systems, such as muscles, vasculature, reproduction, cellular growth and differentiation, malignancy, and the immune system. Vitamin D deficiency in adults can precipitate or exacerbate osteopenia and osteoporosis, cause osteomalacia and muscle weakness, and increase the risk of fracture[3-5]. Identification of the vitamin D receptor (VDR) in most tissues and cells, including peripheral blood mononuclear cells[6,7], and the ability for several of these to convert the primary circulating form, 25-hydroxy vitamin D, into the active form 1,25-dihydroxy vitamin D, has provided insights into the function of this vitamin, particularly regarding its immunoregulatory effects. Vitamin D seems to be a physiological regulator of T cell development. Identification of VDR in Th cells suggests that vitamin D might have a role in the function or the development of Th cells[8,9]. Moreover, vitamin D itself is a transcription factor[10]. Epidemiological evidence indicates a significant association between vitamin D deficiency and an increased incidence of several autoimmune diseases like multiple sclerosis (MS), insulin-dependent diabetes mellitus (IDDM), inflammatory bowel diseases (IBD)[11], rheumatoid arthritis (RA)[12,13], and systemic lupus erythematosus (SLE)[14,15]. On the other hand, increased vitamin D intake may have been associated with a decreased risk of IDDM[16], MS[17], and RA in the Iowa Women's Health Study[18]; but no relationship was found between vitamin D supplementation and the relative risk of developing either SLE or RA in the large prospective cohorts of the Nurses' Health Study[19].

Low vitamin D levels have also been reported in patients with systemic sclerosis (SSc)[20,21], but no data are available on the relationship between a deficient vitamin D status and

SSc disease activity and severity, or on the clinical consequences that such deficiency might cause. Whether vitamin D could have a role in the complex pathogenesis of SSc[22,23] remains unclear.

Studying 2 independent populations of European Caucasian patients with SSc from France and Italy, our aims were to determine the prevalence of vitamin D deficiency and insufficiency, to establish the potential influence of such a condition on the disease phenotype, and to evaluate the effects of a standard dose of vitamin D supplementation.

MATERIALS AND METHODS

Patients. We included consecutive SSc patients attending the Department of Rheumatology A of Hôpital Cochin, Paris, and the Rheumatology Unit of the Azienda Ospedaliero-Universitaria, Cagliari, from September 2007 to May 2008. All patients gave informed consent for all procedures, which were carried out with the local ethics committee's approval. We performed a complete evaluation of all patients for the following data: age, sex, cutaneous SSc subtype as defined by LeRoy, *et al*[24], and disease duration. All subjects completed the Health Assessment Questionnaire (HAQ), disease activity was evaluated with the Disease Activity Score (DAS) according to the European Scleroderma Study Group guidelines[25], and disease severity was assessed by Medsger's severity score[26]. Routine clinical assessment was performed including blood tests and serological testing for antinuclear, anticentromere, and antitopoisomerase I antibodies; 25-OH vitamin D was measured with the RIA Diasorin kit or expressed as "RIA Diasorin equivalent" (Octeia; IDS Ltd., Boldon, UK); intact parathyroid hormone (PTH) was assessed with a chemiluminescent enzyme-labeled immunometric assay (Immulite 2000; Siemens Diagnostic, Los Angeles, CA, USA), and calcium and phosphorus were determined in all SSc patients. Systemic assessment included Doppler echocardiography, lung computed tomography (CT) scan, and pulmonary function tests.

According to current recommendations, vitamin D concentrations < 30 ng/ml were considered as indicating insufficiency[27,28], while values < 10 ng/ml were classified as deficiency[3].

Statistical analysis. All data are presented as mean (standard deviation, SD), unless stated otherwise. The chi-square test was used to compare categorical variables. Correlations between vitamin D levels and various quantitative SSc disease variables were assessed using Spearman's rank correlation test. A comparison between vitamin D and continuous variables was performed by nonparametric Mann-Whitney test. Multiple logistic regression analysis was used to examine the relationship between vitamin D deficiency and clinical or laboratory data. A probability value $p < 0.05$ was considered statistically significant.

RESULTS

Study population. We included 156 consecutive patients with SSc, 90 from Northern France (Paris) and 66 from Southern Italy (Cagliari). Demographic characteristics were comparable for the 2 groups of patients in terms of sex, age, and disease duration; only the cutaneous subset category (i.e., limited vs diffuse) was found to differ, with more frequent diffuse cutaneous subtype in the French cohort — 36% vs 13% in the Italian cohort (p = 0.002). Detailed characteristics of both cohorts are provided in Table 1.

