ORIGINAL ARTICLE

# Increased serum fibroblast growth factor-23 and decreased bone turnover in patients with systemic lupus erythematosus under treatment with cyclosporine and steroid but not steroid only

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## Abstract

*Summary* In patients with systemic lupus erythematosus (SLE), low bone mineral density (BMD) is associated with increased age, prolonged disease, low body mass index (BMI), and overlap with rheumatoid arthritis (RA). Elevated fibroblast growth factor (FGF)-23 in cyclosporine A (CsA) users with SLE are associated with decreased active vitamin D and osteocalcin.

*Introduction* The objective of this study was to investigate the steroid and CsA effect on bone metabolism and serum FGF-23 in SLE patients.

*Methods* Seventy-two SLE patients and 10 age- and sex-matched healthy individuals underwent blood tests for bone metabolic biomarkers and FGF-23, and lumbar spine dual-energy X-ray absorptiometry for BMD.

*Results* Comparisons between patients and controls were made in premenopausal women/men younger than 50 years and postmenopausal women/men older than 50 years separately. SLE patients had more frequent low Z-score ($\leq -2.0$, 8.5 vs. 0 %), osteopenia ($-2.5 <$ T-score $< -1.0$, 52 vs. 50 %), and osteoporosis (T-score $\leq -2.5$, 12 vs. 0 %), than the healthy age-compatible counterparts. BMD was significantly lower in patients with advanced age, longer disease duration, lower BMI, and overlap with RA (all $p < 0.05$ by multiple linear regression analyses). Serum FGF-23 was significantly higher and 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$) lower in SLE patients treated with glucocorticoid and CsA than in those not taking both of them ($p = 0.027$ and 0.002, respectively). The cumulative dose of glucocorticoid was inversely correlated with serum intact parathyroid hormone ($r = -0.299$, $p = 0.011$), C-terminal telopeptide of type I collagen ($r = -0.581$, $p < 0.001$), and osteocalcin ($r = -0.648$, $p < 0.001$). FGF-23 and the cumulative dose of CsA were positively correlated ($r = 0.38$, $p = 0.001$) and both were negatively correlated with $1,25(OH)_2D_3$ ($r = -0.266$, $p = 0.016$ and $r = -0.55$, $p < 0.001$) and osteocalcin ($r = -0.234$, $p = 0.034$ and $r = -0.274$, $p = 0.02$).

*Conclusion* SLE patients treated with glucocorticoid and CsA exhibited markedly decreased bone turnover. Those taking CsA had higher serum FGF-23 associated with suppression of $1,25(OH)_2D_3$ and bone formation. Such high-risk patients necessitate regular screening of osteoporosis.

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## Introduction

Glucocorticoids (GC) and cyclosporine A (CsA) are widely used to control autoimmunity in patients with systemic lupus erythematosus (SLE). They are responsible for drug-induced osteoporosis by markedly increasing bone resorption and impairing osteoblast maturation as well as mineralization

[1–3]. Bone mineral density (BMD) rapidly declines within the first 3 months of GC use, nadirs at 6 months, and then goes through a gradual but steady course of loss [2]. Long-term or high-dose GC treatment is a definite risk factor for GC-induced osteoporosis and fractures [4]. A recent cohort study revealed a significantly higher chance of osteoporotic fractures in SLE patients, especially when they take GC longer than 6 months [5].

Fibroblast growth factor-23 (FGF-23), derived from osteocytes and osteoblasts, has been found to facilitate urine phosphate excretion by inhibition of sodium/phosphate exchangers in renal epithelial cells [6, 7]. FGF-23 exhibits potential roles in bone metabolism. It can decrease the level of active vitamin D (1,25-dihydroxyvitamin $D_3$, $1,25(OH)_2D_3$) and increase its inactive metabolites, $24,25(OH)_2D_3$, by inhibiting $1\alpha$-hydroxylase and enhancing 24-hydroxylase, respectively [8]. In addition, FGF-23 has been proven to inhibit the production of parathyroid hormone (PTH) in vitro [9]. Recent studies have shown the relationship between FGF-23 and osteoporosis. In women with postmenopausal osteoporosis, the serum level of FGF-23 is significantly higher than in normal controls [10]. Elevated FGF-23 is associated with increased overall osteoporotic fracture risk in elderly men [11]. The relationships among GC treatment, BMD, PTH, and vitamin D level in SLE patients have been elucidated in recent years [12–15]. However, the effects of GC and CsA treatment on bone metabolic profiles, especially the FGF-23 level, and their relationships to BMD in SLE patients have not been investigated, and the role of FGF-23 and other markers of bone metabolism in drug-induced osteoporosis are less described.

The aim of this study was to compare the markers of bone and mineral metabolism, including FGF-23, PTH, $1,25(OH)_2D_3$, and BMD in SLE patients and healthy controls, focusing especially on the effect of GC and CsA treatments on bone metabolism in lupus patients.

## Patients and methods

### Study populations

The study was approved by the Institutional Review Board of Taipei Veterans General Hospital (VGHIRB No. 2011-07-007IC). Consecutive SLE patients from outpatient clinics of the rheumatology department in the hospital were screened. Patients fulfilling all of the following were recruited: (1) the classification criteria of SLE [16, 17], (2) adult men or women above 20 years of age, and (3) taking either GC or CsA longer than 3 months. Ten age- and gender-matched healthy controls were enrolled from among the hospital's staffs.

