International Journal of Rheumatic Diseases

The association between systemic sclerosis and bone mineral density based on twelve observational studies

Journal:	*International Journal of Rheumatic Diseases*
Manuscript ID:	IJRD-2013-0466
Manuscript Type:	Review
Date Submitted by the Author:	02-Nov-2013
Complete List of Authors:	Wan, Ya-Nan Wang, Yu-Jie Yan, Jun-Wei Tao, Jing-Hui Li, Xiang-Pei Wang, Jing
Keywords:	Epidemiology < Systemic sclerosis

The association between systemic sclerosis and bone mineral density based on twelve observational studies

Ya-nan Wan[1,a], Yu-Jie Wang[1,a], Jun-Wei Yan[1], Jing-Hui Tao[2], Xiang-Pei Li[2], Jing Wang[1*]

[1] Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, Hefei, China;

[2] Anhui Medical Uniersity Provincial Hospital, Hefei, China;

[a] The authors contributed equally to this work and are acted as co-first authors;

*Correspondence: Jing Wang, M.D, Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, 81 Meishan Road, Hefei, Anhui, 230032, PR China, E-mail: jwang2006@126.com; Tel.: +86 551 65161175; Fax: +86 551 65161126.

Abstract

Previous researches show inconsistency about the effect of systemic sclerosis (SSc) on bone mineral density (BMD). The objective of this study was to perform a meta-analysis of the previous articles to investigate the difference in BMD (g/cm^2) between SSc and non-SSc populations and to estimate potential underlying mechanisms. Twelve full text articles (including an outlier study and two studies with duplicate data) with 662 SSc patients and 886 controls were identified by searching Medline prior to September 10th, 2013 using search terms such as "Systemic sclerosis", "scleroderma", "osteoporosis", "bone density" and "bone mass". It is extracted that BMD (mean and standard deviation), T-score and Z-score at lumbar spine, femoral neck and total hip measured by dual energy x-ray absorptiometry. A lower bone mineral density was found in SSc patients at femoral neck and total hip, but the result of lumbar is inconsistent. The result of meta-analysis showed that BMD in SSc was significantly lower, with weighted mean difference of -0.104 (95% CI: -0.135, -0.073) at the lumbar spine. We conclude that patients with SSc may have a lower BMD level.

Keywords: systemic sclerosis, bone mineral density, meta-analysis

1. Introduction

Osteoporosis is a skeletal disorder characterized by low bone mineral density (BMD) and loss of normal bony architecture and mineralization that increased the risk of fracture [1]. BMD is influenced by a cluster of factors, including race, advanced age, decreased sex-steroid activity, corticosteroid use, certain chronic diseases that affect absorption or vitamin D metabolism, smoking, and excessive alcohol use [2]. It is a significant public health problem worldwide on account of its high morbidity and mortality and its huge economic costs [3, 4]. Considering increased life expectancy and a growing population of elderly people with a high risk of fractures, the economic burden of osteoporosis may aggravate in the future.

Systemic sclerosis (SSc) is an autoimmune connective tissue disease characterized by excessive collagen deposition and damage to skin and internal organs resulted from the production of inflammatory cells and antibodies. As the survival of patients with SSc has improved over the past decades, the prevention of the chronic non-fatal complications, low BMD for instance, has aroused more and more concern. It is reported that the prevalence of osteoporosis and fracture, the most severe result of low bone mineral density, was higher than that in healthy controls [5, 6]. Patients with SSc had a trend for some increased risk factors such as immobility, alcohol drinking, and less exercise [7]. However, whether the presence of osteoporosis in SSc patients is higher than that in healthy people is still inconclusive. Thus, the evidence should be provided first for further research is whether the lower BMD, the internal risk of osteoporosis, exists in SSc patients.

In the article, we will review the current researches about the level of BMD and discuss the possible risk of lower BMD, in patients with SSc. According to the World Health Organization international reference standard for osteoporosis diagnosis [8], we regarded the interest sites as lumbar spine, femoral and total hip and extracted the data of the measured values, T-score and Z-score accessed by dual-energy X-ray absorptiometry.

2. Methods

Literature search and inclusion criteria

All the articles were searched from Medline before September 10th, 2013 using the following terms: "systemic sclerosis", "scleroderma", "osteoporosis", "bone density" and "bone mass". There was no restriction on language. Studies considered eligible for the meta-analysis must meet the following inclusion criteria: (1) research on human; (2) case-control study or cross-section study; (3) including subjects without SSc or similar disease as controls; (4) data reported on at least one of the three sites (lumbar spine, femoral neck and total hip) of BMD; (3) BMD was measured by DXA and expressed by g/cm^2; (4) mean BMD and standard deviations (SDs) were available.

Data extraction

There were two reviewers who participated in the extract of the date with the uniform format. Once a potential disagreement came, it would be solved by discussion. Irrelevant studies were excluded. As for studies with overlapping data, the most complete one was included. The following information was extracted: the first authors, year of publication, type of studies, country, age, duration of the disease, menopause status, steroid treatment, vitamin D supplements and BMD of subjects (measured at lumbar spine, femoral neck or total hip with or without SSc).