Prevalence of vitamin D insufficiency and deficiency. The prevalence for the whole population was 131/156 (84%) for insufficiency and 44/156 (28%) for deficiency. Mean (SD) 25-OH vitamin D value was similar in the 2 cohorts: 19 ± 11

ng/ml. Vitamin D insufficiency and deficiency rates were comparable between the 2 populations: 74/90 (82%) versus 57/66 (86%) for insufficiency and 29/90 (32%) versus 15/66 (23%) for deficiency, respectively, in the French and Italian SSc patients (Figure 1), and they were not influenced by vitamin D supplementation, which at usual dosage (cholecalciferol 800 IU/day) was not statistically different in the 2 groups (30% vs 45%, respectively; p = 0.1).

There was a trend for higher PTH values in patients with reduced vitamin D: for the French and Italian populations, respectively, 44.7 ± 25.1 versus 53.5 ± 29.1 pg/ml (p = 0.06) for insufficiency and 38.2 ± 22.7 versus 49.9 ± 27 pg/ml (p = 0.06) for deficiency.

Influence of vitamin D deficiency on disease phenotype of SSc patients. Vitamin D deficiency was not associated with other markers that could be impaired in malabsorption syndrome, such as hemoglobin, ferritinemia, albuminemia, vitamin B12, or folates, or with autoimmune markers such as prevalence and levels of antinuclear antibodies. However, a slight association was seen for anticentromere antibodies (p = 0.04). A significant negative correlation was found between low serum vitamin D levels and European Disease Activity Score results (p = 0.04, r = –0.17; Figure 2); and an even more significant correlation was found with acute-phase reactants [p = 0.004, r = –0.23 for erythrocyte sedimentation rate (ESR); p = 0.01, r = –0.22 for C-reactive protein (CRP); Figure 3]. Vitamin D deficiency was associated with systolic pulmonary artery pressure (sPAP) estimated by echocardiography (p = 0.004), pulmonary fibrosis (p = 0.04), and ESR (p = 0.008).

No associations were found between vitamin D deficiency and acroosteolysis, calcinosis, HAQ, or Medsger disease severity score.

In multiple logistic regression analysis with the model including sPAP, ESR, and pulmonary fibrosis on CT scan, we found that the value of sPAP (p = 0.02) was associated with vitamin D deficiency as the dependent variable.

DISCUSSION

Our results showed that low vitamin D levels are very common in patients with SSc (84% had values < 30 ng/ml and 28% had values < 10 ng/ml), and that vitamin D deficiency is associated with active disease and inflammatory activity and more strongly with the sPAP value. Moreover, usual vitamin D supplementation in both study populations did not completely protect against deficiency. It is well established that vitamin D levels fluctuate during the year with seasonality[29,30], but we could not observe any differences in vitamin D levels in our patients with regard to their dates of inclusion, which ranged from September to May. Although our data did not support any differences between the 2 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

Table 1. Characteristics of French and Italian patients with SSc.

Feature	SSc Patients from France, n = 90	SSc Patients from Italy, n = 66	p	Combined Population
Sex, F/M	85/5	57/9	NS	142/14
Age, yrs, mean ± SD	56 ± 13	57 ± 9	NS	57 ± 13
Cutaneous subtype (limited/diffuse), n (%)	58 (64)/32 (36)	57 (87)/9 (13)	0.02	115 (73)/41 (26)
Disease duration, yrs, mean ± SD	7 ± 5	7 ± 9	NS	7 ± 6
History of digital ulcers, n (%)	13 (14)	6 (9)	NS	19 (12)
Acroosteolysis, n (%)	15 (16)	6 (9)	NS	21 (13)
Calcinosis, n (%)	13 (14)	8 (12)	NS	21 (13)
Esophagitis, n (%)	20 (21)	17 (26)	NS	37 (23)
Pulmonary arterial hypertension, n (%)	16 (17)	8 (12)	NS	24 (15)
Estimated sPAP, mm Hg, mean ± SD	33.5 ± 10	29.1 ± 10	NS	31 ± 10
Pulmonary fibrosis, n (%)	39 (41)	37 (57)	NS	76 (76)
25-OH D, ng/ml, mean ± SD	18 ± 10	20 ± 12	NS	19 ± 11
25-OH D < 30 ng/ml, n (%)	74 (82)	57 (86)	NS	131 (84)
25-OH D < 10 ng/ml, n (%)	29 (32)	15 (23)	NS	44 (28)
Calcium, mmol/l, mean ± SD	2.3 ± 1	3 ± 3	NS	2.6 ± 2
Phosphorus, mmol/l, mean ± SD	1.2 ± 0.1	1.2 ± 0.1	NS	1.2
ESR, mm/h, mean ± SD	17 ± 11	25 ± 22	0.002	21 ± 17
CRP, mg/dl, mean ± SD	11 ± 25	11 ± 10	NS	5.6 ± 15
Antinuclear antibodies > 1/160, n (%)	56 (59)	53 (81)	0.005	109 (68)
Antitopoisomerase I antibodies, n (%)	20 (21)	22 (34)	NS	42 (26)
Anticentromere antibodies, n (%)	12 (13)	18 (27)	NS	30 (19)
EDAS score > 3, n (%)	18 (19)	13 (20)	NS	31 (19)
Medsger score 3–4, n (%)	16 (17)	13 (20)	NS	29 (18)
HAQ score, mean ± SD	0.9 ± 0.7	1 ± 0.9	NS	0.9 ± 0.8
Decreased FVC (< 75% of normal value), n (%)	15 (16)	7 (10)	NS	22 (14)
Decreased DLCO/AV (< 75% of normal value), n (%)	25 (26)	46 (71)	NS	71 (44)

