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Serum 25-OH vitamin D levels in systemic sclerosis: analysis of 140 patients and review of the literature

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ABSTRACT

Objective. Hypovitaminosis D is increasingly reported in autoimmune diseases. We aimed to investigate the 25OH-vitamin D (25OH-vitD) levels in SSc patients and to correlate 25OH-vitD levels with disease's features.

Methods. We measured the 25OH-vitD serum levels in 140 consecutive patients (F/M 126/15; mean age 61±15.1 years), 91 without (group A) and 49 with (group B) 25OH-cholecalciferol supplementation.

Results. Patients of group A invariably showed low 25OH-vitD levels (9.8±4.1 ng/ml vs 26±8.1 ng/ml of group B); in particular, 88/91 (97%) patients showed vitamin D deficiency (<20 ng/ml), with very low vitamin D levels (<10 ng/ml) in 40 (44%) subjects.

Only 15/49 (30.6%) patients of group B reached the normal levels of 25OH-vitD (≥30 ng/ml), whereas vitamin D deficiency persisted in 12/49 (24.5%) individuals. Parathormone levels inversely correlated with 25OH-vitD ($r = -0.3$, $p<0.0001$), as expected.

Of interest, hypovitaminosis D was statistically associated with autoimmune thyroiditis ($p=0.008$), while calcinosis was more frequently observed in patients of group A ($p=0.057$). Moreover, we found significantly higher percentage of serum anticentromere antibodies in group B patients with 25OH-vitD level ≥30 ng/ml (8/15 vs. 6/34; Fisher's $p=0.017$).

In literature, hypovitaminosis D is very frequent in SSc patients. Moreover, an association with disease duration, calcinosis, or severity of pulmonary involvement was occasionally recognized.

Conclusions. Hypovitaminosis D is very frequent in SSc, particularly severe in a relevant percentage of patients; furthermore, less than 1/3 of supplemented subjects reached normal levels of 25OH-VitD. The evaluation of 25OH-vitD levels should be included in the routine clinical work-up of SSc patients.

Introduction

Systemic sclerosis (SSc) is an immune-mediated connective tissue disorder characterized by multiple organ fibrotic alterations, immune dysfunction and diffuse microangiopathy [1]. The etiology of SSc is still obscure, it is attributed to multifactorial causes such as genetic predisposition, infectious, and environmental factors. While the complex pathogenesis of SSc is characterized by T- and B-cell activation, and autoantibodies production leading to cytokine production responsible for immune-mediated microvascular damage, inflammation, and fibrosis [2].

Vitamin D (VitD) refers to a group of fat-soluble secosteroids (cholecalciferol, calcifediol, calcitriol) that plays a significant role in calcium homeostasis and bone metabolism. Since very few foods contain VitD, the synthesis in the skin is its major natural source, totally correlated to sun exposure. The active metabolite of VitD, the calcitriol (1,25OH-VitD) may be considered a hormone as its synthesis and activity occur in different tissues (skin, liver, kidney, bone, gastrointestinal tract, etc.) [3].

Low vitamin D status and inadequate calcium nutrition are highly prevalent in the general population (30–80%), affecting both genders [4]. In recent years a role of VitD as immune modulator factor has been proposed [5] and its deficiency has been observed in many autoimmune diseases, including type 1 diabetes mellitus, multiple sclerosis, inflammatory bowel disease [6-8], and systemic rheumatic disorders such as rheumatoid arthritis, SLE, and SSc [9-12]. Interestingly, more and more studies demonstrated an high frequency of low VitD levels in SSc [13-32].

The aim of our study was to evaluate the prevalence of hypovitaminosis D in a large cohort of SSc patients, along with its possible relationship with SSc clinical features, compared to previously published findings in the world literature.

Patients and Methods

The present cross-sectional study included 140 consecutive SSc patients (F/M 126/15; mean age 61±15.1SD years), referring to our Rheumatology Unit from 1st November 2015 to 31 May 2016. All patients fulfilled the 2013 ACR/EULAR criteria for SSc and were classified according to the extent of skin involvement in limited and diffuse SSc [33].

The study was approved by the local Ethical Committee (protocol n. 282/15); moreover, all patients gave their written consents.

