Low trabecular bone score is associated with high C-reactive protein levels in systemic sclerosis

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Research article

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Abstract

Objectives

To evaluate trabecular bone score (TBS) in patients with systemic sclerosis (SSc) and to identify risk factors related to low TBS in SSc.

Methods

TBS and areal bone mineral density (aBMD) were assessed in patients with SSc (n = 57), rheumatoid arthritis (RA) (n = 47), and hand osteoarthritis (OA) (n = 37) using DXA. Osteoporosis risk factors, laboratory findings, SSc-specific organ involvement, and patterns of nailfold capillaroscopy (NFC) were also assessed. Multivariate linear regression analysis was performed to identify the risk factors associated with TBS in SSc patients.

Results

The median TBS (Q1, Q3) value was 1.378 (1.322, 1.425) in SSc patients, 1.336 (1.261, 1.396) for RA patients, and 1.430 (1.387, 1.438) for controls ($p < 0.001$). No significant differences were observed in the median lumbar spine TBS and aBMD at the lumbar spine, femoral neck, and total hip between the SSc and RA groups. The TBS was negatively correlated with the erythrocyte sedimentation rate ($p = 0.042$) and C-reactive protein (CRP) ($p = 0.005$) in the SSc group only and with cumulative glucocorticoid doses in the RA group only ($p = 0.031$). We found no association between TBS and SSc cutaneous subtype, internal organ involvement, autoantibody profile, NFC patterns, and use of immunosuppressive agents, such as cyclophosphamide. In the multivariate analyses, age, female sex, current, and average CRP were significantly associated with TBS.

Conclusions

TBS assessment revealed poor bone quality in patients with SSc, similar to those with RA. CRP levels were negatively correlated with TBS in patients with SSc, and higher CRP levels were independently associated with low TBS.

Background

Systemic sclerosis (SSc) is an autoimmune disease characterised by microangiopathy, fibrosis of the skin and internal organs, and disturbances in the immune system [1]. As SSc affects various organs and systems, patients with SSc are at an increased risk of bone loss. Previous studies have shown that patients with SSc have a lower areal bone mineral density (aBMD) and a higher prevalence of fractures than healthy controls [2–4].

The persistent inflammatory process and immobility can play a role in the development of osteoporosis (OP) in rheumatic diseases [5]. The inflammatory phase, characterised by a Th1- and Th17-dependent immune response, is involved in the pathogenesis of SSc [6]. Moreover, malabsorption secondary to gastrointestinal involvement, premature ovarian failure related to treatment with cyclophosphamide (CYC), and use of glucocorticoids (GCs) could increase the risk of OP in patients with SSc [5, 7, 8].

Bone mass and microarchitecture are major determinants of bone strength [9]. The trabecular bone score (TBS) is a texture tool that can be computed from dual-energy X-ray absorptiometry (DXA) images. TBS provides information on bone microarchitecture, and a high TBS value suggests dense and good connectivity. TBS is an independent risk factor for OP fractures [10]. Compared to OP studies using BMD, fewer data are available on bone quality in patients with SSc. Two studies demonstrated that patients with SSc had lower TBS values than controls, which were similar to those in patients with rheumatoid arthritis (RA) [11, 12]. However, the risk factors associated with bone microarchitecture in SSc have not been well studied. In particular, the possible role of inflammation in bone quality needs to be evaluated in patients with SSc.

This study aimed to evaluate TBS in patients with SSc compared with those with RA and hand osteoarthritis (OA) and to identify risk factors related to low TBS in SSc.

Methods

Study population

A single-center cross-sectional study was conducted at the Soonchunhyang University Seoul Hospital (Seoul, South Korea) between July 2019 and December 2020. Patients with SSc, RA, and hand OA were enrolled as controls. The definitive diagnosis of SSc, RA, and hand OA was made in accordance with the 2013 American College of Rheumatology/European League against Rheumatism (ACR/EULAR) classification criteria [13], 2010 ACR/EULAR classification criteria [14], and 1990 ACR classification criteria [15], respectively. Exclusion criteria included patients with inflammatory rheumatic diseases other than SSc and RA, premenopausal status, thyroid or parathyroid disorders, presence of chronic renal or liver disease, gastrectomy, bariatric surgery, malabsorption syndrome, and chronic obstructive pulmonary disease. The study was approved by the Institutional Review Board (IRB) for Human Research (2020-10-008) at Soonchunhyang University Seoul Hospital. Written consent was obtained from all participants.