The exclusion criteria included patients who (1) smoked or drank alcohol above three glasses (about 42 g of alcohol) per day; (2) were pregnant or lactating; (3) had taken thyroxine, aromatase inhibitors (such as anatrozole, letrozole), drug inhibiting gonadal functions (such as leuprolide, medroxyprogesterone acetate), thiazolidinediones, selective serotonin reuptake inhibitors, anticonvulsants, heparin, or warfarin within the past 1 year; or (4) had associated end-stage renal disease, malignant tumor, cirrhosis, hyperthyroidism, hyperparathyroidism, Cushing's syndrome, type 1 diabetes, anorexia nervosa, Turner syndrome, or osteogenesis imperfecta. The same exclusion criteria were applied to the control group.

### Clinical profiles, bone status, and sample collections

Basic demographic data including age, sex, medication history, prior history of nontraumatic fracture, smoking status, body mass index (BMI), SLE disease activity index (SLEDAI [18]), and cumulative dose of GC and/or CsA per kilogram of body weight before enrollment were collected from medical records and questionnaires. BMI was calculated as body weight (kg) divided by the square of height (m). BMD of the lumbar spine (g/cm$^2$) was evaluated using dual-energy X-ray absorptiometry (DXA, Discovery A, Hologic®, MA, USA, coefficient of variation 1 %) for each participant. Z-score or T-score data were interpreted according to the 2013 International Society for Clinical Densitometry consensus [19].

Serum, plasma, and spot urine samples were collected in the morning after at least 8 h of fasting, and were immediately centrifuged at $500 \times g$ and $1500 \times g$, respectively. Plasma-free calcium was detected within 2 h after sampling, and all the other samples were stored at −80 °C before assay.

### Laboratory assays

Plasma free calcium, serum phosphate, creatinine, and urine calcium, phosphate, and creatinine were analyzed using the Clinical Analyzer 7600-310 (Hitachi High Technologies, Tokyo, Japan). The amount of proteinuria (g/day) was surrogated by the spot urine protein/creatinine ratio. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation and represented as milliliters per minute per 1.73 m$^2$ of body surface area [20]. Renal fractional excretion of inorganic phosphate ($FE_{Pi}$) was calculated as previously described [21]. Serum $1,25(OH)_2D_3$ was measured using an $I^{125}$ radioimmunoassay kit (DiaSorin, MN, USA) according to the manufacturer's instructions. Serum intact PTH (iPTH) was quantified with an ARCHITEC® iPTH chemiluminescent microparticle assay kit (Abott, Weisbaden, Germany), and calcitonin was measured with a commercial two-site chemiluminescent immunometric assay (IMMULITE 2000 Calcitonin, the Siemens Laboratory Diagnostics, Germany).

The following serum assays were performed using ELISA methods: FGF-23 (Millipore, Billerica, MA), C-terminal telopeptide (CTX, Serum CrossLaps® ELISA, Immunodiagnostics Systems, UK), and osteocalcin (N-MID® Osteocalcin ELISA, Immunodiagnostic Systems).

Statistical analysis

Categorical data were presented as number (%) and were compared with the Pearson's $\chi^2$ test; numerical data were presented as median (interquartile range) and were analyzed by Kruskal-Wallis test or Mann-Whitney $U$ test. To survey the determinant of BMD levels, we performed multiple linear regression analyses by selecting all the clinical parameters into the model 1 and using stepwise method in the model 2. A receiver operating characteristic (ROC) curve analysis was conducted to determine a BMI cutoff that was properly associated with a higher BMD. To investigate the effects on bone metabolism-related markers, further subgroup analyses based on the utility of CsA and the cutoff dose of GC equivalent to 7.5 mg of prednisolone were used. Spearman's correlation test was used to analyze the relationship between cumulative dose of GC and CsA and markers of bone and mineral metabolism in SLE patients. A $p$ value of <0.05 was considered statistically significant. All tests were two-tailed and adjusted for multiple comparisons. All statistical analyses were performed using SPSS for Windows, version 19.0 (Chicago, IL, USA).

## Results

Characteristics of the study population

In all, 72 SLE patients (97.2 % female; median age, 43.6 years) and 10 healthy volunteers (80 % female; median age, 33.2 years) were enrolled in this study. We classified the SLE patients into three different subgroups: those without GC or CsA treatment, those treated with GC, and those treated with both GC and CsA (as an add-on therapy to GC). Except for higher age, higher rates of postmenopausal woman and rheumatoid arthritis (RA) in SLE patients without GC or CsA treatment, and higher rates of peptic ulcer disease (PUD) or gastroesophageal reflux disease (GERD) in SLE patients with GC and CsA treatment, no other statistical difference was found in gender, BMI, smoking status, comorbidity with endocrine diseases, prior history of nontraumatic fracture, treatment with calcium, vitamin D, or bisphosphonates, and lumbar spine BMD (Table 1).

Compared to the other subgroups, SLE patients treated with both GC and CsA had a higher amount of proteinuria, higher daily dose and treatment duration of GC, higher rate of prior pulse methylprednisolone therapy, and lower rate of medication with hydroxychloroquine (all $p<0.05$, Table 1).