sPAP: systolic pulmonary artery pressure, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, EDAS: European Disease Activity Score, HAQ: Health Assessment Questionnaire, FVC: forced vital capacity, DLCO/AV: decrease in carbon monoxide diffusion capacity divided by alveolar volume.

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.

It has been established that vitamin D deficiency is more common in northern areas where sun exposure is less; nevertheless we did not find a "sun" effect as expected, but we observed a similar prevalence of vitamin D deficiency in both populations (32% in the French cohort, 23% in the Italian). Thus other factors, probably linked to the disease itself or lifestyle, must be taken into consideration.

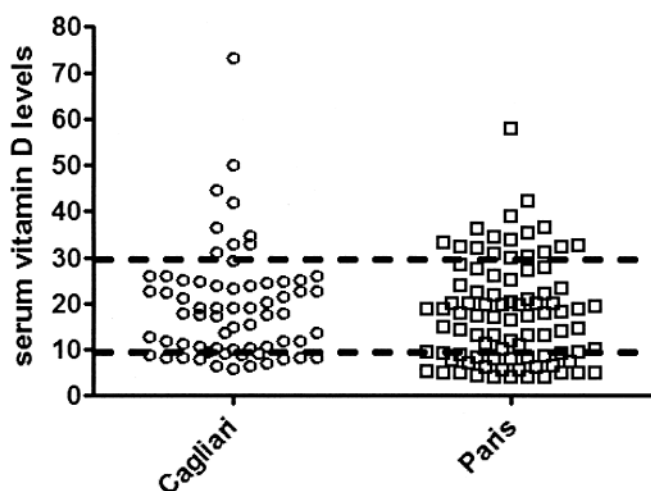The correlation between the frequency of IDDM, MS,

Figure 1. Comparison of vitamin D levels in the French (Paris) and Italian (Cagliari) patients with SSc.
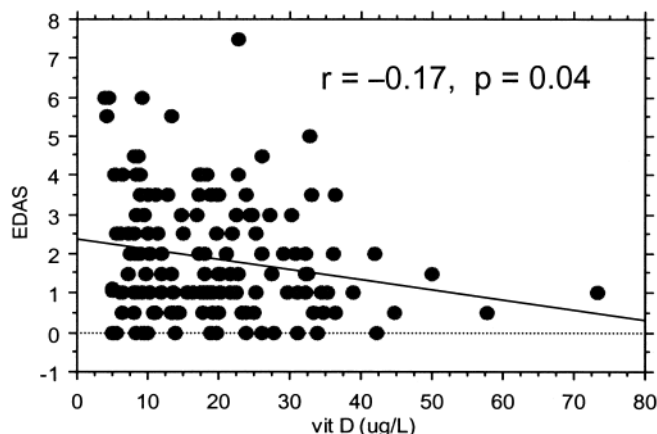

Figure 2. Relationship between vitamin D values (μg/l) and European Disease Activity Score (EDAS) results.
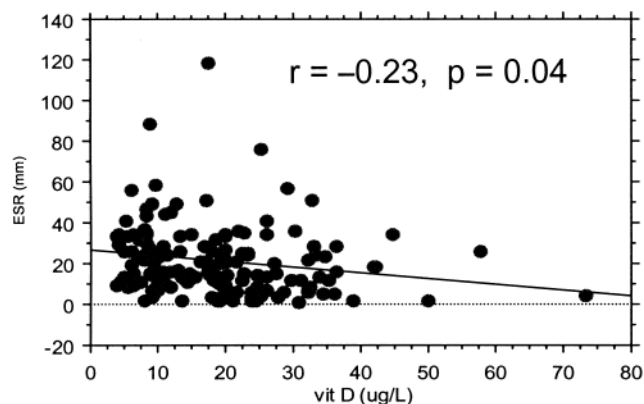
Figure 3. Relationship between vitamin D values (μg/l) and ESR (mm/h).