25OH-vitamin D (25OH-vit D), intact parathyroid hormone (PTH), serum total calcium and phosphorus, and alkaline phosphatase were measured, along with thyroid function assessment (fT3, fT4, anti-thyroglobulin and thyroperoxidase antibodies), serum creatinine, blood cells count, and transam-

inases. Patients' serology as regards antinuclear antibodies and anti-extractable nuclear antigens antibodies was available. The disease duration (mean 8.1±6.6SD years) was referred to the time between SSc onset (first SSc symptom other than Raynaud's phenomenon) and blood test conduction.

The assessment of SSc visceral involvements included spirometric and DLCO tests, high resolution computed tomography, echocardiography with pulmonary arterial pressure estimation, barium oesophagus X-ray, nailfold videocapillaroscopy, and thyroid ultrasound examination. On the basis of anamnestic, physical, and instrumental findings, organ involvement was defined as follows: 'heart involvement': presence of arrhythmias and/or right/left heart failure; 'kidney involvement': renal function deficiency (creatinine-based approximation of the glomerular filtration rate at least < 50 mg/ml/min); 'gastrointestinal involvement': presence of dyspepsia, motility dysfunctions, and/or signs of small intestinal bacterial overgrowth; 'interstitial lung disease': occurrence of typical lung fibrosis at radiological examination.

Dual-energy x-ray absorptiometry (DEXA) measurements were performed in 70 post-menopausal patients using a total body scanner (QDR 4500 Acclaim Series Elite, Hologic Inc., Bedford, MA, USA) to evaluate BMD and T-score. BMD was evaluated both at lumbar spine (L1-L4) and hip (femoral neck and total hip) and was expressed as g/cm2; osteoporosis was defined as −2.5 or lower T-score values and osteopenia as -1 to -2.5 T-score values.

Commercially available radioimmuno-assay (Immuno diagnostic Systems Holdings, Boldon, UK) was used to measure serum levels of 25OH-vit D, the major circulating form and standard indicator of vitD status, defined as normal (≥30 ng/ml), vitD insufficiency (20-29 ng/ml), or deficiency (<20 ng/ml) [34].

The previous reports on vitD serum levels observed in SSc patients of the world literature (PubMed, Scopus, Web of Science, Scientific Information Database, IndMed and Index Medicus for the World Health Organization Eastern Mediterranean Region) were investigated using the key words: scleroderma, systemic sclerosis, and vitamin D in various combinations.

Results

The table 1 summarizes the main clinical and laboratory features of our SSc patients consecutively evaluated regardless the ongoing vitD supplementation. Among 140 SSc patients included in the study 91 were never supplemented with vitD (group A) while 49 (group B) were taking orally 25OH-cholecalciferol 8,000-12,500 UI weekly during a time period of at least 6 months.

The 91 patients not supplemented with 25OH-cholecalciferol (group A) showed serum levels of 25OH-vit D significantly lower compared with the group B (9.8±4.1SD vs 26±8.1 ng/ml; $p<0.0001$; Fig. 1); similarly, subjects supplemented presented lower parathormone (PTH) levels if compared with the patients of group A (44±20.5 vs. 61.8±37; $p=0.002$), while PTH levels inversely correlated

with 25OH-vit D levels (Spearman's r= -0.3, p<0.0001). Of interest, among supplemented patients the 25OH-vit D values reached the normal range (≥30 ng/ml) in only 15/49 (30.6%) cases. As regards the clinical SSc features, statistical difference between the 2 groups was found for just gastrointestinal involvement, which was rather frequently recorded in supplemented patients (group B).

Focusing on the 91 patients of group A (subjects not supplemented), abnormally low 25OH-vit D serum levels were invariably recorded; namely, 88/91 (97%) patients showed vitD deficiency, with very low (<10 ng/ml) vitD levels in 40/91 (44%) patients (mean 25OH-vit D level 6.9±2 ng/ml). The subgroup of not supplemented patients did not present statistical differences as regards clinical features, with the exception of higher PTH levels in patients with severe vitD deficiency (68.5±36.2 vs. 53.3±36.7 pg/ml, Mann-Whitney's p=0.012). Of note, calcinosis, observed in 9/91 cases, tends to be more frequently found in patients with lower 25OH-vit D levels (Mann Whitney's p=0.057). Moreover, vitD deficiency was not correlated to disease duration; in particular, considering only not supplemented SSc patients with disease duration ≤5 years, the 25OH-vit D serum levels were comparable to those found in the whole group A (8.8±4.8 ng/ml and 6.9±2 ng/ml, respectively).