Statistical analysis

If information was acquired from more than one subgroup (for instance, menopausal subjects or fertile subjects) in one study, each subgroup was treated as a separate comparison in our meta-analysis. Because the outcomes of BMD at measured sites were continuous variables and were presented on the same measurement unit (g/cm^2), weighted mean difference (WMD) with 95% confidenceintervals (CIs) calculated using the final follow-up P values obtained from the SSc (+) and SSc (-) groups was used to figure out the strength of the association between SSc and BMD. Our meta-analysis was preformed with a fixed effects model initially. If heterogeneity presented, the analysis should be redone by a random effects model. P value less than 0.05 was considered statistically significant.

Chi-square-based Q statistics was used to assess the heterogeneity of the effect across studies. Besides, I^2 statistics were provided to measure the severe degree of heterogeneity. Effect sizes with a corresponding I^2 value of < 50% were considered to have low heterogeneity, those with 50-75% were

considered moderate and I^2 values of > 75% were considered highly heterogeneous. Sensitivity analyses were performed to indentify the outlier studies in case of the presence of high heterogeneity and the impact of outliers was also assessed to evaluate the influence of their removal. Publication bias was tested by visual inspection of funnel plot. The funnel plot should be asymmetric, if there is a publication bias. Egger and Begg tests were performed to assess the funnel plot asymmetry with a significance level of $P < 0.05$. All statistical analyses were performed by using version STATA 11.0 (Stata Corporation, College Station, TX).

3. Result

Search results and characteristics of studies

The detailed processes of the study selection are shown in Fig. 1. 157 potentially eligible studies were identified. 24 out of them reserved after reading the titles or abstracts (34 studies were ignored due to they are reviews and 99 studies are excluded because they are irrelevant to BMD and SSc). These were no controls in four of them. The results in three of them were not available. Two of them were neither the case-control study nor the cross-sectional study. Other two researches were ignored because the BMD was not acquired by DXA. The last one research excluded is on account of the rheumatoid arthritis control, which may own the similar pathogeny with SSc patients. Finally, 12 researches fulfilled inclusion criteria after excluding 12 studies above. The major studies characteristics are showed in Table 1.

Out of the 12 studies, 7 were the case-control study, and 5 were the cross-sectional study. The population of the study above came from Africa (included 1 study), Europe (included 5 studies), the Americas (included 5 studies) and Asia (included 1 study). There were 6 studies published in recent three years.

From the Table 2 we found all the studies included pointed out that there was a significantly lower BMD, T score and Z score (measured at femoral neck and total hip) in patients with SSc than that in controls without. Besides, we found that the result at the lumbar spine of the study included is inconsistent. Whether the SSc patients have a lower level BMD at the lumbar than controls, it does remain to be confirmed by further research.

Meta-analysis

Because the data of Carbone L et al. and Cheng S et al. was completely consistent from the same institution and population. We remained the study of Cheng S et al. with more complete data. All 11 studies with a fixed effects model were performed initially. On account of significant heterogeneity (I^2 = 99.9 %), the analysis was redone with a random effects model. The results did not show the significant association between SSc and BMD of lumbar spine (WMD= -0.405, 95 % CI [-0.891, 0.082], P = 0.103; I^2 = 99.9 %, P < 0.001 for Q test). Bescause the value of I^2 was very high, the results above were not available.

Hence, outlier studies should be identified and then be omitted. Sensitivity analyses showed that the ID of the outlier study was Ibn Yacoub Y [16]. There were 10 studies included in the final meta-analysis after omitting the outlier study. The heterogeneity was decreased and the results suggested the significant association between the SSc and decreased BMD of lumbar spine (WMD= -0.104, 95 % CI [-0.135, -0.073], P < 0.001; I^2 = 67.0 %, P = 0.001 for Q test).

There was no publication bias by performing Egger's regression (P = 0.892) and Begg's methods (P = 1.000) in the 10 studies (with an outlier study excluded) focus on BMD of lumbar spine.

4. Discussion

In this review, we focus more attention on data measured by DXA at lumbar spine, femoral neck and total hip. Because the diagnose criteria of osteoporosis defined by WHO based on DXA and interested in three sites above. T-score and Z-score (standardized indexes) were figured out based on the baseline level of BMD in a certain population. Thus, the ideal data to be merged should be T-score or Z-score to eliminate the impact of race. However, the available result of T-score and Z-score was few and was not satisfied for merging. Pooled measured result can reflect the difference of BMD level between SSc patients and health controls, but that may be influenced by the effect of race.

We excluded an outlier study when we performed the meta-analysis on the association of SSc with BMD of lumbar spine by sensitivity analyses. In this study, the level of BMD in lumbar spine was significantly lower in women with SSc than women without. Unlike other nine studies, the patients of this research came from Africa. This might be the main reason for the detection of the outlier study.

A significant overall association of SSc with increased BMD of lumbar spine was detected in our

meta-analysis. Since SSc is a lot of conditions interacting with each other, the pathological mechanism behind the effects of SSc on BMD is complex and has not been investigated clearly. Considerable heterogeneity was found which can impact the association as reflected by a high I^2 statistic. This heterogeneity may probably stem from different study design, menopause status and individual characteristics that were not considered by each study. Owing to complex conditions of limited studies included, subgroup analysis was not performed.