BMD in SLE patients

Although no statistical significance was reached, 8.5 % of the premenopausal female and male SLE patients aged <50 years had a Z-score≤−2.0, compared to the age-matched healthy controls, who all presented with normal BMD (Fig. 1a). In the postmenopausal female and male SLE patients aged ≥50 years, the frequency of osteopenia and osteoporosis was higher than in the matched controls (52 vs. 50 % and 12 vs. 0 %, respectively, Fig. 1b). BMD as related to the age and the disease duration of the SLE patients is shown in Fig. 1c, d, respectively. Adjusted for postmenopausal status, presence of RA, PUD/GERD, SLE duration, SLEDAI score, and cumulative dose of GC and CsA in model 1, the SLE patients exhibited a steady and significant decrement in the BMD level with aging ($\beta=-0.004$, $p=0.013$). The BMD was also significantly associated with BMI ($\beta=-0.013$, $p=0.040$). Subsequent ROC analysis for a cutoff of BMI associated with higher BMD indicated a value of 23, which was associated with a BMD above its median, with sensitivity of 65.6 %, specificity of 63.2 %, and area under the curve of 0.626. Thus, BMD was significantly higher in patients with a BMI≥23 than in those with a BMI<23 (0.996 [0.932–1.069] vs. 0.918 [0.806–1.003] g/cm$^2$, $p=0.014$). In model 2, a significantly lower BMD was observed in SLE patients with overlap with RA and in those with a longer disease duration ($\beta=-0.120$, $p=0.031$; $\beta=-0.004$, $p=0.045$, respectively in Table 2).

GC, CsA, and bone metabolism

Regarding the effect of GC on bone metabolism in SLE patients, significantly lower serum CTX (57.06 vs. 59.95 pg/mL, $p<0.001$, Fig. 2f) and osteocalcin (38.69 vs. 564.95 pg/mL, $p<0.001$) levels were found in SLE patients treated with GC than in those without GC or CsA treatment. The cumulative dose of GC was inversely correlated with serum CTX ($r=-0.581$, $p<0.001$) and osteocalcin ($r=-0.648$, $p<0.001$, Table 3). Furthermore, a lower serum iPTH level was found in SLE patients treated with GC than in those without GC or CsA treatment (38.0 [16.4–53.4] vs. 50.0 [36.9–65.9] pg/mL, Fig. 2c). The cumulative dose of GC was negatively correlated with the serum iPTH ($r=-0.299$, $p=0.011$) and spot urine calcium/creatinine ratio ($r=-0.251$, $p=0.033$, Table 3). The serum-free calcium did not differ in SLE patients treated with and without GC (Fig. 2d and Table 3).

To analyze the effect of CsA on bone metabolism, we minimized the effect of GC in SLE patients by inspecting patients who received prednisolone equivalent to ≤7.5 mg/day and neglecting those who received prednisolone equivalent to >7.5 mg/day. After this adjustment, CsA therapy was found to

**Table 1** Characteristics of SLE patients and healthy controls

| Clinical parameter | Healthy control (N=10) | SLE without GC or CsA (N=20) | SLE with GC (N=32) | SLE with GC and CsA (N=20) | p value |
|---|---|---|---|---|---|
| Age, year | 33.2 (31.8–53.2) | 58.2 (46.7–63.7) | 41.9 (33.9–52.1) | 34.3 (27.2–45.4) | 0.004 |
| Female | 8 (80) | 20 (100) | 30 (93.8) | 20 (100) | 0.068 |
| Postmenopausal woman | 3 (30) | 12 (60) | 8 (25) | 4 (20) | 0.028 |
| Height, cm | 160 (155–169) | 157 (155–161) | 158 (155–163) | 158 (154–160) | 0.694 |
| Body weight, kg | 57.5 (52–61) | 53.5 (49.3–68.3) | 54.3 (49.2–63.8) | 53 (47.5–61.5) | 0.763 |
| BMI, kg/m2 | 21.6 (20.1–24.0) | 22.1 (19.8–26.2) | 22.9 (19.9–24.0) | 21.7 (18.9–23.9) | 0.784 |
| eGFR, mL/min/1.73 m2 | 122 (110–129) | 102 (91–111) | 112 (85–124) | 91 (49–119) | 0.061 |
| Hemoglobin, g/dl | NA | 13.1 (12.2–13.8) | 12.8 (11.5–13.3) | 11.3 (8.5–12.2) | <0.001 |
| Smoking, ever | 0 | 2 (10) | 4 (12.5) | 3 (15) | 0.646 |
| Comorbidity | | | | | |
|   RA[b] | 0 | 5 (25) | 1 (3.1) | 1 (5) | 0.024 |
|   PUD/GERD | 0 | 0 | 1 (3.1) | 4 (20) | 0.027 |
| Ca/Vit D supplement | 2 (20) | 5 (25) | 13 (40.6) | 8 (40) | 0.469 |
| Bisphosphonate, prior | 0 | 0 | 2 (6.3) | 0 | 0.361 |
| BMD of L spine, g/cm2 | 0.918 (0.868–1.025) | 0.912 (0.818–0.996) | 0.972 (0.838–1.047) | 0.979 (0.826–1.062) | 0.666 |
| Fracture of L spine | 0 | 0 | 3 (9.4) | 1 (5) | 0.402 |
| SLE duration, year | NA | 5.5 (1.1–19.5) | 7.0 (1.7–13.8) | 12.5 (6.0–20.3) | 0.072[a] |
| SLEDAI | NA | 4 (4–8) | 7 (2–9) | 8 (4–14) | 0.185[a] |
| Urine protein, g/day | NA | 0.1 (0.1–0.1) | 0.1 (0.1–0.1) | 0.2 (0.1–0.4) | 0.001[a] |
| Medication history | | | | | |
|   Hydroxychloroquine | NA | 13 (65) | 18 (56) | 6 (30) | 0.002[a] |
|   Daily dose of GC[c], mg | NA | 0 | 7.5 (5.0–10.0) | 10 (6.6–12.5) | <0.001[a] |
|   Duration of GC, month | NA | 0 | 19 (7–64) | 43 (23–69) | <0.001[a] |
|   Prior pulse MTP therapy | NA | 1 (5) | 9 (28.1) | 14 (70) | <0.001[a] |
|   Rituximab | NA | 1 (5) | 1 (3.1) | 3 (15) | 0.241[a] |
|   Anti-TNF α therapy | NA | 2 (10)[d] | 0 | 0 | 0.069[a] |