TBS and aBMD assessment

aBMD was measured at the lumbar spine (L1-L4) and left hip (femoral neck and total proximal femur) using DXA (Hologic Horizon W; Hologic Inc., Danbury, CT, USA). All measurements were taken by experienced operators using the same machine and standardised procedures for participant positioning. TBS was evaluated at the lumbar spine (L1–L4) on the same DEX acquisition used for aBMD assessment using TBS iNsight software (version 3.0; Med-Imaps, Merignac, France). Patients with SSc were divided into three TBS groups according to the risk of fracture in a recent meta-analysis: high risk, TBS < 1.23; intermediate risk, TBS 1.23-1.31; low risk, TBS above 1.31 [16].

Clinical and laboratory evaluation

For each patient, the following data were collected at the time of TBS assessment: demographics, disease duration, defined as the time elapsed between the onset of the first disease-related symptoms (except for Raynaud phenomenon in SSc) and enrollment, OP risk factors (smoking status, alcohol consumption, menopausal status, and family history of OP fracture).

The daily glucocorticoid (GC) dose and cumulative GC dose were documented, including the use of immunosuppressive agents (CYC, mycophenolate mofetil [MMF], and methotrexate), and OP medications (bisphosphonate [BP], selective oestrogen receptor modulator [SERM], vitamin D, and calcium). Vertebral fractures were evaluated using radiographs of the thoracic and lumbar spines.

Laboratory tests were performed to determine the erythrocyte sedimentation rate (ESR) and the levels of C-reactive protein (CRP), 25 hydroxyvitamin D (25(OH)D), and bone turnover markers (bone alkaline phosphatase [ALP], serum alkaline phosphatase, procollagen type 1 intact N-terminal propeptide [P1NP], C-terminal telopeptide of type 1 collagen [CTX], and osteocalcin). CRP serum levels were also documented at every visit from the first visit to our rheumatology clinic. Patients with SSc were classified into three groups according to CRP concentration: inflammatory, intermediate, and non-inflammatory SSc. Patients with SSc displaying elevated CRP levels (≥0.5 mg/dL) at more than 80% of visits were defined as inflammatory SSc, while patients with normal CRP levels on all visits were defined as non-inflammatory SSc [6]. CRP elevations related to infections or medical interventions, as evidenced by a retrospective chart review, were excluded from the analysis. Laboratory findings, including the presence of serum anti-nuclear antibody, anti-centromere, anti-topoisomerase, and anti- ribonucleoprotein, were also recorded.

Additionally, internal organ involvement in patients with SSc, including interstitial lung disease, gastrointestinal involvement [17], scleroderma renal crisis [18], and pulmonary arterial hypertension, were investigated [19]. The skin thickness score was obtained using the modified Rodnan total skin score [20]. Patterns of nailfold capillaroscopy (NFC) were obtained for patients with SSc as previously reported by Cutolo et al.: early, active, and late patterns [21].

Statistical analysis

Statistical analyses were performed using the SPSS software package (SPSS Inc., Chicago, IL, USA) for Windows v. 22.0. Differences among three groups were analysed using the Kruskal-Wallis test with Bonferroni post-hoc test. Data were compared using the chi-square test and Mann-Whitney U-test, as appropriate. The Spearman rank correlation test was used to assess the relationship between the TBS and continuous variables. Partial correlation analyses, adjusted for age, sex, and cumulative GC dose, were performed to assess the relationship between TBS and CRP levels. Generalised estimating equations (GEE) models were used to determine the independent variables associated with TBS. All items that were associated with TBS by univariate analysis with *p*-values less than 0.05 were included in the multivariate model. In the multivariate analysis, separate models for current CRP and average CRP were performed due to multicollinearity. The results were considered statistically significant at $p<0.05$.