These were some limitations that should be considered in the present study. First of all, three sites (lumbar spine, femoral neck and total hip) for BMD measurement have been researched in studies included. This meta-analysis only included BMD of lumbar spine because that site was most extensively studied with inconsistent result. Second, subgroup analyses were not performed because of the limited number of studies included in our meta-analysis. That enhanced the reliability of the statistical power, but was helpless to detect the source of heterogeneity. Third, osteoporosis was due to change of both bone density and quality, leading to increased risk of fractures. However, we only evaluated the effects of SSc on BMD, which was one-sided. Fracture risk might be increased in spite of normal or decreased BMD.

Mechanisms responsible for an association between SSc and BMD are plentiful and complicated. We discuss the most important factors which can impact the association between SSc and BMD from a clinical perspective.

Low body mass index

It was reported low body mass index (BMI) is a significant risk factor for low bone density and increased bone loss in early postmenopausal women in a randomized trial of alendronate for prevention of osteoporosis in recently postmenopausal women with normal bone mass (n = 1609) [9]. Souza RB et al [10] and Marighela TF et al.[11] reported that SSc patients had a lower BMI than controls[10, 11]. Besides, a decreased serum leptin (adipo-cytokines) level was found in patients with systemic sclerosis, which was correlated with lower BMI [13], and there is evidence that leptin regulates production of osteoprotegerin (OPG) and receptor activator of nuclear factor-kappa B ligand (RANKL) to result in diminished recruitment of osteoclasts and reduced bone resorption [14]. Mok CC et al. [15] and Sampaio-Barros PD et al. [5] pointed out that low BMI was independently associated with low BMD of

the total hip and femoral neck. However, the result is inconsistent with that of Ibn Yacoub Y et al [16]. Besides, Pehlivan Y et al [17] recently reported that leptin was found to be higher in SSc in contrast to the control group matched age and BMI, which is inconsistent with the result of Kotulska A et al [13].

Inflammation

Statistically significant negative correlations were found between inflammation (e.g. Interleukin-1beta (IL-1β), Interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α)), character of SSc, and lumbar spine BMD, while serum IL-6 was also negatively correlated with BMD at the hip (femoral neck) among postmenopausal women [18, 19]. Giuliani N et al. [20] have provided evidence that these was a significant negative correlations between lumbar spine BMD and serum IL-6 soluble receptor, which enhances IL-6 bioactivity [21]. However, Hustmyer FG et al. [22] and Kania DM et al. [23] observed no associations of IL-1β production with lumbar spine BMD, plasma IL-6 concentration with lumbar spine or femoral neck BMD. The patients of that study included both fertile and postmenopausal women. Higher sRANKL levels and sRANKL/OPG ratio were found in patients with SSc [24, 25] and Turk N et al. pointed out that TNF-α has a strong association with the osteoclastogenic mediator RNKL, and an inverse association with BMD, in patients with Crohn's disease (a kind of inflammatory bowel disease may caused by abnormal autoimmune status) [26]. Besides, it was reported that increased C-reactive protein (CRP, a sensitive marker of systemic inflammation), which was regulated by IL-1, IL-6, and TNF-α [27, 28], was associated with poor bone health [29, 30]. But that effect of CRP was not found in the research conducted by Frediani B et al [31], which included 55 patients with SSc and 60 age-matched healthy controls.

Menopause

Menopause acts a significant role in the presence of lower BMD in woman. It was found that the frequency of menopause was higher in SSc patients than in age-matched controls [11, 12]. Di Munno et al. [32] reported reduced BMD in SSc patients and observed a negative correlation between BMD and the duration of menopause. Sampaio-Barros PD. et al [5] further pointed out that SSc patients presented an early menopause. Besides, a high level of prolactin was found in SSc patients [33-35], which may

result in early menopause. The secretion of prolactin by the pituitary is affected by interleukin 2 [36]. Larrea F et al. recently put forward a possibility that mononuclear cells from systemic lupus erythematosus patients can secrete prolactin directly [37]. These data are in accord with the presence of bidirectional interaction between the neuroendocrine and immune systems [38] and can explain the increased prolactin levels as a result of disease activity. Although evidence was provided that SSc patients had an earlier age [12, 16] (different from research of Atteritano M et al. [39]) and a longer duration of menopause [16], but either of them was associated with the decrease in BMD [16]. Similar results were reported by other authors [14, 33, 40]. According to the data of Mok CC et al. [15], menopause was not significantly associated with lower BMD at lumbar spine, total hip and femoral neck.

The severity of the disease

Frediani et al [31, 41] pointed out that SSc patients have reduced BMD of lumbar spine and femoral neck mainly in the diffuse form and in those with internal organ involvement. Another study found BMD to be related to the extent of skin involvement, in that patients with the diffuse form presented the lowest mean percentage of the expected BMD values of the lumbar spine [32].Besides, It is reported that reduced BMD of lumbar spine can be seen in a majority of patients with lung involvement (advanced pulmonary arterial hypertension) [42]. As a matter of fact, the extent of skin involvement is not only a useful tool to assess prognosis, but is also directly related to the extent of visceral involvement and to the severity of the disease [43]. Ibn Yacoub Y et al. [16] found significant association between bone loss (at lumbar spine and femoral neck) and severity of joint involvement in particular the severity of joint inflammatory pain and the presence of an erosive arthropathy. But In their patients, there were no correlations between internal organs involvement (skin or lung involvement) and the reduction in BMD. In addition, the duration of the disease can reflect the severity of the disease to some extent. In the sample of Ibn Yacoub Y et al [16], the loss of bone mass appeared to be associated with longer disease duration, while Mok CC et al [15] and Frediani B et al [31] had a contrary conclusion against it.