Data in parentheses mean percentage or interquartile range

*SLE* systemic lupus erythematosus, *GC* glucocorticoid, *CsA* cyclosporine, *BMI* body mass index, *eGFR* estimated glomerular filtration rate, *RA* rheumatoid arthritis, *PUD/GERD* peptic ulcer disease or gastroesophageal reflux disease, *Ca/Vit D* calcium or vitamin D, *BMD* bone mineral density, *L* lumbar, *SLEDAI* systemic lupus erythematosus disease activity index, *MTP* methylprednisolone

[a] Comparison was made among SLE patients but not healthy controls

[b] These patients also fulfill the 2010 classification for RA

[c] Prednisolone equivalent

[d] These treatment were for the control of RA activity rather than lupus activity in patients with SLE and RA overlap

be associated with higher CTX and iPTH (58.3 [57.7–59.7] vs. 56.9 [56.0–57.6] pg/mL in Fig. 3f and 64.8 [46.7–94.0] vs. 38.0 [10.7–51.4] pg/mL in Fig. 3c, respectively). The cumulative dose of CsA was inversely correlated with osteocalcin ($r=-0.274$, $p=0.020$, Table 3). In addition, CsA therapy was significantly associated with an increased serum FGF-23 level (56.2 [46.4–67.4] vs. 25.4 [14.0–43.1] pg/mL, $p=0.033$ in Fig. 3a) and was also positively correlated with serum FGF-23 and renal FE$_{Pi}$ ($r=0.380$, $p=0.001$; $r=0.234$, $p=0.048$, respectively, Table 3). On the other hand, CsA treatment was significantly associated with a lower serum $1,25(OH)_2D_3$ (21.0 [16.3–38.7] vs. 70.1 [56.5–85.0] pg/mL, $p=0.011$ in Fig. 3b) and spot urine calcium/creatinine ratio (0.012

[0.003–0.028] vs. 0.073 [0.025–0.137], $p=0.008$ in Fig. 3e). Individual values of both of these parameters were also negatively correlated with the cumulative dose of CsA ($r=-0.550$, $p<0.001$ and $r=-0.360$, $p=0.002$, respectively). The serum-free calcium was not associated with CsA treatment (Fig. 3d and Table 3).

FGF-23, bone metabolism, and FGF-23 determinants

To understand the general effect of FGF-23 on bone metabolism, its levels in sera from SLE patients and normal individuals were analyzed as a whole. FGF-23 in serum was inversely correlated with osteocalcin ($r=-0.234$, $p=0.034$) but not

**Fig. 1** Bone mineral density (BMD) of lumbar spine in SLE patients and healthy controls. **a** Distribution of Z-score in premenopausal women or men aged below 50 years. Four of the 47 (8.5 %) SLE patients had a Z-score≤−2.0. **b** Distribution of T-score in postmenopausal women or men aged of 50 years or more. Thirteen and 3 of the 25 SLE patients (52 and 12 %) had osteopenia and osteoporosis, respectively. Only two of the four healthy controls had osteopenia. Scattered plot of the data of lumbar spine BMD by age (**c**) and SLE duration (**d**). *Dash* and *dotted lines* indicate the regression lines of the patients with body weight ≥23 and <23 kg, respectively
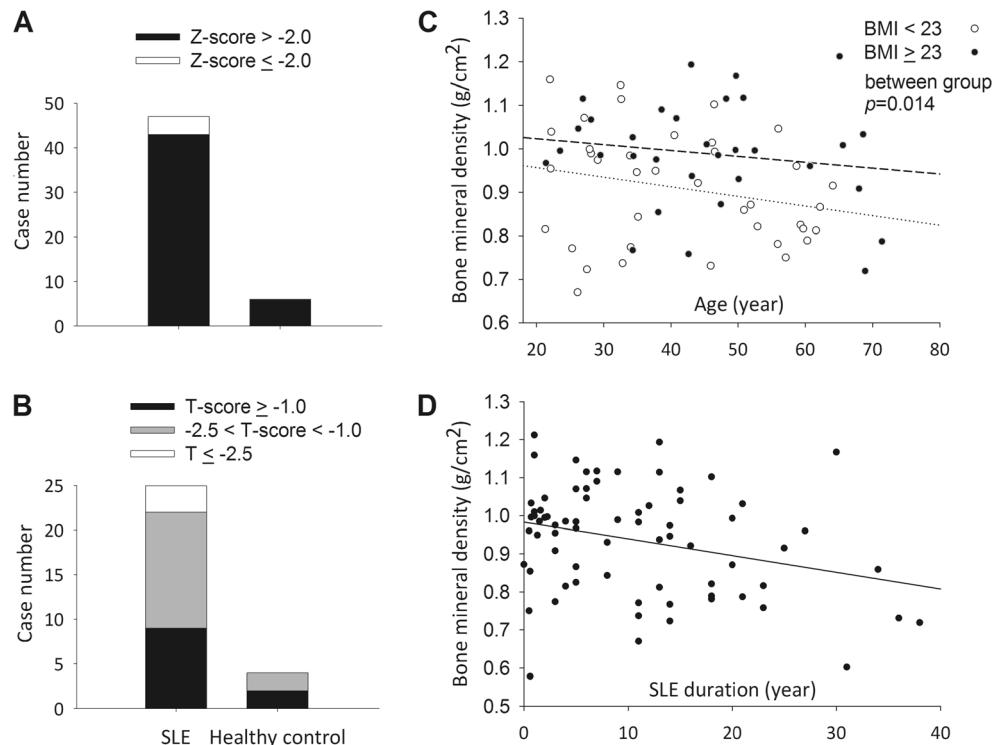


