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Bone Loss in Iliac Biopsies: A Comparison Between Rheumatoid Arthritis and Postmenopausal Osteoporosis Using a Histomorphometric Study

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Summary. **To assess the mechanism that causes generalized osteoporosis in rheumatoid arthritis (RA) and to clarify the role of low-dose corticosteroid therapy, 41 postmenopausal women with RA underwent iliac biopsies after double-labeling with tetracycline. Twenty-two patients (group P) were treated with prednisolone (mean daily dose: 7.5±3.0 mg), and the remaining 19 patients (group N) were untreated. Using histomorphometry, parameters of bone status data in group N were compared with those in 28 age-matched Japanese postmenopausal women (controls) and group P. The bone volume, wall thickness (W. Th), and trabecular number in the controls correlated negatively with age, but those in group N did not. The mean value of trabecular thickness (Tb. Th) in the controls was constant, but that in group N decreased with age. The mean values of W. Th, osteoid thickness, and osteoblast surface were significantly lower, while mineral apposition rate was higher in group N than in the controls. Both resorption and formation periods tended to be prolonged. In group P, a decrease in the adjusted apposition rate and a prolongation of the formation period were noted, although both osteoclast and osteoblast surfaces were more extended than in group N. The cumulative dose of prednisolone significantly correlated with the decline in W. Th. One mechanism responsible for the generalized osteoporosis observed in RA patients without corticosteroid therapy is a reduction in bone formation, leading to an imbalance in the formation phase from the bone resorption phase. Corticosteroid therapy, even in low doses, suppresses osteoblast activity and causes a decline in W. Th; since trabeculae are already thinned or disconnected in patients with RA, corticosteroids can cause a progression of osteoporosis.**

Key words—histomorphometry, osteoporosis, rheumatoid arthritis, ilium, corticosteroids.

INTRODUCTION

Osteoporosis of the juxta-articular bone is a recognized complication of rheumatoid arthritis (RA) which occurs in the early stages of disease and is caused by local inflammatory factors[1,2]. A more generalized form of osteoporosis is also described in association with RA[3,4]. Whether the osteoporosis results from the disease itself[5,6] or from related factors such as corticosteroid therapy[7] or inactivity[8,9] is uncertain. An imbalance between bone resorption and formation results in osteoporosis, but data have been conflicting regarding the influence of these two processes in RA patients in whom osteoporosis has been attributed to increased bone resorption[10,11], decreased bone formation[12], or both[13].

Since Saville et al.[7] found that corticosteroids play a significant role in bone loss in RA, numerous studies have examined long-term prednisolone therapy in postmenopausal RA patients; in some studies, therapy was associated with the development of spinal osteoporosis[14,15], even at low doses, while others did not find any association[16-18].

The above studies regarding juxta-articular and generalized osteoporosis in RA patients used x-ray absorptiometry or static histomorphometric analysis.

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Table 1. Characteristics of the study groups*

	n	Age (mean±SD)	45 to 54 years	55 to 64 years	65 to 74 years
Rheumatoid arthritis	41	60±7	10	19	12
Group N	(19)	60±7	5	8	6
Group P	(22)	59±8	5	11	6
Age-matched controls	28	61±6	7	11	10
Recker's healthy controls	34	60±8	11	12	11

*There were no significant differences in age between study groups (Mann-Whitney U-test).

However, few reports have included dynamic parameters of histomorphometric analysis to evaluate generalized osteoporosis in RA patients as compared with age-matched controls.

It is postulated that generalized osteoporosis in patients with RA can be caused by both the disease itself and corticosteroid therapy. The present study examined this hypothesis by evaluating the mechanism of bone loss using histomorphometric analysis of the ilium after double-labeling with tetracycline in postmenopausal RA patients with and without corticosteroid therapy and in age-matched controls; the effects of low-dose corticosteroids over the long-term were assessed.