Results

Baseline characteristics of the study population

A total of 141 patients were included in the study: SSc (n = 57), RA (n = 47), and hand OA (n = 37). **Table 1** summarises the baseline characteristics of the study population. The current GC doses were comparable between patients with SSc and those with RA. Cumulative doses of GC were significantly lower in patients with SSc than in those with RA (adjusted $p<0.001$). The use of BP was higher in the RA group than in the SSc and OA groups ($p<0.001$). The median (Q1, Q3) duration of BP use in the RA group was 1.9 (0.8, 4.15).

Assessment of TBS and BMD

The median TBS (Q1, Q3) value was 1.378 (1.322, 1.425) for patients with SSc, 1.336 (1.261, 1.396) for patients with RA, and 1.430 (1.387, 1.438) for patients with hand OA ($p<0.001$) (**Table 2**). No significant differences were observed in the median lumbar spine TBS and aBMD at the lumbar spine, femoral neck, and total hip between the SSc and RA groups.

Correlation between clinical parameters, inflammatory markers, aBMD, and TBS

In all groups, the TBS showed a significantly negative correlation with age and a positive correlation with the aBMD of the femoral neck and total hip (**Table 3**). The TBS was negatively correlated with ESR (current and average) and current CRP in the SSc group only and with cumulative GC doses in the RA group only. The average CRP was negatively correlated with TBS in both the SSc and RA groups. However, partial correlation analysis for TBS and average CRP levels, adjusted for age, sex, and cumulative GC doses, showed a negative correlation in the SSc group ($r=-0.316$, $p=0.020$) and non-significant correlation in the RA group ($r=-0.197$, $p=0.200$). In both SSc and RA groups, no significant correlation between CRP (average and current) and aBMD (femoral neck, total hip, lumbar spine) was found.

Characteristics of patients with SSc stratified according to TBS fracture risk groups

Table 4 shows the clinical and laboratory characteristics of the TBS subgroups in patients with SSc according to the risk of fracture: high risk (n=3), intermediate risk (n=8), and low risk (n=46). Patients with intermediate to high risk of fracture were older and had higher cumulative GC doses for the last three months than those in the low-risk groups ($p=0.014$ and $p=0.046$, respectively). According to the CRP status, inflammatory and intermediate SSc were more prevalent in the intermediate-risk to high-risk TBS group than in the low-risk TBS group (27.3% and 63.6% vs. 2.2% and 28.3%, respectively) ($p=0.003$). About 70% of patients in the low-risk TBS group had non-inflammatory SSc.

The use of BP and supplemental vitamin D was more frequent in the intermediate-risk to high-risk group than in the low-risk group. There were no significant differences among the TBS subgroups of patients in terms of fracture risk with respect to smoking status, alcohol consumption, disease duration, autoantibody profiles, internal organ involvement, mRSS, and the use of immunosuppressive agents. When the three categories of NFC patterns were compared for the patients with SSc, no significant association was observed between NFC patterns and TBS, aBMD, and laboratory findings (**see Additional fie 1**).

Linear regression analysis for TBS among patients with SSc

Table 5 shows the results of the univariate and multivariate analyses of TBS in patients with SSc. Univariate analysis revealed that TBS was associated with age, female sex, ESR, CRP, average ESR, average CRP, and alcohol consumption. In the multivariate analyses, age, female sex, CRP, and average CRP were significantly associated with TBS.

Discussion

In the present study, we investigated the bone microarchitecture based on TBS and risk factors affecting poor bone quality in patients with SSc. Decreased TBS and BMD values were observed in patients with SSc and RA compared to those with hand OA. Inflammatory markers, such as CRP and ESR, were negatively correlated with TBS in patients with SSc, but not in those with RA. Age, female sex, current, and average CRP were independent risk factors associated with low TBS.