CTX ($r=−0.112$, $p=0.317$), regardless of what treatment group, patients or normal controls. It was also inversely correlated with serum $1,25(OH)_2D_3$ ($r=−0.266$, $p=0.016$) but not iPTH ($r=−0.018$, $p=0.871$). To analyze the determinants of FGF-23, we performed a stepwise multiple linear regression by adjusting age, postmenopausal status, presence of RA, PUD/GERD, hemoglobin level, eGFR, urine protein, serum iPTH, $1,25(OH)_2D_3$, and cumulative dose of GC and found that the cumulative dose of CsA per kilogram of body weight was significantly associated with FGF-23 ($\beta=−0.005$, $p=0.008$).

## Discussion

This cross-sectional, case-control study demonstrated that premenopausal female and male SLE patients aged <50 years

**Table 2** Multiple linear regression of lumbar spine BMD with clinical parameters and treatment of SLE patients

| Clinical parameter | Model 1 | | Model 2 | |
|---|---|---|---|---|
| | $\beta$ (95 % CI) | $p$ value | $\beta$ (95 % CI) | $p$ value |
| Age, year | −0.004 (−0.007 to −0.001) | 0.013 | – | – |
| Postmenopausal woman | −0.087 (−0.182 to 0.009) | 0.073 | – | – |
| Overlap with RA | 0.061 (−0.053 to 0.175) | 0.288 | −0.120 (−0.230 to −0.011) | 0.031 |
| PUD/GERD | −0.100 (−0.258 to 0.058) | 0.210 | – | – |
| BMI | 0.013 (0.000 to 0.018) | 0.040 | – | – |
| SLE duration, year | −0.004 (−0.008 to 0.000) | 0.062 | −0.004 (−0.007 to 0.000) | 0.045 |
| SLEDAI score | 0.003 (−0.003 to 0.009) | 0.284 | – | – |
| Cumulative dose of GC[a] | 0.000 (0.000 to 0.000) | 0.306 | – | – |
| Cumulative dose of CsA[b] | $−4.5×10^{−6}$ ($−2.3×10^{−5}$ to $1.4×10^{−5}$) | 0.631 | – | – |

*BMD* bone mineral density, *SLE* systemic lupus erythematosus, $\beta$ unstandardized coefficient, *CI* confidence interval, *RA* rheumatoid arthritis, *PUD/GERD* peptic ulcer disease or gastroesophageal reflux disease, *BMI* body mass index, *SLEDAI* SLE disease activity index, *GC* glucocorticoid, *CsA* cyclosporine A