Among 91 not supplemented SSc patients, 66 (73%) presented autoimmune thyroiditis (defined by the presence of anti-thyroglobulin and/or anti-thyroid peroxydase antibodies plus parenchymal inhomogeneity at ultrasound examination); these patients showed serum 25OH-vitD levels significantly lower compared to those without autoimmune thyroiditis (14.4±8.5SD and 19.9±9.5 ng/ml, respectively; p=0.008). Interestingly, autoimmune thyroiditis was less frequently found in the 49 supplemented patients (51% in group B vs 73% in group A; p=0.015).

The BMD of patients not supplemented with vitD did not correlated with 25OH-vit D serum levels; on the whole, the frequency of osteoporosis among two groups was not associated with the severity of hypovitaminosis D.

Finally, considering only the group B (supplemented SSc patients), we found a increased frequency of anticentromere (ACA) antibodies in subjects with normal 25OH-vit D serum levels (8/15 vs. 6/34; Fisher's p=0.017).

Review of the literature

The main clinical studies on SSc patients with regards to serum levels of 25OH-vit D are summarized in the Tab. 2. Previously published papers were prevalently carried out on small European SSc patient's series [13-15, 17-21, 23, 24, 27-29], often in comparison with healthy controls [14, 17, 18, 21-24, 28-30]; only few studies included more than 100 SSc patients [15, 18, 21, 25]. On the whole, female gender was clearly prevalent, accordingly to the epidemiology of SSc; while patients' mean age ranged between 50-60 years, except for the study by Shinjo SK et al. regarding Brazilian cases of juvenile SSc [26].

The presence of severe hypovitaminosis D (<10 ng/ml) resulted quite frequent, ranging from 6.7% [17] up to 46% of our patients' series, with mean 25OH-vitD values always under the cut-off of sufficiency (30 ng/ml) and significantly lower than that observed in healthy controls. Although the limited number of published studies, there were not valuable relationships between the observed prevalence of hypovitaminosis D and the geographical provenience of SSc patients series investigated.
Some correlations between hypovitaminosis D and SSc clinical features were observed even if extremely variable and discordant among different studies [13, 16-18, 20-22, 29-32]. In particular, lower 25OH-vit D levels were occasionally observed in patients with longer disease duration, calcinosis, severity of skin and/or pulmonary involvement. While the correlations with PTH levels (inversely) or bone mass density (directly) were reported as predictable in only few studies [16, 22, 27, 28].
Overall, it is presumable that several factors may have influenced these findings, including SSc-related and non SSc-related parameters, thus leading to heterogeneous outcomes; anyway, the high frequency of hypovitaminosis D in SSc patients represents the most relevant result.

Discussion

The results of the present study emphasize the high prevalence and severity of hypovitaminosis D in SSc patients; abnormally low levels of 25OH-Vit D were invariably recorded in not supplemented individuals with clear-cut deficiency values (<10 ng/ml) in 44% of cases. Moreover, over 2/3 of supplemented patients showed 25OH-Vit D insufficiency/deficiency despite the therapy, even though significantly higher levels were medially recorded in comparison with not supplemented patients. In patients of group A the PTH serum levels were inversely correlated with hypovitaminosis D, as expected. More interestingly, autoimmune thyroiditis was detected in at least half cases with a significantly higher percentage in individuals without compared to those with 25OH-Vit D supplementation; while gastrointestinal involvement was statistically more frequent in supplemented patients; this finding might at least in part explain the low rate of serum 25OH-cholecalciferol normalization in supplemented individuals. Finally, the presence of anticentromere antibodies correlates with the normalization of 25OH-Vit D levels after supplementation, suggesting that anticentromere-positive SSc patients might be more responsive to vitamin D supplementation; this hypothesis is consistent with the well-known better disease outcome.
The prevalence of hypovitaminosis D is quite frequent also in the general population, even if with largely variable percentages among different epidemiological studies (4); in this respect, the prevalence of vitamin D deficiency (<20 ng/ml) observed in our SSc patients is clearly higher compared to that observed in Italian general population of the same geographical area, namely 97% vs 37% (35). Few correlations between hypovitaminosis D and SSc clinical features were reported in the previous literature, often with discordant findings among different studies [13, 16, 18, 20-22, 29-32].

