

ORIGINAL CONTRIBUTION

The Effect of Long-term Glucocorticoids on Bone Metabolism in Systemic Lupus Erythematosus Patients: The Prevalence of Its Anti-inflammatory Action upon Bone Resorption

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The study was made to evaluate bone turnover in systemic lupus erythematosus (SLE) patients undergoing long-term glucocorticoid therapy. Thirty-eight female patients with established SLE were compared with a control group consisting from 160 age-matched healthy women. Serum concentrations of proinflammatory cytokines: interleukin-1 α , interleukin-6, tumor necrosis factor- α , granulocyte-macrophage colony stimulating factor (GM-CSF) and some biochemical markers of osteoporosis (osteocalcin, total and bone alkaline phosphatase, procollagen type I carboxyterminal propeptide, carboxyterminal telopeptides of type I collagen - CTx) were measured. Additionally, morning urine excretions of deoxypyridinoline and calcium/creatinin ratios were determined. The forearm densitometry (DXA) was performed in all patients. Bone mineral content (BMC) and bone mineral density (BMD) in the SLE group was not significantly different from the controls, and no relationship was found between the glucocorticoid exposure and the BMC/BMD. However, biochemical markers of bone resorption — CTx and calcium/creatinin ratio — were significantly increased in the patient group. Our results suggest that BMD/BMC is preserved in glucocorticoid-treated SLE patients despite accelerated bone turnover.

INTRODUCTION

Systemic Lupus Erythematosus (SLE)^b is a chronic, inflammatory disease characterized by a wide range of immunological changes and autoimmune phenomena [1, 2]. Glucocorticoids (gcs) still remain the first-choice treatment option for SLE.

They possess strong anti-inflammatory and immunosuppressive properties, mainly due to their profound effect on the production of proinflammatory cytokines [3-5]. Although very effective, gcs treatment causes a number of adverse events. For instance, gcs have an inhibitory effect on the bone turnover and may induce osteo-

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^b Abbreviations: ACT, α -1-chymotrypsine; AGP, α -1-acid-glycoprotein; BGP, osteocalcin; BMC, bone mineral content; BMD, bone mineral density; CTx, carboxy-terminal collagen crosslinks; Dpd, deoxypyridinoline; DXA, densitometry; gcs, glucocorticoids; GM-CSF, granulocyte-macrophage colony stimulating factor; IL-1- α , interleukin-1- α ; IL-6, interleukin-6; SLE, systemic lupus erythematosus; TNF- α , tumor necrosis factor- α

porosis, delay healing of fractures, inhibit bone growth, and cause osteonecrosis via reduction of osteoblast activity [4, 6, 7]. The risk of glucocorticoid-induced osteoporosis may be especially high in patients who need long-term, high-dose gcs treatment, like those with SLE. The aim of the study presented here was to evaluate the bone tissue metabolism in SLE patients based on the measurement of biochemical markers of osteoporosis and densitometry results in relation to the glucocorticoid exposure.

MATERIALS

Patients: Thirty-eight female patients with established SLE treated with gcs (age 46.4 ± 12.8 years, disease duration 108 ± 82 months; total gcs dose equivalent to prednisone 25.3 ± 28.1 g per patient, duration of treatment 90.8 ± 78.5 months; all values are mean \pm SD) The control group consisted of 160 healthy women (age 52 ± 12 years). Sixteen patients (age 35.9 ± 10 years; disease duration 82 ± 69 months; total dose of gcs-equivalent to prednisone 14.7 ± 12.6 g per patient) and 58 healthy controls (age 42 ± 10 years) were premenopausal. Twenty-two patients (age 53.9 ± 8.5 years, disease duration 126 ± 87 months, total dose of gcs-equivalent to prednisone 33.1 ± 33.6 g per patient) and 102 controls (age 52 ± 8 years) were postmenopausal. Beside gcs, 10 patients were treated with cyclophosphamide, four of them orally (50 to 100 mg daily), and six of them intravenously (800 mg every 4th to 8th week). One patient received azathioprine (100 mg per day orally). Twenty-four of SLE patients were treated with non-steroidal anti-inflammatory drugs. SLE patients and healthy subjects who underwent estrogen, calcitonin or bisphosphonate therapy were not included.