[a] Prednisolone equivalent per kilogram of body weight

[b] CsA per kilogram of body weight
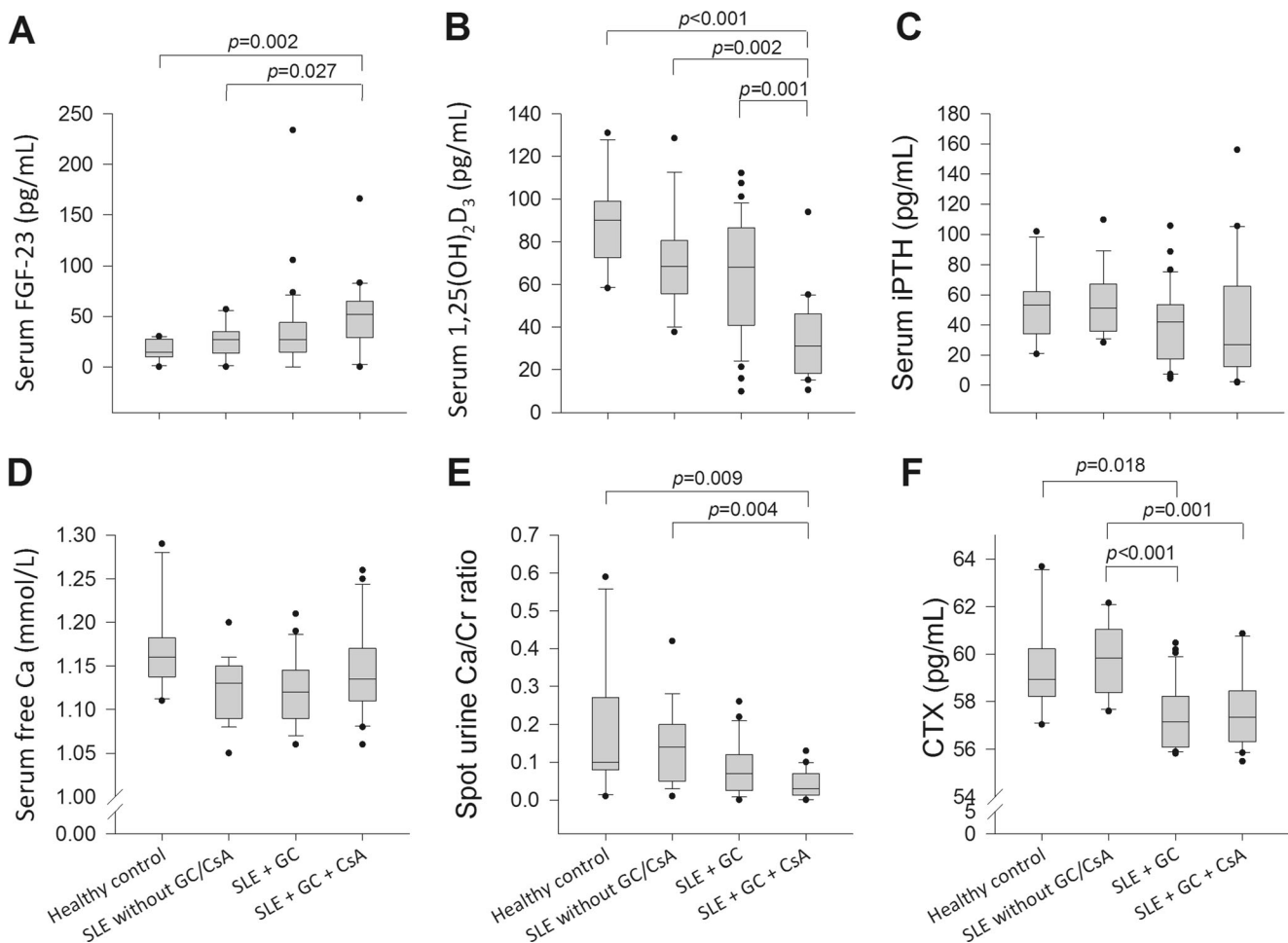
**Fig. 2** Box and whisker plot of the markers of bone and mineral metabolism in the healthy controls and SLE patients according to GC and CsA treatment. *FGF-23* fibroblast growth factor-23, *iPTH* intact parathyroid hormone, *Ca* calcium, *Cr* creatinine, *CTX* C-terminal telopeptide of type I collagen, *GC* glucocorticoid, *CsA* cyclosporine A

more frequently had a lumbar spine Z-score≤−2.0, while postmenopausal female SLE and male SLE patients aged ≥50 years had higher rates of osteopenia and osteoporosis than the age-matched healthy individuals. SLE patients exhibited a conspicuous decreasing in BMD parallel with aging and prolonged disease duration. A low BMD is also significantly associated with a low BMI or overlap with RA in SLE patients. A cumulative dose of GC was negatively correlated with serum iPTH and CTX. A cumulative dose of CsA was positively correlated with serum FGF-23, but negatively with serum 1,25(OH)$_2$D$_3$. Both cumulative doses of GC and CsA were inversely correlated with serum osteocalcin. These results imply that sufficient nutrition to improved BMI and regular check of osteoporosis after the diagnosis of SLE is a feasible strategy to ameliorate the bone loss effect of GC in SLE patients undergoing regular GC therapy. The osteoporotic effect of GC may originate partly from the inhibition of PTH and osteocalcin, and that of CsA originates instead from increasing FGF-23 and decreasing 1,25(OH)$_2$D$_3$ as well as osteocalcin production.

Up to now, the exact role of FGF-23 in bone metabolism and GC- or CsA-induced osteoporosis has not been well documented. The present investigation is probably the first to evaluate the effect of GC and CsA on FGF-23, together with its interaction in the bone metabolism of patients with SLE. Our results have provided a more comprehensive view of the changes in bone and mineral metabolism exerted by drug treatments and autoimmune process *per se* in SLE patients. Most of the potential confounding factors affecting osteoporosis showed no statistical differences between subgroups. Although the advanced age, postmenopausal status, and overlap syndrome of SLE and RA appeared to be most frequent in SLE patients without GC or CsA treatment, compared to those occurred in other subgroups, our data are still qualified, without exclusion, for the analyses to determine the differences of BMD, FGF-23, and the effect of GC and CsA on bone metabolism in SLE, rendering our deduction more straightforward.

Decreased BMD is a common comorbidity of SLE. As shown in the present investigation, the prevalence rate of Z-

**Table 3** Correlations of GC and CsA treatments with bone metabolism-related markers in SLE patients

| Marker | Cumulative dose of GC[a] | | Cumulative dose of CsA[a] | |
|---|---|---|---|---|
| | $r$ | $p$ value | $r$ | $p$ value |
| Serum FGF-23 | 0.232 | 0.050 | 0.380 | 0.001 |
| Serum iPTH | −0.299 | 0.011 | −0.076 | 0.527 |
| Serum 1,25(OH)$_2$D$_3$ | −0.199 | 0.094 | −0.550 | <0.001 |
| Serum CTX | −0.581 | <0.001 | −0.163 | 0.170 |
| Serum osteocalcin | −0.648 | <0.001 | −0.274 | 0.020 |
| Renal FE$_{Pi}$ | 0.109 | 0.363 | 0.234 | 0.048 |
| Spot urine protein/Cr | 0.083 | 0.486 | 0.442 | <0.001 |
| Spot urine Ca/Cr ratio | −0.251 | 0.033 | −0.360 | 0.002 |
| Serum free Ca | 0.038 | 0.748 | 0.085 | 0.479 |
| Serum phosphate | 0.013 | 0.911 | 0.080 | 0.504 |