In particular, the observed association with longer disease duration, calcinosis, and in particular with extent and severity of skin fibrosis suggested an impaired cholecalciferol synthesis in the sclerodermic cutis. In our cohort, a trend to a direct correlation between hypovitaminosis and calcinosis was found; whereas the low number of SSc patients with diffuse skin subset did not permit a statistical correlation of 25OH-Vit D levels with the severity of skin sclerosis. Overall, it is presumable that the high rate of hypovitaminosis D observed in SSc patents may be multifactorial. Several SSc-related (disease duration, severity of skin sclerosis reduced cholecalciferol synthesis, gastrointestinal involvement malabsorption, etc.) and non SSc-related factors (genetic, environmental, climatic with insufficient sun exposure work-related, insufficient intake, drug-related VitD malabsorption, vitD receptor tissue resistance, etc.) may be variably involved in the pathogenesis of hypovitaminosis D, leading to heterogeneous clinical patterns among different study populations. In the majority of patients we measured vit D levels during autumn-winter; however, no seasonal differences were observed considering patients evaluated during spring-summer. This finding might be explained considering that SSc patients are very little and rarely exposed to the sun in order to prevent the worsening of their skin involvement. Of some interest is our observation that whole-body UVB irradiation over 6 months did not improve the hypovitaminosis D in a small series of SSc patients (unpublished data). Moreover, clinically overt malabsorption syndrome was never observed in SSc patients included in the present study, as well as we did not find significant differences of 25OH-vit D levels between diffuse and limited cutaneous SSc subsets. Similarly, low 25OH-vit D levels were not correlated with the disease duration (≤5 years) suggesting that probably more severe, long-lasting SSc organ damage is not the main cause of hypovitaminosis D, even if this aspect should be further investigated. An intriguing hypothesis was proposed by Carmel NN et al [36] who reported the presence of anti-vitamin D antibodies in SSc patients possibly responsible for hypovitaminosis D; this first clue should be confirmed by further studies.

Anyway, the high frequency of hypovitaminosis D in various SSc patients' populations, regardless their genetic and epidemiological differences, might suggest a prominent role of the disease *per se*.

Considering the group B (supplemented SSc patients), we found an increased frequency of ACA antibodies in subjects with higher 25OH-vit D serum levels; this finding could identify a subset of patients with a better response to Vit D supplementation, at least in part related to better prognosis of the disease that characterizes ACA-positive patients [1].

A possible relationship between low levels of 25OH-vit D and autoimmune thyroiditis has been previously suggested by other cross-sectional studies [37]; this finding was further supported by the statistically significant correlation between autoimmune thyroiditis and vitD deficiency in our SSc patients.

Apart from its function on calcium and bone homeostasis, vitD is recognized to contribute to the immune system regulation by preserving its homeostasis [38, 39]. It has been documented that vitD receptors are present on the surface of antigen presenting cells, natural killer cells, as well as B- and T-lymphocytes, explaining multiple immunomodulation effects on both innate and adaptive immune responses [5, 38-40]. Physiopathology investigations confirm that hypovitaminosis D, in genetically predisposed subjects, can impair self-tolerance by compromising the regulation of dendritic cells, regulatory T-lymphocytes and Th1 cells [40]. Furthermore, epidemiological studies indicate a significant association between vitamin D deficiency and an increased incidence of several autoimmune diseases [7, 34]. In SSc, this pathogenetic relationship has not been completely understood. The high prevalence of vitamin D deficiency is clearly evidenced by numerous studies [13-32], always in patients series with overt disease; therefore, the evaluation of vitD status in individuals with very early phase of SSc and/or pre-scleroderma Raynaud's phenomenon could be decisive in order to better explain the actual role of hypovitaminosis D. This latter might represents a predisposing condition for the development of the SSc and/or disease progression cofactor. Considering the SSc outcome, low levels of VitD with impaired immunomodulation action could be associated with a more aggressive SSc subset. Consistently, treatment with vitamin D in SSc patients might produce a beneficial effect, reducing both disease activity and severity through its multiple immunomodulation activity [42, 43]. In addition, recent studies on murine fibroblasts have shown a direct anti-fibrotic effect of vitamin D, by the impairment of TGFβ1 expression, the myofibroblast transdifferentiation, and the concomitant reduction of collagen I and III synthesis [44-46].