Other risks

As we known that the use of corticosteroids may have an impact on BMD. A meta-analysis reported that subgroup analysis of BMD data performed on a change-from-baseline basis showed that corticosteroids had a clear effect on both lumbar and femoral BMDs in patients with rheumatoid arthritis (SMD = -0.354; 95% CI, -0.620 to -0.088, P = 0.009; SMD = -0.488; 95% CI, -0.911 to -0.065, P = 0.024, respectively) [44]. But, the studies we included haven't found any exact evidence of the same effect in SSc patients [5, 6, 15]. Vitamin D is known for the excellent protective factor of bone loss. It was reported that SSc patients had lower levels of 25-hydroxyvitamin D3 than controls [15, 39] and there is a high prevalence of vitamin D deficiency. Atteritano M et al. [39] found lower levels of vitamin D may a role in the risk of osteoporosis and vertebral fractures, while Rios-Fernández R et al. [45] could not demonstrate the relationship between vitamin D and BMD.

5. Conclusion and outlook for further studies

SSc patients may have a lower BMD than healthy controls by analyzing all studies above at femoral neck and total hip. Further more studies should be performed to confirm the validity of lower BMD at lumbar in SSc patients. The possible risk of lower BMD could be BMI, the severity of disease (organ involvement and diffuse form), inflammation (IL-1β, IL-6, TNF-α and CRP) and menopause (duration of menopause and early age of menopause).

According to the previous studies, further researches should pay more attention on issues as follows. (1) Matching: age and gender are the common matching element. Considering that most of the female patients of the researches included were menopausal, the status of menopause, an important risk of bone loss in women [45], should be considered. (2) Data collection: either protective factors (the use of vitamin D, vitamin D metabolites and analogs) or adverse factors (the use of steroids, such as prednisone) of bone metabolism should be collected. For vitamin D, the level of serum vitamin D, intake of vitamin D from diet, the frequency and time of sun exposure were significant to evaluate the impact of vitamin D. (3) Measurement: For consider that DXA is an important method to measure bone density (sensitivity and specificity were 70.0% and 98.3% on a lesion-based analysis, 73.1% and 90.5% on a patient-based analysis in vertebral fracture assessment [46]), it is convenient to compare with other researches if the instrument of the researches is DXA. According to official position of

International Society for Clinical Densitometry on densitometry [8], the interested site should be focus on lumbar spine, femoral neck and total hip. Osteoporosis is the worst result of low BMD. Though most result of the articles included is quantitative, either quantitative or qualitative data is needed. Because whether the qualitative change of BMD occurs in SSc patients is more notable.

Acknowledgments

This work was partly supported by grants from the Academic Leader Foundation of Anhui Medical University, the Natural Science Foundation of Anhui Province in 2013 (Code: 1308085MH169) and the Key Project of the Education Department of Anhui Province Natural Science Research (Code: KJ2012A165).

Conflict of Interest

None.

References

1. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy (2001) Osteoporosis prevention, diagnosis, and therapy. JAMA 285:785-795.
2. Russell G (2003) Pathogenesis of osteoporosis. In: Hochberg MC, Silman AJ, Smolen JS, Weinblatt ME, Weisman MH (ed) Rheumatology. 3rd edn. Philadelphia: Mosby, pp 2075-80.
3. Reginster JY, Gillet P, Ben Sedrine W et al (1999) Direct costs of hip fractures in patients over 60 years of age in Belgium. Pharmacoeconomics 15:507-514.
4. Gazzaruso C (2012) An increased risk for fractures: another cause of frailty in HIV-infected subjects. Endocrine 41:347-9.
5. Sampaio-Barros PD, Costa-Paiva L, Filardi S et al (2005) Prognostic factors of low bone mineral density in systemic sclerosis. Clin Exp Rheumatol 23:180-4.
6. Avouac J, Koumakis E, Toth E et al (2012) Increased risk of osteoporosis and fracture in women with systemic sclerosis: a comparative study with rheumatoid arthritis.

Arthritis Care Res (Hoboken) 64:1871-8.