Laboratory tests: Blood samples were taken from all subjects in fasting condition, without anticoagulants, centrifuged, and

stored at -70° C. Urine samples were obtained after a 24-hour calcium- and collagen-free diet, centrifuged, and stored at -70° C.

Serum levels of acute-phase reactants: α -1-acid-glycoprotein (AGP) and α -1-chymotrypsine (ACT) were measured by rocket immunoelectrophoresis [8]. Glycosylation profile of α -1-acid-glycoprotein was determined using affinity immunoelectrophoresis with the lectin concanavalin A as described by Bøgg-Hansen [9] and the results were expressed as reactivity coefficients (AGP-RC). Bone isoenzyme of alkaline phosphatase (AP-B), deoxypyridynoline (Dpd), osteocalcin (BGP), and carboxy-terminal propeptide of type 1 collagen (PICP) were measured by ELISA using commercially available kits (Alkphase-B, Pylilinks-D, Novocalcin and Prolagen-C, respectively) obtained from Metra Biosystems (USA). Serum levels of carboxy-terminal collagen crosslinks (CTx) were measured with Serum CrossLaps One Step ELISA kits from Osteometer Biotech (Denmark). Serum tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), interleukin-1 α (IL-1 α), and granulocyte/monocyte colony stimulating factor (GM-CSF) were measured by ELISA using the Quantikine kit purchased from R&D System (USA).

Densitometry: The bone mineral density (BMD) and bone mineral content (BMC) of the forearm were measured using standard DXA technique (DTX-200, Osteometer, Denmark).

Statistical analysis: The Mann-Whitney U, the Kruskal-Wallis ANOVA, and the Fisher's exact test were used to compare variables (differences were considered significant at $p < .05$). To determine the correlation between the variables the Spearman test was used (results were considered positive for $r > 0.5$ at $p < .05$).

RESULTS

The differences in BMC and BMD between SLE patients and age-matched healthy controls were not statistically significant (Table 1). We found eight SLE patient with osteoporosis (age, 60.3 ± 9.4 years) and 22 woman with osteoporosis in healthy group (63.5 ± 6.5 years), but there were no differences in BMC and BMD between this two group.

The BMC and BMD were decreased in postmenopausal women in either patient and control groups (Table 2). Because of correlation between BMC and age of menopause the changes in BMC depending on age have been analyzed in the group of SLE patients and healthy subjects. The correlation between BMC and age suggests faster bone loss in SLE patients, however it was not found to be statistically significant (Figure 1).

Table 1. Bone mineral content (BMC) and bone mineral density (BMD) in systemic lupus erythematosus (SLE) patients treated with glucocorticoids and in the control group, (all values are mean \pm SD).

	SLE	Control group
BMC (g)	2.888 ± 0.584 (1.613 – 4.368)	2.901 ± 0.501 (1.788 – 5.160)
BMC T-score	-1.0 ± 1.2 (-3.7 – 2.2)	-0.9 ± 1.0 (-3.3 – 1.9)
BMD (g/cm²)	0.423 ± 0.080 (0.239 – 0.594)	0.427 ± 0.065 (0.238 – 0.639)
BMD T-score	-1.2 ± 1.4 (-4.3 – 1.8)	-1.1 ± 1.1 (-4.3 – 1.3)

* – Statistically significant vs. control group ($p < .01$)

– Statistically significant vs. control group ($p < .05$)

Table 2. Bone mineral content (BMC) and bone mineral density (BMD) in systemic lupus erythematosus (SLE) patients and in the control group before (Menop-) and after menopause (Menop+), (all values are mean \pm SD).