Although it is one of the most large case series of SSc patients evaluated for hypovitaminosis D, the present study have some limitations; in particular, considering the high clinical heterogeneity of SSc, larger patients' series should be recruited in order to correlate low 25OH-Vit D levels with specific disease's clinical variants.

In conclusion, our study underlines the very high frequency of hypovitaminosis D in SSc patients, with severe deficiency in a significant number of individuals; furthermore, normal levels of 25OH-Vit D were reached in less than one third of supplemented patients. Several reasons of these findings could be hypothesized, mainly impaired cutaneous vitD synthesis and/or cholecalciferol malabsorption, needing further pathogenetic investigations. In the clinical practice, the measurement of 25OH-vit D serum levels should be routinely included at the patient's first assessment and during the follow-up, with adequate supplementation in individuals with documented hypovitaminosis D.

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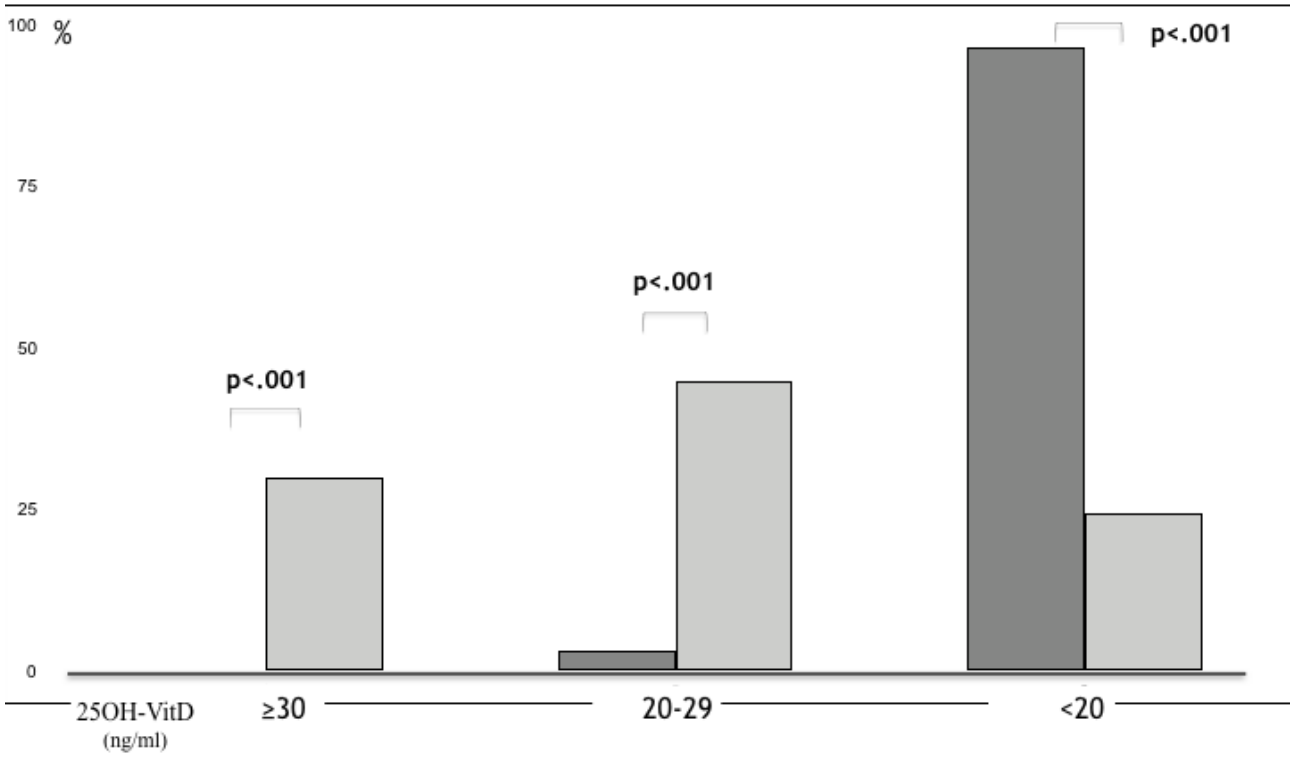
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Legend to Figure 1