7. Yuen SY, Rochwerg B, Ouimet J et al (2008) Patients with scleroderma may have increased risk of osteoporosis. A comparison to rheumatoid arthritis and noninflammatory musculoskeletal conditions. J Rheumatol 35:1073-8.
8. Lewiecki EM, Gordon CM, Baim S et al (2008) International Society for Clinical Densitometry 2007 Adult and Pediatric Official Positions. Bone 43:1115-21.
9. Ravn P, Cizza G, Bjarnason NH et al (1999) Low body mass index is an important risk factor for low bone mass and increased bone loss in early postmenopausal women. Early Postmenopausal Intervention Cohort (EPIC) study group. J Bone Miner Res 14:1622-7.
10. Souza RB, Borges CT, Takayama L et al (2006) Systemic sclerosis and bone loss: the role of the disease and body composition. Scand J Rheumatol 35:384-7.
11. Marighela TF, Genaro PD, Pinheiro MM et al (2013) Risk factors for body composition abnormalities in systemic sclerosis. Clin Rheumatol. doi: 10.1007/s10067-013-2235-1.
12. La Montagna G, Vatti M, Valentini G et al (1991) Osteopenia in systemic sclerosis. Evidence of a participating role of earlier menopause. Clin Rheumatol 10:18-22.
13. Kotulska A, Kucharz EJ, Brzezińska-Wcisło L et al (2001) A decreased serum leptin level in patients with systemic sclerosis. Clin Rheumatol 20:300-2.
14. Reid IR (2002) Relationships among body mass, its components, and bone. Bone 31:547-55.
15. Mok CC, Chan PT, Chan KL et al (2013) Prevalence and risk factors of low bone mineral density in Chinese patients with systemic. Rheumatology (Oxford) 52:296-303.
16. Ibn Yacoub Y, Amine B, Laatiris A et al (2012) Bone density in Moroccan women with systemic scleroderma and its relationships with disease-related parameters and vitamin D status. Rheumatol Int 32:3143-8.
17. Pehlivan Y, Onat AM, Ceylan N et al (2012) Serum leptin, resistin and TNF-α levels in patients with systemic sclerosis: the role of adipokines in scleroderma. Int J Rheum Dis 15:374-9.
18. Zheng SX, Vrindts Y, Lopez M et al (1997) Increase in cytokine production (IL-1 beta, IL-6, TNF-alpha but not IFN-gamma, GM-CSF or LIF) by stimulated whole blood cells in postmenopausal osteoporosis. Maturitas 26:63-71.
19. Papadopoulos NG, Georganas K, Skoutellas V et al (1997) Correlation of interleukin-6 serum levels with bone density in postmenopausal women. Clin Rheumatol 16:162-5.

20. Giuliani N, Sansoni P, Girasole G et al (2001) Serum interleukin-6, soluble interleukin-6 receptor and soluble gp130 exhibit different patterns of age- and menopause-related changes. Exp Gerontol 36:547-57.

21. Tamura T, Udagawa N, Takahashi N et al (1993) Soluble interleukin-6 receptor triggers osteoclast formation by interleukin 6. Proc Natl Acad Sci U S A 90:11924-8.

22. Hustmyer FG, Walker E, Yu XP et al (1993) Cytokine production and surface antigen expression by peripheral blood mononuclear cells in postmenopausal osteoporosis. J Bone Miner Res 8:51-9.

23. Kania DM, Binkley N, Checovich M et al (1995) Elevated plasma levels of interleukin-6 in postmenopausal women do not correlate with bone density. J Am Geriatr Soc 43:236-9.

24. Dovio A, Data V, Carignola R et al (2008) Circulating osteoprotegerin and soluble RANK ligand in systemic sclerosis. J Rheumatol 35:2206-13.

25. Lewiecki EM (2006) RANK ligand inhibition with denosumab for the management of osteoporosis. Expert Opin Biol Ther 6:1041-50.

26. Turk N, Cukovic-Cavka S, Korsic M et al (2009) Proinflammatory cytokines and receptor activator of nuclear factor kappaB-ligand/osteoprotegerin associated with bone deterioration in patients with Crohn's disease. Eur J Gastroenterol Hepatol 21:159-66.

27. Weinhold B, Rüther U (1997) Interleukin-6-dependent and -independent regulation of the human C-reactive protein gene. Biochem J 327 (Pt 2):425-9.

28. Moshage H (1997) Cytokines and the hepatic acute phase response. J Pathol 181: 257-66.

29. Ding C, Parameswaran V, Udayan R et al (2008) Circulating levels of inflammatory markers predict change in bone mineral density and resorption in older adults: a longitudinal study. J Clin Endocrinol Metab 93:1952-8.

30. Koh JM, Khang YH, Jung CH (2005) Higher circulating hsCRP levels are associated with lower bone mineral density in healthy pre- and postmenopausal women: evidence for a link between systemic inflammation and osteoporosis. Osteoporos Int 16:1263-71.

31. Frediani B, Baldi F, Falsetti P et al (2004) Clinical determinants of bone mass and bone ultrasonometry in patients with systemic sclerosis. Clin Exp Rheumatol 22:313-18.

32. Di Munno O, Mazzantini M, Massei P et al (1995) Reduced bone mass and normal calcium metabolism in systemic sclerosis with and without calcinosis. Clin Rheumatol 14:407-12.

33. La Montagna G, Baruffo A, Pasquali D et al (2001) Assessment of pituitary gonadotropin release

to gonadotropin releasing hormone/thyroid-stimulating hormone stimulation in women with systemic sclerosis. Rheumatology (Oxford) 40:310-4.