	SLE		Control group	
	Menop-	Menop+	Menop-	Menop+
BMC (g)	3.086 ± 0.377 (2.494 – 3.880)	2.762 ± 0.661 # (1.613 – 4.368)	3.103 ± 0.459 (2.360 – 5.160)	2.781 ± 0.489 (1.788 – 3.913)
BMC T-score	-0.6 ± 0.8 (-1.8 – 1.2)	-1.2 ± 1.4 (-3.7 – 2.2)	-0.54 ± 0.81 (-2.1 – 1.9)	-1.2 ± 1.0 (-3.3 – 1.3)
BMD (g/cm²)	0.466 ± 0.046 (0.404 – 0.579)	0.395 ± 0.085 * (0.239 – 0.594)	0.456 ± 0.050 (0.378 – 0.639)	0.408 ± 0.065 # (0.238 – 0.566)
BMD T-score	-0.4 ± 0.8 (-1.5 – 1.5)	-1.7 ± 1.5 * (-4.3 – 1.8)	-0.57 ± 0.78 (-1.9 – 1.3)	-1.4 ± 1.1 (-4.3 – 1.3)

* – Statistically significant vs. group before menopause ($p < .01$)

– Statistically significant vs. group before menopause ($p < .05$)

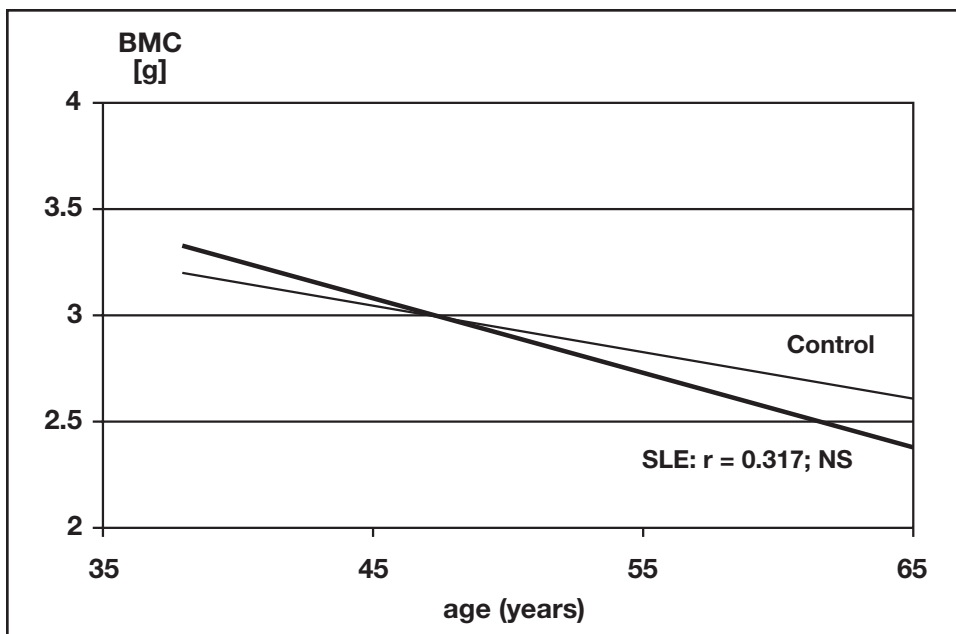


Figure 1. Correlation between bone mineral content (BMC) and the age in post-menopausal patients after menopause and controls.