Prevalence of hypovitaminosis D in 91 not supplemented (grey, group A) vs 49 supplemented



(black, group B) SSc patients.

Grup A: SSc patients invariably show abnormally low 25OH-vitD levels (<30 ng/ml), while vitD insufficiency (20-29 ng/ml) or deficiency (<20 ng/ml) were detected in 3.3% and 96.7% of patients, respectively.
Group B: normal 25OH-vitD levels (≥30 ng/ml) were found in only 30.5% of supplemented patients, while 45% of patients show vitD insufficiency and 24.5% vitD deficiency.

Tab. 1. Clinical and laboratory features of 140 SSc patients with/without 25OH-cholecalciferol supplementation.

	Group A - 91 pts not supplemented	Group B - 49 pts supplemented	p values A vs B
25OH-vitD levels (ng/ml)	9.8 ± 4.1	26 ± 8.1	**<0.001**
25OH-vitD ≥30 (normal vitD)	0%	30.6%	**<0.001**
25OH-vitD 20-29 (vitD insufficiency)	3.3%	44.9%	**<0.001**
25OH-vitD <20 (vitD deficiency)	96.7%	24.5%	**<0.001**
25OH-vitD < 10 (vitD severe deficiency)	44%	0%	**<0.001**
Age (years)	60.4±16.4	62.6±12.4	0.41
Gender (M/F)	13/78	8/41	0.80
Disease duration (years)	7.5±6.2	9.5±7.3	0.09
Diffuse skin subset	15%	20%	0.48
Calcinosis	10%	12%	0.77
Anti-Scl70	44%	37%	0.47
Anticentromere	26%	29%	0.84
Anti-nucleolar antibodies	16%	8%	0.20
Heart involvement	36%	33%	0.71
Gastrointestinal involvement	47%	69%	**0.013**
Kidney involvement	10%	8%	1
Interstitial lung disease	68%	78%	0.33
FVC<70%	9%	14%	0.39
DLCO<50%	25%	33%	0.43
Pulm. arterial hypertension	8%	12%	0.38
Autoimmune thyroiditis	73%	51%	**0.015**
Osteoporosis	23%	32%	0.54
Parathormone (pg/ml)	61.8±37	44±20.5	**0.002**

Table 2. 25OH-cholecalciferol levels in systemic sclerosis: review of the literature.