Chronic inflammatory diseases are also associated with OP. Bone loss is related to increased osteoclast activity induced by pro-inflammatory cytokines, such as interleukin 6 (IL-6), IL-1, and tumour necrosis factor [22]. CRP is a general inflammatory marker produced by hepatocytes upon stimulation by IL-6. An inverse association between CRP levels and BMD has also been reported in healthy women [23]. In previous SSc studies, an association between elevated CRP levels and disease activity, mRSS, ILD progression, and high mortality has been demonstrated [6, 24, 25]. We found a positive correlation of CRP (current and average) with TBS, and higher CRP was independently associated with low bone quality in patients with SSc. Interestingly, CRP level was not associated with aBMD in patients with SSc. These results show the direct interaction between inflammation and bone microarchitecture in patients with SSc. Surveillance of OP, including bone quality, is needed to reduce OP complications, especially in patients with SSc with increased CRP levels.

Although the RA group had significantly older age and higher cumulative GC doses than the SSc group, the TBS did not differ between the two groups. Cumulative GC doses were marginally higher in patients with SSc with intermediate to high TBS risk scores than in those with low-risk scores. However, a correlation between cumulative GC dose and TBS was observed in the RA group, but not in the SSc group. Although GCs may alleviate bone resorption related to inflammation, GCs are known risk factors for bone loss [26]. Due to the higher surface area and rate of bone turnover, GCs affect the trabecular bone compartment more than the cortical bone compartment [27]. Koumakis et al. showed that daily GC doses were independently associated with low TBS in patients with SSc. Koumakis et al. reported that the mean cumulative GC doses and disease duration of patients with SSc were 8630 mg and 10.2 years, respectively [11]. In contrast, the median cumulative GC doses and disease duration in our study were 885 mg and 2.9 years, respectively. A much lower cumulative GC dose and shorter disease duration could have affected our observation that GC had little impact on bone quality in patients with SSc. In line with this, Koumakis et al. reported much lower absolute TBS values compared to our study. This could also be explained by the large difference in the cumulative GC doses. The proportion of BP users, vitamin D supplementation, and postmenopausal women were similar between the two studies [11].

We found no association of TBS with SSc cutaneous subtype and internal organ involvement pattern, which was consistent with a previously reported study [11]. According to the NFC pattern, we observed no significant differences in patients with SSc. Ruaro et al. reported that patients with SSc with late pattern had lower TBS than those with early and active patterns [12]. Ruaro et al. reported that 25(OH)D levels were significantly lower in patients with SSc with a late pattern. However, the three SSc groups according to the NFC pattern did not show a difference in 25(OH)D levels in our study. Ruaro et al. excluded patients with SSc on drug regimens that could influence bone turnover, but we included patients with SSc undergoing OP treatment such as BP, calcium, and vitamin D. These distinct study designs could lead to differences in the results between the two studies.

CYC administration is related to premature ovarian failure, and an animal study has shown the suppressive effects of CYC on osteoblastogenesis [5, 28], which leads to concerns about the development of OP in patients with SSc. One study showed that CYC use was associated with an increased risk of femoral neck fractures in patients with systemic lupus erythematosus (SLE) [29]. However, a cross-sectional study on a large SLE cohort failed to show an association between immunosuppression and BMD [30]. We could not find an association between immunosuppressive agents and TBS in patients with SSc. Among the CYC users in our SSc population (n = 17), only 5 patients were premenopausal at the time of CYC administration, 8 patients were postmenopausal, and 4 patients were male. Postmenopausal status at CYC administration or reduction in the inflammatory

response of CYC could affect the results of our study [31]. Further large studies are needed to reveal the relationship between CYC treatment and the development of OP in patients with SSc.

Our study had several limitations. First, this study was a cross-sectional observational design, and we could not determine a causal relationship between CRP and TBS in patients with SSc. Second, due to the relatively small number of patients with SSc with intermediate-to high-risk TBS scores, the regression analysis could be underpowered. Third, there was a lack of data on the history of non-vertebral fractures. Finally, we included patients with SSc who were taking OP medication. Patients with SSc with intermediate to high TBS risk used more BP and supplemental vitamin D, reflecting those already diagnosed with OP. Further studies on the relationship between TBS and CRP are needed after excluding the OP drug influence in patients with SSc.