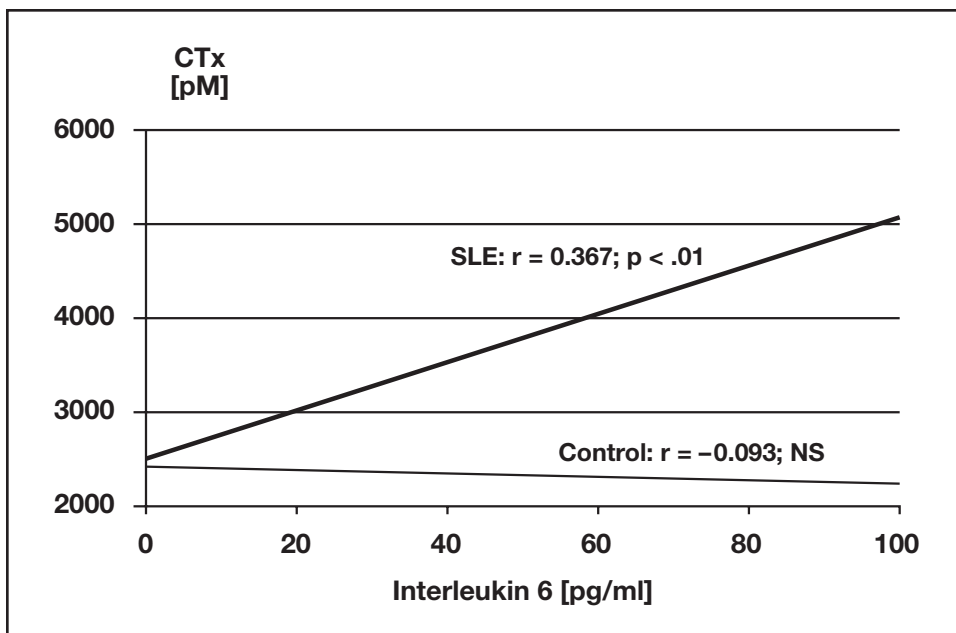


Figure 2. The correlation between the levels of carboxy – terminal collagen crosslinks (CTx) and interleukin 6 (IL-6) in SLE patients and in the control.

Table 3. The level of erythrocyte sedimentation rate (ESR), α 1-acid-glycoprotein (AGP), α 1-acid-glycoprotein-reactivity-coefficient (AGP-RC) and α -chymotripsine (ACT), antibodies to dsDNA and C3, C4 complement in systemic lupus erythematosus (SLE) patients and in the control group, (all values are mean \pm SD).

	SLE	Control group
ESR (mm/h)	41 \pm 30 * (4 – 138)	10 \pm 4 (4 – 18)
AGP (mg/l)	1717 \pm 811 * (195 – 4040)	696 \pm 299 (351 – 1386)
AGP-RC	1.16 \pm 0.33 (0.70 – 2.30)	1.31 \pm 0.24 (1.00 – 1.70)
ACT (mg/l)	677 \pm 360 # (166 – 2028)	303 \pm 81 (189 – 420)
dsDNA (IU/ml)	392 \pm 479 * (33 – 1925)	23 \pm 24 (0 – 72)
C3 (mg/l)	1226 \pm 318 (653 – 1848)	1148 \pm 257 (696 – 1695)
C4 (mg/l)	176 \pm 81 # (55 – 372)	243 \pm 69 (117 – 360)

* – Statistically significant vs. control group ($p < .01$)

– Statistically significant vs. control group ($p < .05$)

Table 4. The levels of cytokines: interleukin 1a (IL-1 α), interleukin 6 (IL-6), tumour necrosis factor alpha (TNF- α) and granulocyte-macrophage colony stimulating factor (GM-CSF) in systemic lupus erythematosus (SLE) patients and in the control group, (all values are mean \pm SD).

	SLE	Control group
IL-1α (pg/ml)	1.9 \pm 2.4 (0.0 – 12.5)	0.7 \pm 1.3 (0.0 – 3.8)
IL-6 (pg/ml)	20.4 \pm 30.6 * (3.1 – 146.7)	1.6 \pm 2.0 (0.0 – 5.8)
TNF-α (pg/ml)	8.1 \pm 5.2 * (2.6 – 29.2)	1.3 \pm 2.0 (0.0 – 5.4)
GM-CSF (pg/ml)	1.7 \pm 1.2 (0.0 – 3.8)	0.8 \pm 1.2 (0.0 – 3.7)

* – Statistically significant vs. control group ($p < .01$)

– Statistically significant vs. control group ($p < .05$)

Serum levels of acute phase proteins are shown on Table 3. In the SLE group, increase in the IL-6 and TNF- α levels was observed. The levels of other cytokines were similar in patients and controls (Table 4). No significant differences were found between the patients and the healthy controls in the levels of BGP, AP and AP-B. Two markers of bone resorption (CTx and Ca/Crea ratio) were significantly increased in SLE compared to control subjects (Table 5). The differences in pre- and postmenopausal patients are shown on Table 6. We divided our patients with SLE into three groups: eight with osteoporosis, 15 with osteopenia, and 15 without osteoporosis and osteopenia. Two markers of

bone metabolism (AP-B and Dpd) and serum levels of IL-6 were significantly increased in SLE with osteoporosis compared to SLE without osteoporosis and osteopenia (Table 7).