MATERIALS AND METHODS

This was a prospective study, patients with RA who fulfilled the 1987 revised criteria of the American College of Rheumatology[19] and had no history of anti-osteoporotic agents were informed of the possibility of generalized osteoporosis and the usefulness of histomorphometry when a first operation on the lower extremity was performed. Forty-one postmenopausal women with RA who gave written consent underwent an iliac crest bone biopsy. The average age at the time of the bone biopsy was 60±7 years (range, 46 to 74 years). Twenty-two patients (group P) had been treated with prednisolone (mean daily dose: 7.5±3.0 mg, mean duration: 9±6 years), and the remaining 19 patients (group N) had not. Twenty-eight Japanese postmenopausal women without osteopenia, at an average age of 61±6 years (range, 50 to 74 years) and who gave us written informed consent, also underwent an iliac crest bone biopsy when they were operated on their hip or lumbar (Table 1). All patients of the age-matched control group had no vertebral compression fracture. Patients with serum calcium, phosphorus, and/or alkaline phosphatase outside the normal range were excluded. Patients with a history of hyper- or hypo-function of the thyroid, parathyroid, adrenal, pituitary glands, or diabetes mellitus were also excluded. We compared the static and tetracycline-based bone histomorphometric data of our age-matched control women with the data from 34 healthy postmenopausal women reported by Recker et al[20].

Disease characteristics of the 41 RA patients between group N and P are shown in Table 2. Using the number of joints with erosion (NJE), Ochi et al.[21] described three groups, a subset with least erosive disease (LES), one with more erosive disease (MES), and one with mutilating disease (MUD). In the present study, we defined the LES group as NJE<20 and erosive articular changes primarily limited to peripheral smaller joints; the MES group was NJE>21 with larger axial joints involved; and the MUD group was NJE>46 with a typical opera-glass hand. There were no statistically significant differences in average duration from the onset of disease, body mass index, functional class, RA type described by Ochi et al., number of positive rheumatoid factor, or erythrocyte sedimentation rate (ESR) (Table 2).

Each subject was given in vivo double tetracycline labeling as follows: oral tetracycline hydrochloride (250 mg 4 times daily) for 3 days (label 1), followed by a 7 to 14-day drug-free interval, and then 3 additional days of oral tetracycline hydrochloride (250 mg 4 times daily) (label 2). From 7 to 14 days after the end of label 2, a transiliac bone biopsy was performed at a standard site approximately 2 cm posterior and inferior to the anterior-superior spine of the ilium, using a trephine of 8 mm inner diameter.

The specimens were fixed in 70% alcohol for processing without decalcification, and were immersed in Villanueva bone stain for 3 days. The specimens were dehydrated by sequential incubation in increasing concentrations of ethanol and acetone, and then embedded in methyl methacrylate. Frontal sections (5 μm) were cut with a Jung Model K microtome (Reichert-Jung, Heidelberg, Germany).

Table 2. Disease characteristics of the 41 rheumatoid arthritis patients taking prednisolone (group P) or not receiving therapy (group N)

	Group P (n=22)	Group N (n=19)	P-value
Age at biopsy (years)	58±8	60±7	ns
Disease duration (years)	13±9	16±9	ns
Body mass index	21.3±3.4	21.6±3.6	ns
Functional class			
2	1	2	
3	20	16	ns
4	1	1	
No. of joints with erosion			
LES	1	1	
MES	18	16	ns
MUD	3	2	
RF positive, no. (%)	82	79	ns
ESR (mm/hour)	56±31	65±30	ns
Dose of prednisolone (mg)	7.5±3.0		
Duration (years)	9±6		