Conclusion

TBS assessment revealed poor bone quality in the lumbar spine in patients with SSc, similar to that in patients with RA. CRP levels were negatively correlated with TBS in patients with SSc, and higher CRP levels were independently associated with low TBS. These findings suggest that inflammation plays a role in the decreased bone quality of patients with SSc.

Declarations

Ethics approval and consent to participate

The study was approved by the Institutional Review Board (IRB) for Human Research (2020-10-008) at Soonchunhyang University Seoul Hospital.

Consent for publication

Written consent was obtained from all participants.

Availability of data and materials

The datasets generated and/or analyzed in this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

KAL, HJK, and HSK were involved in study conception and design. KAL, JSK, and WHC were involved in data acquisition. KAL and HSK performed data analysis and interpretation. All authors were involved in the drafting or critical revision of the article, and all authors approved the final version for publication. HSK had full access to all of the data in the study and takes

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Tables

Table 1 Characteristics of the study population included in the study

	SSc (n=57)	RA (n=47)	Controls (n=37)	*P*-value	Post hoc analysis[a]		
					P-value (SSc vs. RA)	*P*-value (SSc vs. controls)	*P*-value (RA vs. controls)
Age, years	58 (49.5, 62)	64 (57, 73)	59 (52, 65)	<0.001	<0.001	0.555	0.027
Female, n (%)	49 (86.0)	38 (80.9)	32 (86.5)	0.713			
BMI, kg/m^2	22.2 (20.0, 24.2)	22.3 (20.7, 24.8)	21.8 (20.1, 24.6)	0.548			
Current smoking	4 (7.0)	9 (19.1)	4 (10.8)	0.026	0.117	>0.999	0.243
Alcohol ≥3 U/day, n (%)	4 (7.0)	4 (8.5)	2 (5.4)	0.155			
Diabetes mellitus, n (%)	1 (1.8)	9 (19.1)	4 (10.)	0.077			
Hypertension, n (%)	0 (0)	19 (40.4)	7 (18.9)	<0.001	<0.001	0.003	0.255
History of vertebral fracture, n (%)	4 (7.0)	6 (12.8)	0 (0)	0.030	>0.999	0.453	0.096
Family history of osteoporotic facture, n (%)	1 (1.8)	1 (2.1)	0 (0)	0.590			
Disease duration, years	2.9 (0.7, 5.0)	3.8 (2.1, 6.4)	2.8 (1.3, 4.6)	0.139			
Cumulative GC dose, mg	885 (0, 3350)	4470 (1132.5, 6805)	0 (0, 0)	<0.001	<0.001	<0.001	<0.001
Current GC dose at time of BMD, mg/day	2.5 (0, 4.38)	2.5 (2.5, 5)	0 (0, 0)	<0.001	0.069	<0.001	<0.001
Hormone replacement therapy, current	4 (7.0)	0 (0)	4 (10.8)	0.137			
Laboratory tests							
ESR, mm/h	33 (19, 51)	35 (20, 55)	16 (11, 27)	0.002	>0.999	0.009	0.006
CRP, mg/dL	0.06 (0.03, 0.11)	0.11 (0.04, 0.25)	0.09 (0.04, 0.10)	0.045	0.048	>0.999	0.408
Bone ALP, mcg/L	11.7 (9.4, 15.2)	12.1 (9, 15.3)	14.6 (10.5, 17.5)	0.599			
25(OH)D, ng/mL	15.7 (10.1, 26.4)	12.2 (19.2, 31.2)	18.5 (14.0, 28.2)	0.186			
C-telopeptide of collagen type I, ng/mL	0.39 (0.28, 0.55)	0.31 (0.19, 0.44)	0.34 (0.19, 0.55)	0.029	0.018	0.774	0.876
Procollagen 1 N-terminal propeptide, ng/mL	45.6 (32.6, 75.5)	29.9 (18.3, 57.9)	53.7 (37.3, 72.3)	0.004	0.054	>0.999	0.003
Medication							
Bisphosphonate, ever	2 (3.5)	16 (34.0)	0 (0)	<0.001	<0.001	>0.999	<0.001
BP duration, years		1.9 (0.8, 4.15)	0 (0, 0)		>0.999	0.009	<0.001
SERM	5 (8.8)	8 (17.0)	0 (0)	0.029	0.693	0.240	0.024
Vitamin D	19 (33.3)	25 (53.2)	7 (18.9)	0.005	0.147	0.342	0.003