Statistically significant correlation between total dose of gcs and the BMC was not observed ($r = -0.065$, NS). However, statistically significant correlation were observed between the levels of CRP and CTx ($r = 0.244$, $p < .01$) and AGP and CTx ($r = 0.272$; $p < .01$). There were also statistically significant correlation between CTx and IL-6 (Figure 2), CTx and BGP, and the AP-B activity and the BGP level in the group of SLE patients and the control group (Figure 3).

Table 5. The levels of markers of bone tissue metabolism: osteocalcin (BGP), alkaline phosphatase (AP) and alkaline phosphatase-bone formation (AP-B), procollagen type I carboxyterminal propeptide (PICP), carboxyterminal telopeptides of type I collagen (CTx), deoxypyridinoline (Dpd) and calcium/creatinin ratio (Ca/Crea) in systemic lupus erythematosus (SLE) patients and in the control group, (all values are mean \pm SD).

	SLE	Control group
BGP (ng/ml)	6.8 \pm 3.7 (1.2 – 21.0)	6.5 \pm 3.7 (1.2 – 13.8)
AP (U/l)	105 \pm 71 (21 – 192)	92 \pm 27 (31 – 146)
AP-B (U/l)	29.4 \pm 14.8 (11.7 – 68.5)	24.0 \pm 11.5 (7.0 – 55.7)
PICP (ng/ml)	118 \pm 55 (70 – 241)	105 \pm 40 (48 – 166)
CTx (pM)	3380 \pm 2541 # (598 – 10231)	2390 \pm 1360 (1146 – 5975)
Dpd (nM)	7.7 \pm 4.8 (1.0 – 19.0)	6.5 \pm 1.6 (4.2 – 9.0)
Ca/Crea	0.31 \pm 0.20 # (0.08 – 0.66)	0.22 \pm 0.11 (0.05 – 0.44)

* – Statistically significant vs. control group ($p < .01$)

– Statistically significant vs. control group ($p < .05$)

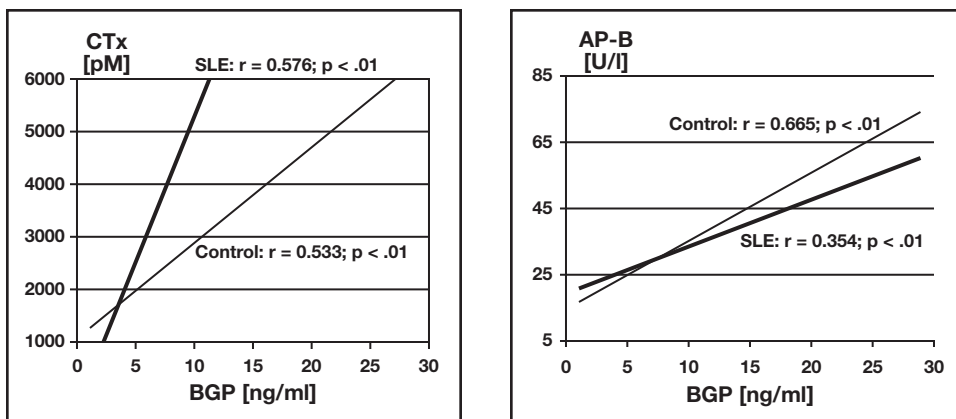


Figure 3. The correlation between the levels of following biochemical markers of osteoporosis: carboxy – terminal collagen crosslinks (CTx), bone alkaline phosphatase (AP-B), and osteocalcin (BGP) in systemic lupus erythematosus (SLE) patients and in the control.