*GC* glucocorticoid, *CsA* cyclosporine A, *r* Spearman's correlation coefficient, *FGF-23* fibroblast growth factor-23, *iPTH* intact parathyroid hormone, *1,25(OH)$_2$D$_3$* 1,25 dihydroxyvitamin D$_3$, *CTX* C-terminal telopeptide of type I collagen, *FE$_{Pi}$* fractional excretion of inorganic phosphate, *Cr* creatinine, *Ca* calcium

[a] Per kilogram of body weight

score≤−2.0 is 8.5 % among the young SLE patients, which is comparable with that reported (8.8 %) by Lee et al. [22]. Also, the prevalence rates of osteopenia (52 %; 39.7 to 54 %) and osteoporosis (12 %; 5 to 18 %) among the elderly SLE patients were compatible with those reported recently [15, 23–27]. Advanced age and low BMI, but not SLE disease activity, were significantly associated with low BMD and were consistent with those of previous investigations [23, 24]. The previous studies of association between low BMD and SLE disease duration were variable and inconsistent due to differences in age and races [28–30]. Herein, we demonstrate the association between disease duration and low BMD in adult SLE patients, showing the cumulative effect of long-term inflammation on BMD, which was in accordance with a study of juvenile SLE patients [30]. Furthermore, we show that overlap with RA is associated with a significantly low BMD in SLE patients and strengthen the evidence for RA as a well-known clinical risk factor for osteoporosis and fracture [31].

Alterations of bone turnover markers have been observed in SLE patients, especially in those taking GC or CsA [32]. GC treatment is associated with the decreased bone formation
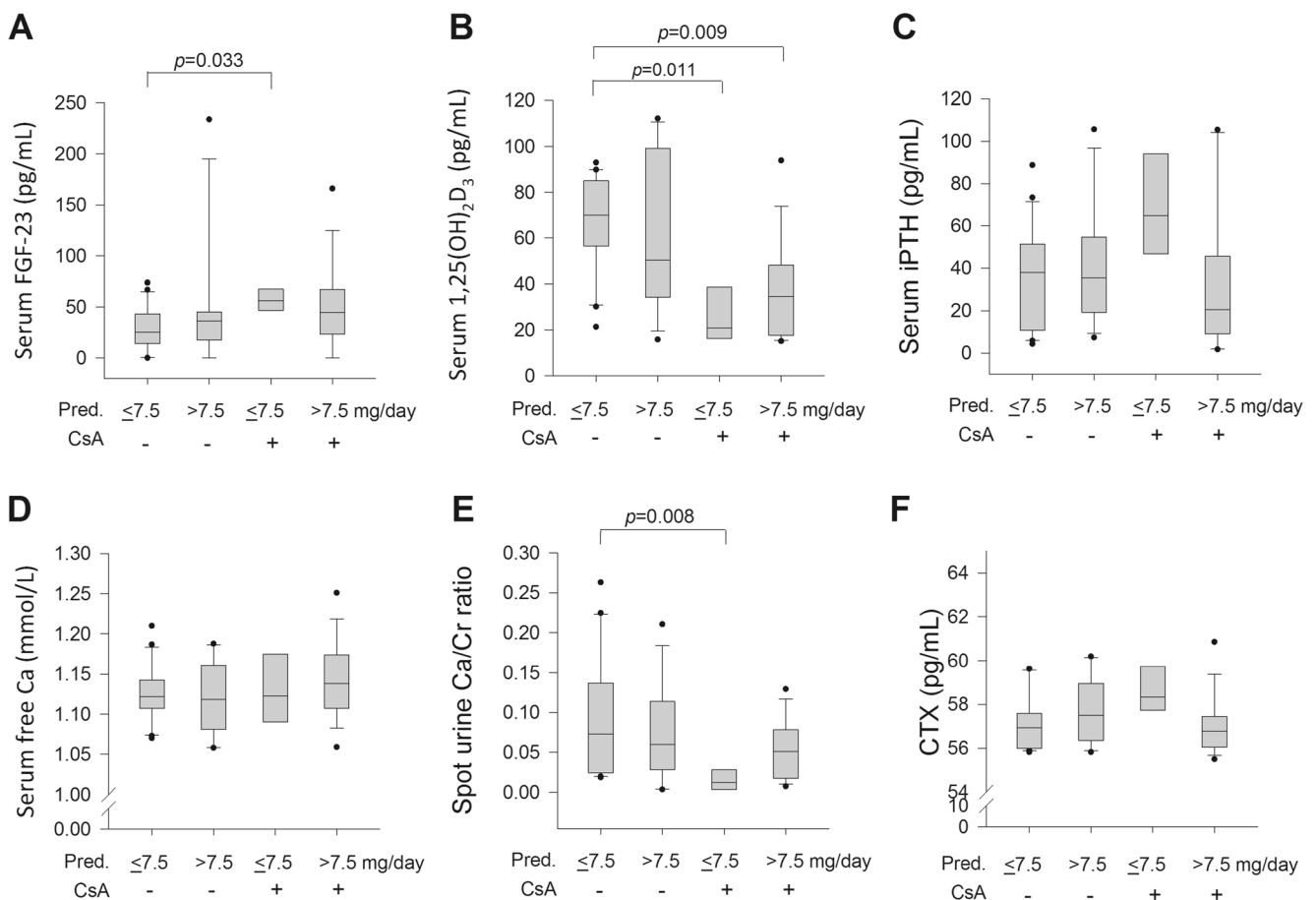