	Authors, year (ref. no.)	Tipe of study	Country	Pts no.	Mean age	M/F	25OH-VitD concentration (ng/ml)	Severe hypovitaminosis (<10 ng/ml)	Controls	25OH-VitD concentration (ng/ml)	Correlations
1	Serup J, 1985 (27)	cohort	Denmark	20	52.8 (31-67)	6/14	15±9.7	n.a.	No		tendence for calcinosis and disease duration (p>0.05)
2	Matsuoka LY, 1991 (28)	open label, controlled	USA (Caucasian)	19	51 (28-69)	4/15	28±3 SEM	n.a.	19	29 ± 3 SEM	n.a.
3	Orbach H, 2007 (29)	cross-sectional	Israel/Hungary	229	n.a.	n.a.	11±5.8	n.a.	No		n.a.
4	Brun-Moscovici Y, 2008 (30)	retrospective	Israel	60 (44 Jews/ 16 Arabic)	55±14	9/51	13.8±7.2	46%	No		acroosteolysis, calcinosis, disease duration, PTH, Arab origin
5	Dovio A, 2008 (31)	open label, controlled	Italy	60	58 (31-72)	12/48	23 (3-92)	6.7%	60	39 (14-138)	osteocalcin and CTX (negative correlations)
6	Vacca A, 2009 (32)	cross-sectional	Italy/France	90 (Sardinia) + 66 (Paris, France)	57±13	14/142	19±11	28%	No		EDAS, acute-phase reactants, sPAP
7	Rios-Fernandez R, 2010 (33)	cohort	Spain	48	59.1±11.7	0/48	n.a.	9.5%	No		No
8	Caramaschi P, 2010 (34)	cross-sectional	Italy	65	58.1±14.8	13/52	15.8±9.1	29.2%	No		disease duration, DLCO, acute-phase reactants, NVC pattern
9	Arnson Y, 2011 (35)	multicenter, cross-sectional	Hungary, Italy, Spain	327	56.7±18.2	20/140	13.5±9	n.a.	141	21.6±9.7	skin involvment, age, DLCO, FR
10	Ibn Yacoub Y, 2011 (36)	case-control	Marocco	30	49.4±13.1	0/30	10.9±2.7	26.7%	30	57.4±4.2	severity of joint pain, anti-Scl70, lumbar/femoral BMD
11	Seriolo B, 2011 (37)	cohort	Italy	53	58.5±7.3	0/53	Winter:19.3±12.3 Summer:21.7±13.4	Winter:60% Summer:64%	53	Winter:32.1±14.1 Summer:39.4±15.4	n.a.
12	Bellolli L, 2011 (38)	cohort	Italy	43	61±14	1/42	18.1±15.2	51.2%	99	17.3±12	No
13	Gambichler T, 2011 (39)	cohort	Germany	137	56±13.6	18/119	median 13.1 range (4.1-47.8)	35.8%	No		No
14	Shinjo SK, 2011 (40)	case-control	Brazil	10	20.9±1.8	1/9	18.1±6.4	0%	10	25.1±6.6	femoral BMAD
15	Rios-Fernandez R, 2012 (41)	multicenter, retrospective, cohort	Spain	90	56.5±13.3	0/90	South:27.4±16.2 North: 20.7±11	11.1%	No		No (with BMD)
16	Atterritano M, 2013 (42)	cross-sectional	Italy	54	54.4±1.7	0/54	18.3±4	n.a.	54	39.6±7.5	PTH, lumbar/femoral BMD, stiffness index, osteocalcin, deoxy-pyridinoline
17	Corrado A, 2015 (43)	cohort	Italy	64	64.5 (42-75)	0/64	15.7±10.2	n.a.	35	22.9±9.1	skin involvment (limited/diffuse)
18	Zhang L, 2015 (44)	case-control	China	60	47.7±12.7	8/52	26.5±6.3	16.7%	60	36.3±14.2	pulmonary involvement

19	Montabone E, 2016 (45)	open label, controlled	Italy	35	61 (36-72)	3/32	18.5	11%	No		worse physical component score (Medical out-comesStudy Short-form 36)
20	Sampaio-Barros MM, 2016 (46)	cross-sectional	Brasil	38	40.2±7.3 (18-50)	0/38	20.66±8.20	11%	No		BMI, BMD-femur, SF-36 (evaluated withpositive correlations). NVC a/devascularization (negative correlations)
21	*Present study*	*cross-sectional*	*Italy*	*140*	*61±15.1*	*15/126*	*supplemented: 26±8.1 not supplemented: 9.8±4.1*	*51/91 (56%)*	*No*		*PTH, ACA (among supplemented pts)*

SSc: sistemic sclerosis; VitD: Vitamin D; mRSS: modified Rodnan skin score; FVC: forced vital capacity; DLCO: diffusing lung capacity for carbon monoxide HRCT: High-resolution computed tomography; NVC: nailfold videocapillaroscopy; sPAP: systolic pulmonary artery pressure; BMI: body mass index; BMD: bone mineral density; BMAD: bone mineral apparent density; EDAS: European Disease Activity Score; FR: rheumatoid factor; SF-36: Short-Form-36 Questionnaire.