34. Karanth S, Marubayashi U, McCann SM (1992) Influence of dopamine on the altered release of prolactin, luteinizing hormone, and follicle-stimulating hormone induced by interleukin-2 in vitro. Neuroendocrinology 56:871-80.
35. Larrea F, Martínez-Castillo A, Cabrera V et al (1997) A bioactive 60-kilodalton prolactin species is preferentially secreted in cultures of mitogen-stimulated and nonstimulated peripheral blood mononuclear cells from subjects with systemic lupus erythematosus. J Clin Endocrinol Metab 82:3664-9.
36. Blalock JE (1994) The syntax of immune-neuroendocrine communication. Immunol Today 15:504-11.
37. Mirone L, Barini A, Barini A (2006) Androgen and prolactin (Prl) levels in systemic sclerosis (SSc) relationship to disease Severiny. Ann NY Acad Sci 1069:257-62.
38. Orbach H, Zandman-Goddard G, Amital H et al (2007) Novel biomarkers in autoimmune diseases: prolactin, ferritin, vitamin D, and TPA levels in autoimmune diseases. Ann N Y Acad Sci 1109:385-400.
39. Atteritano M, Sorbara S, Bagnato G et al (2013) Bone mineral density, bone turnover markers and fractures in patients with systemic sclerosis: a case control study. PLoS One 8:e66991. doi: 10.1371/journal.pone.0066991.
40. Deluca HF, Cantorna MT (2001) Vitamin D: its role and uses in immunology. FASEB J 15:2579-85.
41. Frediani B, Baldi F, Falsetti P et al (2004) Bone mineral density in patients with systemic sclerosis. Ann Rheum Dis 63:326-7.
42. Malik N, McCarthy K, Minai OA et al (2012) Prevalence and significance of decreased bone density in pulmonary arterial hypertension. South Med J 105:344-9.
43. Krieg T, Takehara K (2009) Skin disease: a cardinal feature of systemic sclerosis. Rheumatology (Oxford) 48 Suppl 3:iii14-8.
44. Lee YH, Woo JH, Choi SJ et al (2008) Effects of low-dose corticosteroids on the bone mineral density of patients with rheumatoid arthritis: a meta-analysis. J Investig Med 56:1011-8.
45. Rios-Fernández R, Callejas-Rubio JL, Fernández-Roldán C et al (2012) Bone mass and vitamin D

in patients with systemic sclerosis from two Spanish regions. Clin Exp Rheumatol 30:905-11.

46. Riis BJ, Hansen MA, Jensen AM et al (1996) Low bone mass and fast rate of bone loss at menopause: equal risk factors for future fracture: a 15-year follow-up study. Bone 19:9-12.

47. Bazzocchi A, Spinnato P, Fuzzi F et al (2012) Vertebral fracture assessment by new dual-energy X-ray absorptiometry. Bone 50:836-41.

48. Carbone L, Tylavsky F, Wan J et al (1999) Bone mineral density in scleroderma. Rheumatology (Oxford) 38:371-2.

49. Cheng S, Tylavsky FA, Orwoll ES et al (1999) The role of collagen abnormalities in ultrasound and densitometry assessment: In vivo evidence. Calcif Tissue Int 64:470-6.

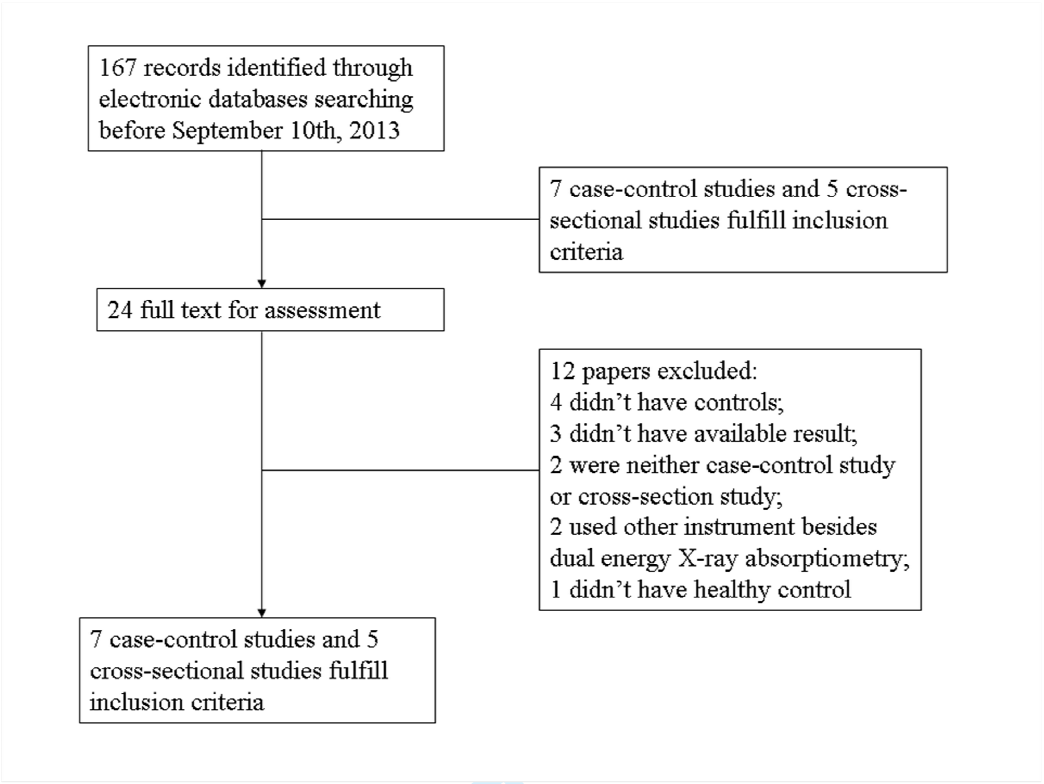

Fig 1. Search and selection of case-control studies and cross-sectional studies.