Calcium	3 (5.3)	13 (27.7)	5 (13.5)	0.007	0.006	0.774	0.351
Methotrexate	4 (7.0)	20 (42.6)	0 (0)	<0.001	<0.001	0.453	<0.001
Cyclophosphamide	17 (29.8)	0 (0)	0 (0)	<0.001	<0.001	<0.001	-
Mycophenolate mofetil	22 (38.6)	0 (0)	0 (0)	<0.001	<0.001	<0.001	>0.999

Data are presented as the median (Q1, Q3), unless otherwise stated. [a]Adjusted *P*-values corrected by Bonferroni adjustment are presented. SSc, systemic sclerosis; RA, rheumatoid arthritis; OA, osteoarthritis; BMI, body mass index; GC, glucocorticoid; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; ALP, alkaline phosphatase; 25(OH)D, 25-hydroxy vitamin D3; SERM, selective oestrogen receptor modulator

Table 2 Comparison of bone involvement assessed by aBMD and TBS in SSc, RA, and controls

	SSc (n=57)	RA (n=47)	controls (n=37)	*P*-value	*Post hoc analysis*[a]		
					P-value (SSc vs. RA)	*P*-value (SSc vs. controls)	*P*-value (RA vs. controls)
Lumbar spine TBS	1.378 (1.322, 1.425)	1.336 (1.261, 1.396)	1.430 (1.387, 1.438)	**<0.001**	0.150	**0.018**	**<0.001**
Lumbar spine aBMD, g/cm^2	0.830 (0.732, 0.930)	0.85 (0.76, 0.913)	0.905 (0.838, 1.006)	**0.016**	>0.999	**0.030**	**0.039**
Femoral neck aBMD, g/cm^2	0.660 (0.575, 0.759)	0.628 (0.501, 0707)	0.674 (0.617, 0.738)	**0.042**	0.153	>0.999	0.051
Total hip aBMD, g/cm^2	0.791 (0.640, 0.877)	0.739 (0.633, 0.824)	0.82 (0.726, 0.890)	0.072	0.762	0.699	0.054

Data are presented as the median (Q1, Q3), unless otherwise stated. [a]Adjusted *P*-values corrected by Bonferroni adjustment are presented. aBMD, areal bone mineral density; TBS, trabecular bone score; SSc, systemic sclerosis; RA, rheumatoid arthritis; OA, osteoarthritis

Table 3 Correlation between clinical data, inflammatory markers, aBMD, and TBS

Group	Age, years	BMI, kg/m^2	Cumulative GC dose, mg	ESR, mm/h	Average ESR, mg/dL	CRP, mg/dL	Average CRP, mg/dL	aBMD, g/cm^2		
								Lumbar spine	Femoral neck	Total hip
SSc, TBS, L1-L4	-0.581 (<0.001)	-0.232 (0.082)	-0.147 (0.275)	-0.280 (0.042)	-0.323 (0.014)	-0.375 (0.005)	-0.381 (0.003)	0.816 (<0.001)	0.540 (<0.001)	0.678 (<0.001)
RA, TBS, L1-L4	-0.595 (<0.001)	-0.112 (0.452)	-0.315 (0.031)	-0.205 (0.167)	-3.332 (0.246)	-0.076 (0.610)	-0.388 (0.007)	0.440 (0.002)	0.626 (<0.001)	0.474 (0.001)
Controls, TBS, L1-L4	-0.461 (0.004)	-0.365 (0.026)	-	-0.073 (0.668)	0.021 (0.789)	0.015 (0.932)	0.004 (0.962)	0.281 (0.092)	0.446 (0.006)	0.345 (0.03)