Table 6. The levels of osteocalcin (BGP), alkaline phosphatase (AP) and alkaline phosphatase-bone formation (AP-B), procollagen type I carboxyterminal propeptide (PICP), carboxyterminal telopeptides of type I collagen (CTx), deoxypyridinoline (Dpd) and calcium/creatinin ratio (Ca/Crea) in systemic lupus erythematosus (SLE) patients and in the control group before (Menop-) and after menopause (Menop+), (all values are mean ± SD).

	SLE		Control group	
	Menop-	Menop+	Menop-	Menop+
BGP (ng/ml)	6.0 ± 2.7 (1.3 – 9.8)	7.3 ± 4.2 (1.2 – 21.0)	5.6 ± 3.6 (1.2 – 11.0)	7.8 ± 3.6 (3.2 – 13.8)
AP (U/l)	81 ± 28 (21 – 145)	122 ± 86 (64 – 473)	87 ± 29 (31 – 146)	106 ± 18 # (89 – 133)
AP-B (U/l)	24.2 ± 11.9 (12.1 – 56.9)	33.1 ± 15.8 (11.7 – 68.5)	26.2 ± 11.5 (7.0 – 42.5)	30.5 ± 11.6 # (18.4 – 55.7)
PICP (ng/ml)	74 ± 6 (70 – 78)	133 ± 56 # (85 – 241)	88 ± 38 (48 – 150)	124 ± 37 # (80 – 166)
CTx (pM)	3862 ± 3072 (978 – 10231)	3086 ± 2200 (598 – 8641)	1660 ± 426 (1146 – 2166)	3241 ± 1615 # (1560 – 5975)
Dpd (nM)	4.3 ± 3.5 (1.0 – 10.0)	9.2 ± 4.5 # (4.0 – 19.0)	5.4 ± 1.3 (4 – 7)	7.6 ± 1.0 # (7.0 – 9.0)
Ca/Crea	0.4 ± 0.27 (0.13 – 0.66)	0.28 ± 0.18 (0.08 – 0.59)	0.17 ± 0.11 (0.05 – 0.43)	0.25 ± 0.10 (0.05 – 0.44)

* – Statistically significant vs. group before menopause (p < .01)

– Statistically significant vs. group before menopause (p < .05)

Table 7. The levels of interleukin 1a (IL-1 α), interleukin 6 (IL-6), tumour necrosis factor alpha (TNF- α) and granulocyte-macrophage colony stimulating factor (GM-CSF) osteocalcin (BGP), alkaline phosphatase (AP) and alkaline phosphatase-bone formation (AP-B), carboxyterminal telopeptides of type I collagen (CTx), deoxypyridinoline (Dpd) and calcium/creatinin ratio (Ca/Crea) in systemic lupus erythematosus (SLE) patients with osteoporosis, with osteopenia and with without osteoporosis and osteopenia (all values are mean \pm SD).

	SLE with osteoporosis	SLE with osteopenia	SLE without osteoporosis and osteopenia
IL-1 (pg/ml)	1.4 \pm 1.3 (0.0 – 3.3)	3.1 \pm 3.3 (0.0 – 12.5)	1.0 \pm 1.7 (0.0 – 5.8)
IL-6 (pg/ml)	42.2 \pm 52.4 # (3.7 – 146.7)	18.7 \pm 27.3 (3.1 – 97.4)	10.8 \pm 9.7 (3.1 – 37.0)
TNF (pg/ml)	9.4 \pm 4.9 (5.7 – 18.5)	8.7 \pm 6.7 (4.8 – 29.2)	6.4 \pm 2.9 (2.6 – 12.4)
GM-CSF (pg/ml)	1.9 \pm 1.3 (1.0 – 3.8)	1.2 \pm 0.8 (0.0 – 2.3)	2.3 \pm 1.1 (1.2 – 3.8)
BGP (ng/ml)	10.2 \pm 4.9 (6.0 – 21.0)	5.8 \pm 3.6 (1.2 – 11.9)	7.1 \pm 3.7 (2.1 – 15.6)
AP (U/l)	112 \pm 41 (70 – 192)	92 \pm 20 (64 – 151)	91 \pm 23 (21 – 138)
AP-B (U/l)	40.7 \pm 18.9 * (18.3 – 68.5)	19.9 \pm 7.1 (11.7 – 37.9)	21.2 \pm 7.9 (12.2 – 40.0)
CTx (pM)	4141 \pm 3068 (732 – 8641)	2805 \pm 3119 (665 – 10231)	3670 \pm 2387 (598 – 7655)
Dpd (nM)	12.1 \pm 0.97 * (2.0 – 19.0)	6.2 \pm 3.4 (2.7 – 9.8)	7.8 \pm 4.9 (1.0 – 13.3)
Ca/Crea	0.31 \pm 0.20 (0.08 – 0.46)	0.25 \pm 0.20 (0.02 – 0.66)	0.28 \pm 0.14 (0.14 – 0.25)