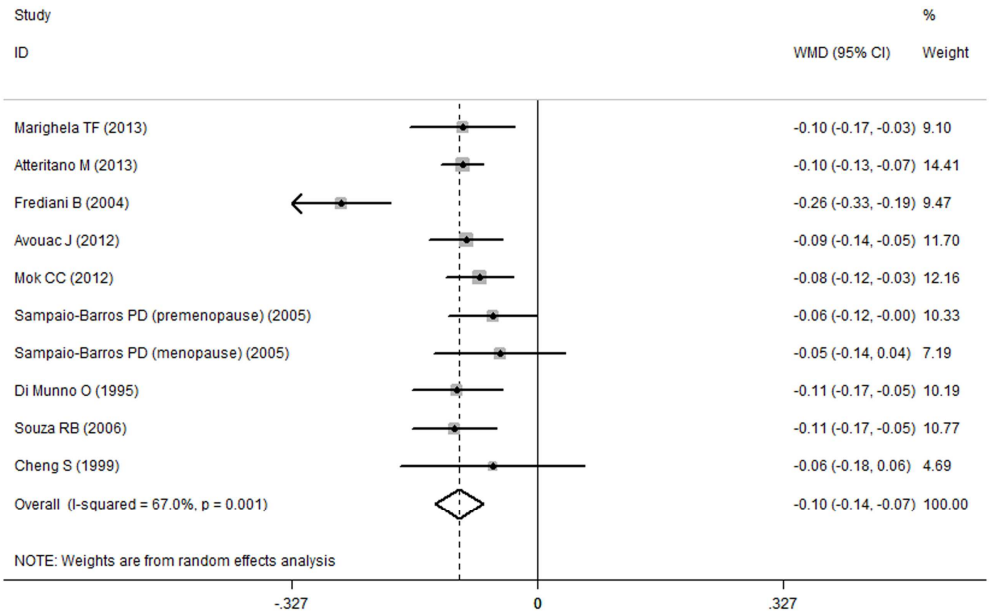

Fig. 2 Forest plot of the association between SSc and BMD of lumbar spine using a random effects model

Table 1 Characteristics of the articles studying the risk of osteoporosis in patients with systemic sclerosis.

First Author	Year	Type of study	Population	Age, year Mean(SD)	Menopause (case/control)	Duration ,year Mean(SD)	Densitometric osteoporosis[b]	Previous fracture	Steroid treatment ever	Vitamin D supplement ever
Ibn Yacoub Y [16]	2011	Case-control	Morocco	49.44 (13.07)	26/24	9.63 (5.9)	SSc: 60% Controls: 25%	SSc:11.66% Controls: 18.33%	None	None
Marighela TF [11]	2013	Cross-sectional	Brazil	59.3 (14.8)	36/27	8.9 (7.1)	NA	NA	The treatment of prednisone or equivalent on patients was no more than 10 mg/day.	NA
Atteritano M [47]	2013	Case-control	Italy	54.43 (1.73)	54/54	5 (2)	SSc: 22% Controls: 8%	SSc:24% Controls: 1.8%	None	None
Rios-Fernández R [44]	2012	Cross-sectional	Spain	56.49 (13.3)	76/76	NA	SSc: 33% Controls: 16%	NA	17% patients have ever received glucocorticosteroids therapy	58% patients have ever received vitamin D supplements
Carbone L [48]	1999	Cross-sectional	USA	NA	15/15	9.87 (NA)	NA	NA	NA	NA
Frediani B [31]	2004	Case-control	Italy	54.1 (14.1)	30/60	10.9 (NA)	SSc:16.4% Controls: NA	NA	None	NA
Avouac J [6]	2012	Cross-sectional	France	62 (12)	63/216	10 (9)	SSc: 30% Controls: 11%	SSc: 35% Controls: 10%	58% patients have ever taken corticosteroids	69% patients and 25% healthy controls have

										ever taken vitamin D supplementation
Mok CC [15]	2012	Case-control	China	49.4 (11.3)	45/37	7.8 (6.4)	NA	SSc: 5% Controls: 6%	23% patients have ever received treatment of prednisolone	35% controls and 23% patients were currently receiving vitamin D supplement.
Sampaio-Barros PD [5]	2005	Cross-sectional	Brazil	Premenopause: 35.79 (6.77) Menopause: 54.21 (6.43)	0/0 28/60	NA	SSc: 42.4% Controls: NA	NA	Premenopause: 36% patients have previously used corticosteroids[a] Menopause: 39% patients have previously used corticosteroids[a]	NA
Di Munno O [32]	1995	Case-control	Italy	54.8 (10.3)	36/32	5.9 (NA)	NA	NA	None	None
Souza RB [10]	2006	Case-control	Brazil	62.2 (7.7)	43/47	13.2 (8.0)	SSc: 32.5% Controls: 14.8% (based on lumbar spine) SSc: 51.1% Controls: 19.1%	SSc: 18.6% Controls: 29.8%	None	NA

							(based on femoral neck)			
Cheng S [49]	1999	Case-control	USA	54.1(12.8)	15/15	NA	NA	NA	None	NA