R coefficients (*P*-values); aBMD, areal bone mineral density; TBS, trabecular bone score; SSc, systemic sclerosis; RA, rheumatoid arthritis; OA, osteoarthritis; BMI, body mass index; GC, glucocorticoid; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

Table 4 Characteristics of SSc group stratified according to the TBS risk group

Variable	Intermediate to high risk (TBS ≤1.31, n=11)	Low risk (TBS>1.31, n=46)	*P*-value
Age, years	61 (58, 66)	56 (48.8, 60)	0.014
Female, n (%)	11 (100)	36 (78.3)	0.332
BMI, mean (S.D.), kg/m^2	22.5 (20.1, 24.9)	22.1 (19.7, 24.2)	0.407
Current smoking, n (%)	0 (0)	4 (8.7)	1.000
Alcohol ≥3 U/day, n (%)	0 (0)	4 (8.7)	1.000
History of vertebral fracture, n (%)	2 (18.2)	2 (4.3)	0.164
Disease duration, years	3.8 (0.7, 5.8)	2.7 (0.5, 4.9)	0.348
Hormone replacement therapy, n (%)	0 (0)	4 (8.7)	1.000
Diabetes, n (%)	0 (0)	1 (2.2)	0.176
Subgroups of SSc			
Limited cutaneous SSc, n (%)	7 (63.6)	21 (45.7)	0.381
According to CRP status, n (%)			0.003
Inflammatory	3 (27.3)	1 (2.2)	
Intermediate	7 (63.6)	13 (28.3)	
Non-inflammatory SSc	1 (9.1)	32 (69.6)	
Laboratory tests			
ESR, mm/h	45 (27, 65)	31.5 (19.5, 48.5)	0.160
CRP, mg/dL	0.14 (0.04, 0.66)	0.05 (0.03, 0.11)	0.079
Average ESR, mm/h	43.3 (22.8, 69.0)	31.1 (20.2, 47.8)	0.192
Average CRP, mg/dL	0.32 (0.6, 0 .68)	0.06 (0.39, 1.56)	0.021
Bone ALP, mcg/L	13.2 (8.5, 26.0)	11.7 (9.4, 14.7)	0.587
25(OH)D, ng/mL	15.7 (12.8, 28.5)	15.8 (10.0, 25.2)	0.785
C-telopeptide of collagen type I, ng/mL	0.6 (0.22, 0.56)	0.41 (0.31, 0.55)	0.194
Procollagen 1 N-terminal propeptide, ng/mL	52.4 (26.9, 79.2)	44.5 (34.1, 75.4)	0.846
ANA≥1:160, n (%)	11 (100)	45 (97.8)	1.000
Anti-centromere, n (%)	8 (72.7)	22 (47.8)	0.176
Anti-topoisomerase, n (%)	0 (0)	13 (28.3)	0.050
Anti-RNP, n (%)	1 (9..1)	2 (4.3)	0.481
Organ involvement			
Interstitial lung disease, n (%)	8 (72.7)	30 (65.2)	1.000
Gastrointestinal involvement, n (%)	8 (72.7)	25 (54.3)	0.495
Scleroderma renal crisis, n (%)	0 (0)	1 (2.2)	1.000