**Fig. 3** Box and whisker plot of the markers of bone and mineral metabolism in SLE patients undergoing GC or CsA treatment. *FGF-23* fibroblast growth factor-23, *iPTH* intact parathyroid hormone, *Ca* calcium, *Cr* creatinine, *CTX* C-terminal telopeptide of type I collagen, *Pred.* prednisolone, *CsA* cyclosporine A

[2]. The present investigation also demonstrated that the cumulative GC dose was negatively correlated with serum osteocalcin in SLE patients, which was consistent with a recent study [33]. However, the effect of GC treatment on bone resorption in SLE patients remains uncertain. Baker-LePain et al. [33] reported that lupus activity, but not GC treatment, was a negative predictor of bone resorption. Banno et al. [34] found that there was no difference in bone resorption markers between osteoporotic and non-osteoporotic SLE patients. We have shown that a marked reduction of serum CTX is associated with GC treatment even in the absence of a different BMD. On the other hand, CsA treatment has been found to be associated with increased bone formation and bone resorption in vivo [1, 35]. Although it was difficult to segregate the contributions of CsA and GC treatment to bone turnover in our SLE patients, we have provided evidence for higher bone turnover (increased CTX in serum) as a result of CsA treatment combined with a low-dose GC. However, the effect of CsA on bone formation may have been abolished by concurrent GC administration.

GC and CsA treatments may lead to fluctuations in the vitamin D level in SLE patients. GC exposure has been reported to be associated with high [12], equivocal [13], or low [14, 36] vitamin D levels. An animal study revealed that CsA treatment is associated with vitamin D resistance through downregulation of vitamin D receptors in spite of an increase in $1,25(OH)_2D_3$ level [37]. On the contrary, the present investigation has shown that CsA therapy is inversely correlated with the level of $1,25(OH)_2D_3$ and is positively correlated with FGF-23 in SLE patients. Since FGF-23 inhibits $1\alpha$-hydroxylase, it is conceivable that CsA may inhibit $1,25(OH)_2D_3$ via the stimulation of FGF-23 excretion. In addition, a higher serum iPTH level was found in CsA users who also received low-dose GC. These results were consistent with those reported by Kovalik et al. [38], who showed a significantly increased serum iPTH level in atopic dogs undergoing CsA therapy. Despite this consistency, we have found an inverse correlation between serum iPTH and the cumulative dose of GC ($r=-0.299$, $p=0.011$), suggesting that GC and CsA are reciprocally antagonizing each other in their interaction with iPTH. Trends toward an increased renal calcium excretion and a decreased iPTH were observed among CsA users who treated with prednisolone equivalent to >7.5 mg/day than those with ≤7.5 mg/day (Fig. 3c–e), suggesting PTH keeps normocalcemia by stimulating renal calcium reabsorption [39].

The production of FGF-23 is increased in conditions such as chronic renal failure, high-phosphate diet, elevated iPTH and $1,25(OH)_2D_3$, and iron-deficiency anemia [40]. Lundberg et al. [41] have reported that high FGF-23 is associated with albuminuria and progression of chronic renal failure in patients with IgA nephropathy. As shown in the present investigation, cumulative dose of CsA was still significantly associated with high FGF-23 even after adjusting these factors. Additionally, a significantly decreased renal calcium excretion and steady serum-free calcium was observed in CsA users than in non-CsA users (Fig. 3a, d, and e), suggesting that FGF-23 maintains calcium homeostasis by stimulating renal calcium reabsorption [42]. Elevated FGF-23 was also associated with suppressed $1,25(OH)_2D_3$ and osteocalcin particularly in SLE patients with the highest degree of proteinuria treated with both GC and CsA. Thus, it can be inferred that the increased FGF-23 parallel to the CsA treatment may synergistically act with GC to result in decreased bone turnover in SLE patients, which in turn leads to osteoporosis in the long run [10, 11].

There are several clinical and research implications from the present study. Since low vitamin D level is common, especially in the SLE patient treated with GC and CsA, adequate vitamin D supplementation has been advocated in recent clinical trials [43, 44]. Vitamin D can offset the phosphate diabetes caused by FGF-23 and hence preclude further bone resorption. Therefore, in patients with advanced age and low body weight, a more important strategy to deter osteoporosis is adequate nutrition rather than a reduced GC or CsA dose.

Although we have demonstrated the role of FGF-23 in the development of drug-induced osteoporosis, especially the CsA-induced osteoporosis in patients with SLE, there are several drawbacks to the present investigation. Since data concerning medications, including GC and CsA, were collected retrospectively from the medical records, the dosing and combination therapy as well as compliance with the prescription could not be controlled. Despite these concerns, a decrease in bone turnover was still observed. On the other hand, the lack of significant differences in BMD among subgroups and data on microarchitectural deterioration impeded our ability to analyze the fracture risk. The independent effects of CsA and GC on bone and mineral metabolism were difficult to assess because calcineurin inhibitors such as CsA seldom serve as monotherapy in lupus nephritis patients [45]. Nevertheless, the present investigation has still shown the alterations and interrelationship of $1,25(OH)_2D_3$, FGF-23, and iPTH in SLE patients treated with GC and CsA. In conclusion, careful screening for osteoporosis is of paramount importance in SLE patients with advanced age, prolonged disease, and low BMI who take various immunosuppressants. The association found between FGF-23 and CsA administration warrants further research.

**Conflicts of interest** None.

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