[a] Corticosteroids treatment (≥ 5 mg daily oral dose of prednisone or equivalent, for at least 60 days)

[b] The diagnosis of densitometric osteoporosis was based on lumbar spine or femoral neck

NA: not available

Table 2. BMD in patients with SSc and controls per skeletal site (mean ± SD g/cm^2)

First author	Case/control[a]	Measured values of BMD			T-score			Z-score		
		Case	Control	*P* value	Case	Control	*P* value	Case	Control	*P* value
Skeletal site of BMD measurement: femoral neck										
Ibn Yacoub Y [16]	60/60	-1.93 (0.32)	-0.81 (0.69)	< 0.001	NA	NA	NA	NA	NA	NA
Marighela TF [11]	61/67	0.89 (0.2)	0.98 (0.2)	0.000	NA	NA	NA	NA	NA	NA
Atteritano M [39]	54/54	0.54 (0.04)	0.72 (0.07)	< 0.001	-2.60 (0.20)	-1.30 (0.30)	<0.001	NA	NA	NA
Rios-Fernández R [45]	100/100	NA	NA	NA	-1.3 (1.4)	-0.9 (1.1)	0.022	-0.3 (1.2)	0.2 (1.1)	0.001
Carbone L [48]	15/15	-0.671 (0.13)	0.820 (0.14)	0.0103	-2.183 (1.31)	-0.76 (1.34)	0.0084	-0.923 (1.45)	0.469 (1.32)	0.0181
Frediani B [31]	55/60	0.832 (0.125)	0.955 (0.095)	<0.05	NA	NA	NA	NA	NA	NA
Mok CC [15]	84/84[b]	0.674 (0.125)	0.733 (0.116)	0.002	NA	NA	NA	-0.82 (0.96)	-0.35 (0.96)	0.002
Sampaio-Barros PD [5]	Premenopause: 33/47 Menopause: 28/60	0.870 (0.176)	0.973 (0.134)	NA	NA	NA	NA	NA	NA	NA
Souza RB [10]	43/47	0.64 (0.11)	0.75 (0.13)	<0.01	NA	NA	NA	NA	NA	NA
Cheng S [49]	15/15	0.671 (0.13)	0.820 (0.14)	0.006	-0.218 (1.3)	-0.72 (1.4)	0.006	-0.92 (1.5)	0.48 (1.3)	0.010
Skeletal site of BMD measurement: total hip										
Carbone L [48]	15/15	0.745 (0.15)	0.907 (0.12)	0.0032	-1.890 (1.22)	-0.540 (0.98)	0.0032	-1.062 (1.27)	0.220 (1.02)	0.0056

Avouac J [6]	71/227	0.817 (0.148)	0.901 (0.136)	<0.001	NA	NA	NA	NA	NA	NA
Mok CC [15]	84/84[b]	0.787 (0.139)	0.858 (0.116)	<0.001	NA	NA	NA	-0.79 (0.99)	-0.25 (0.90)	<0.001
Skeletal site of BMD measurement: lumbar										
Ibn Yacoub Y [16]	60/60	-2.97 (0.25)	0.46 (0.11)	<0.001	NA	NA	NA	NA	NA	NA
Marighela TF [11]	61/67	1.03 (0.2)	1.13 (0.2)	0.001	NA	NA	NA	NA	NA	NA
Atteritano M [39]	54/54	0.78 (0.08)	0.88 (0.07)	<0.001	-2.20 (0.30)	-1.20 (0.30)	<0.001	NA	NA	NA
Rios-Fernández R [45]	100/100	NA	NA	NA	-1.4 (1.4)	-0.7 (1.4)	0.000	-0.3 (1.5)	0.3 (1.2)	0.001
Carbone L [48]	15/15	0.98 (0.15)	1.047 (0.19)	0.2769	0.870 (1.35)	0.320 (1.74)	0.3894	0.243 (1.30)	0.718 (1.85)	0.4212
Frediani B [31]	55/60	0.980 (0.174)	1.241 (0.118)	<0.01	NA	NA	NA	NA	NA	NA
Avouac J [6]	71/227	0.963 (0.188)	1.058 (0.169)	<0.001	NA	NA	NA	NA	NA	NA
Mok CC [15]	84/84[b]	0.893 (0.154)	0.970 (0.147)	0.001	NA	NA	NA	-0.93 (1.21)	-0.29 (1.27)	0.001
Sampaio-Barros PD [5]	Premenopause: 33/47 Menopause: 28/60	1.081 (0.183)	1.12 (0.175)	NA	NA	NA	NA	NA	NA	NA
Di Munno O [32]	43/50	0.974 (0.143)	1.081 (0.154)	<0.005	NA	NA	NA	NA	NA	NA
Souza RB [10]	43/47	0.83 (0.12)	0.94 (0.15)	<0.01	NA	NA	NA	NA	NA	NA
Cheng S [49]	15/15	0.983 (0.15)	1.043 (0.19)	0.341	-0.94 (0.14)	-0.35 (1.7)	0.341	0.20 (1.28)	0.70 (1.81)	0.384

[a] It will be footnoted if there is male in case or control.

[b] 9 man patients and 9 health men were included in both case and control

NA: not available