Digital ulcer, n (%)	2 (18.2)	8 (17.4)	1.000
Pulmonary arterial hypertension, n (%)	2 (18.2)	2 (4.3)	0.175
Arthritis, n (%)	3 (27.3)	13 (28.3)	1.000
mRSS	5 (2, 8)	8 (4, 13.25)	0.179
Medication			
Cumulative GC dose, mg	2365 (780, 3800)	470 (0, 2890)	0.076
GC dose at time of BMD, mg/day	2.5 (1.87, 5.62)	0 (0, 2.5)	0.056
Cumulative GC dose for recent 3 months, mg	225 (105, 450)	26.25 (0, 225)	0.046
Bisphosphonates, ever	2 (18.2)	0 (0)	0.034
BP duration, years			
SERM, n (%)	2 (18.2)	3 (6.5)	0.244
Vitamin D, n (%)	7 (63.6)	12 (26.1)	0.022
Calcium, n (%)	2 (18.2)	1 (2.2)	0.079
Tramadol, n (%)	2 (18.2)	3 (6.5)	0.244
Methotrexate, n (%)	1 (9.1)	3 (6.5)	1.000
CYC, n (%)	2 (18.2)	15 (32.6)	0.476
Cumulative dose of CYC	0 (0, 0)	0 (0, 1187.5)	0.308
Mycophenolate mofetil	3 (27.3)	20 (43.5)	0.582
TBS, L1-4	1.255 (1.212, 1.278)	1.39 (1.357, 1.448)	0.001
aBMD, g/cm^2			
Lumbar spine	0.660 (0.613, 0.786)	0.867 (0.79, 0.997)	<0.001
Femoral neck	0.536 (0.476, 0.697)	0.682 (0.604, 0.777)	0.032
Total hip	0.638 (0.497, 0.770)	0.808 (0.709, 0.879)	0.003

Data are presented as the median (Q1, Q3) unless otherwise stated.

SSc, systemic sclerosis; TBS, trabecular bone score; BMI, body mass index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; ALP, alkaline phosphatase; 25(OH)D, 25-hydroxy vitamin D3; ANA, antinuclear antibody; RNP, ribonucleoprotein; mRSS, modified Rodnan total skin score; GC, glucocorticoid; SERM, selective oestrogen receptor modulator; CYC: cyclophosphamide; aBMD: areal bone mineral density

Table 5 Independent variables associated with TBS among patients with SSc according to GEE models

Variable	Univariate B (95% CI)	*p*-value	Multivariate average CRP model		Multivariate CRP model	
			B (95% CI)	*p*-value	B (95% CI)	*p*-value
Age	-0.005 (-0.008, -0.003)	<0.001	-0.005 (-0.007, -0.003)	<0.001	-0.005 (-0.007, -0.003)	<0.001
Sex, female	-0.068 (-0.107, -0.028)	0.001	-0.062 (-0.091, -0.033)	<0.001	-0.062 (-0.092, -0.032)	<0.001
BMI	-0.007 (-0.015, 0.001)	0.079				
Disease duration, years	0.001 (-0.006, 0.008)	0.755				
ESR, mm/h	-0.001 (-0.002	0.041				
CRP, mg/dL	-0.055 (-0.092, -0.018)	0.004			-0.039 (-0.071, -0.007)	0.018
Average ESR, mm/h	-0.001 (-0.002, 0.000)	0.021				
Average CRP, mg/dL	-0.057 (-0.102, -0.012)	0.013	-0.042 (-0.077, 0.007)	0.019		
25(OH)D, ng/mL	0.001 (-0.002, 0.003)	0.461				
Cumulative GC dose, g	-0.004 (-0.011, 0.003)	0.297				
GC dose at time of BMD, mg/day	-0.007 (-0.017, 0.003)	0.170				
Cumulative GC dose for recent 3 months, g	-0.084 (-0.188, 0.019)	0.110				
Use of cyclophosphamide	-0.006 (-0.047, 0.036)	0.783				
Use of mycophenolate mofetil	-0.003 (-0.031, 0.026)	0.862				
Smoking	-0.031 (-0.068, 0.007)	0.109				
Alcohol ≥3 U/day	-0.076 (-0.109, -0.043)	<0.001				

TBS, trabecular bone score; SSc, systemic sclerosis; GEE, generalized estimating equation; CRP, C-reactive protein; BMI, body mass index; ESR, erythrocyte sedimentation rate; 25(OH)D, 25-hydroxy vitamin D3; GC, glucocorticoid.

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