RA, SLE, and IBD and the geographic latitude, UV irradiation, and vitamin D levels is well known[16,31], but it is unclear whether vitamin D deficiency is a cause or a result of autoimmunity or of its treatment. However, it seems that high vitamin D intake is associated with a reduced risk of developing IDDM, RA, and MS[16-18,32].

Vitamin D deficiency has been reported in RA and SLE. One study demonstrated an inverse cross-sectional association between vitamin D metabolite levels and disease activity in patients with early inflammatory polyarthritis[33]; 2 other studies found an association just between $1,25(OH)_2$ vitamin D level and CRP or ESR in patients with long-duration RA[34,35].

Some reports have demonstrated lower vitamin D values in patients with SLE compared with osteoarthritis[14], fibromyalgia[36], and healthy controls[14,15]. Kamen, *et al* reported an association between vitamin D deficiency and recently diagnosed SLE as well as with lupus nephritis[15]. By contrast, in a study by Ruiz-Irastorza, *et al*, none of these associations was found, particularly with SLE activity and severity, as measured by the SLE Disease Activity Index and the Systemic Lupus International Collaborating Clinics-American College of Rheumatology index, while vitamin D deficiency was correlated with fatigue[37].

Recently, Zold, *et al* suggested that vitamin D deficiency in patients with undifferentiated connective tissue disease may play a role in the subsequent development into well defined connective tissue disease[38].

Thus, all these studies show a high prevalence of vitamin D deficiency in several autoimmune diseases, but epidemiological studies have also found low vitamin D levels in about 30% of healthy young subjects[39]. A multicenter study of 43 osteoporosis centers from all regions of Italy revealed that vitamin D deficiency is extremely common among elderly women, particularly in those with a lower education level, those living in central Italy, smokers, and those with lower vitamin D intake[40]. A high prevalence of insufficiency was also found in French women of different ages[41].

Our study is the first to show, in patients with SSc, some degree of association between vitamin D deficiency and disease activity, particularly with inflammation measures and with sPAP. We can hypothesize that patients with active disease and vascular complications have less chance of being exposed to UV and they may have insufficient intake of dietary vitamin D. Even though we did not find a significant correlation with markers that could be impaired in malabsorption and vitamin D deficiency, and the glucose breath test was not performed on all patients, we did not completely exclude the presence of intestinal involvement, which in any case is mostly asymptomatic in SSc patients. On the other hand, vitamin D affects the immune system at many levels and with different mechanisms, thus deficiency might influence the development of a more active disease that would also be complicated by higher sPAP values. Other factors could be the use of steroids, although high doses are rarely used in SSc, unlike treatment in lupus[37].

Of major importance, we observed that a standard dose of vitamin D supplement does not prevent or correct the deficiency, suggesting that dosages should be increased with regard to the disease and its activity or severity. This could have practical repercussions, as our results strongly suggest the need for a higher supplement dosage, but this remains to be validated together with potential effects on the clinical phenotype of vitamin D normalization.

In our patients, in contrast with a study by Braun-Moscovici, *et al*[21], we found no correlation between vitamin D deficiency and the occurrence of acroosteolysis and calcinosis, and similarly no correlations were seen between vitamin D deficiency and disease severity, corresponding to findings in patients with SLE[37].

Our study has some limitations. The design was cross-sectional, and therefore the direction of associations could not be conclusively determined. Moreover, the data could be confounded by the fact that patients with high disease activity or higher sPAP had worse health status, and thus had a diet lacking in vitamin D or had less UV exposure than they should have. Another point is that we did not assess the dietary intake of vitamin D, usually evaluated with a food-frequency questionnaire, even though this is not as reliable an indicator of vitamin D status as measurement of serum levels[42]. Moreover, we did not compare the 2 SSc populations with healthy controls, but we consider that a great deal of data were already available and that we could not assemble a larger cohort of healthy controls who would improve the data for controls.

In summary, we found a high frequency of vitamin D deficiency in 2 SSc populations from different geographic areas, even when standard substitution was undertaken, and this vitamin D deficiency correlated with disease activity, acute-phase reactants, and estimated sPAP values.

Vitamin D deficiency in SSc may be linked to multiple risk factors: insufficient sun exposure due to disability, or

insufficient intake and malabsorption. The role of vitamin D deficiency in the pathogenesis of autoimmune disease remains unclear[43], thus the advice to widely prescribe vitamin D integration to prevent development of such diseases requires clarifying data; it is advised to regularly assess vitamin D levels in these patients, even in sunny countries. A further step would be to investigate any changes in the disease activity or phenotype after stronger vitamin D supplementation.

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