* – Statistically significant vs. group without osteoporosis and osteopenia ($p < .01$)

– Statistically significant vs. group without osteoporosis and osteopenia ($p < .05$)

DISCUSSION

The bone loss may occur in about 25 to 30 percent of SLE patients [10]. Gcs treatment is thought to be the main cause of bone loss in SLE and its effect is dose dependent [11-13]. Moreover, the degree of bone loss is also connected with the course of SLE as a consequence of the activity of the inflammatory process. The changes are found especially in trabecular bone and to lesser degree in cortical bone

[14]. The most important and the most serious results of osteoporosis are fractures. The risk of fractures is determined by peak bone mass, which people reach in their twenties [15] and which is influenced by hormonal function, diet, and some environmental factors [16, 17]. In SLE, fractures are observed less frequently than in some other rheumatic conditions, e.g., rheumatoid arthritis

Glucocorticoids inhibit bone formation by blocking proliferation of osteoblasts.

They also intensify the osteoclast activity [18]. The largest bone loss is observed during the first six to 12 months of gcs therapy [19, 20]. The degree of bone loss depends on the duration of treatment, daily and total dose of gcs and the way of gcs administration (e.g., systemic or local, continuous or alternate dosing). In our SLE group, the gcs dosage was being adjusted to disease activity and severity and ranged from 0 to 60 mg daily for a given patient. Thus, gcs dosage profile over time may have some influence on the degree of bone loss in individual patients.

In the investigated group of SLE patients the changes in BMC and BMD were not significant in comparison to control. However, the statistical analysis of correlation between BMC and age showed slightly accelerated bone loss in the patient group (Figure 1). This matches well with previous observations [21, 22]. Several recent studies have convincingly demonstrated that BMD measured in vertebral region and distal forearm was not significantly different in SLE compared to healthy subjects. However, a decrease in BMD was observed in femoral neck of SLE patients. Moreover, no correlation between BMD and the gcs dosage was found [22-24].

The levels of bone formation markers (BGP, AP, AP-B, and PIPC) were not significantly different in comparison to controls. However, the bone resorption was escalated. The levels of two (CTx, Ca/Crea) of three analyzed parameters were increased. It is in agreement with earlier studies, which confirmed a link between osteoporosis and increased bone resorption in SLE, while bone formation is only slightly decreased or even normal [22].

Levels of IL-6 and TNF- α were elevated in SLE, as it was previously described [3, 17, 25]. It has been suggested that serum TNF- α may be used as a marker of the SLE activity [26] and its performance is comparable to the SLEDAI (Systemic Lupus Erythematosus Disease

Active Index). We did not find any significant relationship between the decrease in BMC/BMD in our SLE group in relation to cytokine activity. Cytokine activity has an important impact on bone turnover. It is known that proinflammatory cytokines like TNF- α or IL-1 are the most potent stimuli of bone resorption, and that gcs strongly restrain the cytokine synthesis [27]. In SLE, the inhibitory effect of gcs treatment on activity of proinflammatory cytokines may counterweigh its deleterious impact on the osteoblast/osteoclast functions.

Our results suggest that osteoporosis does not seem to be a frequent complication of long-term gcs treatment in SLE despite the increased bone resorption. Thus, long-term gcs treatment appears safe with respect to bone metabolism in patients with SLE.

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