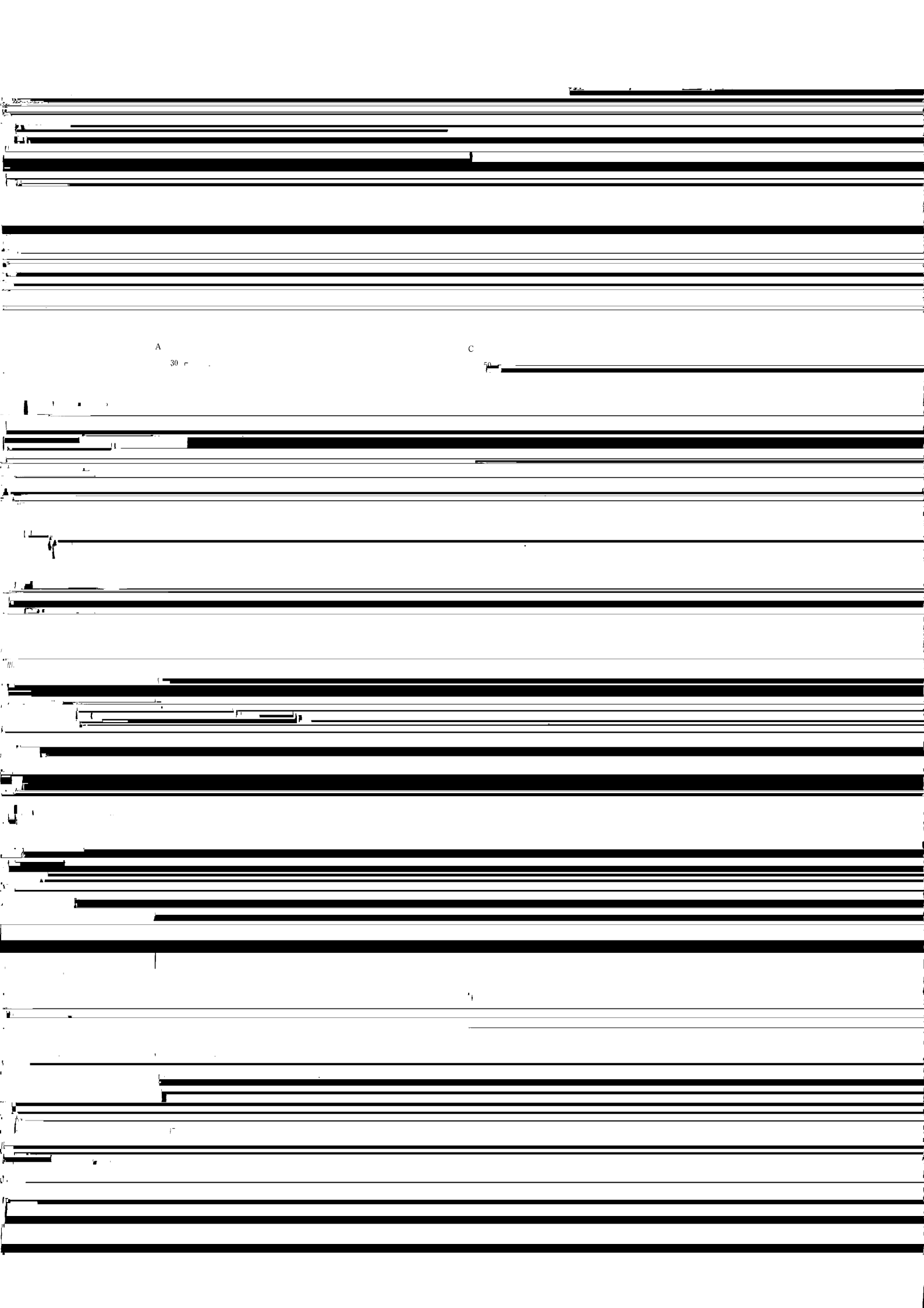
Values are the mean±SD. ns, not significant; Body mass index=body weight/(height)^2; LES, least erosive subset; MES, more erosive subset; MUD, mutilating disease; RF, rheumatoid factor; ESR, erythrocyte sedimenation rate (Westergren).

At least 15 adjacent fields were quantified at a magnification of ×160 in each section after processing by a semiautomatic digitizer (System Supply

rate (MAR) calculated as the distance between double labels divided by interval labeling time; bone formation rate per surface reference (BFR/BS) and

Table 2. [illegible]

Abbreviation	Unit	Terminology
Structural data		
W.Th	μm	Wall thickness
Tb.N	/mm	Trabecular number
Tb.S		Trabecular separation


A
30
C
50

	(28)	C vs. N	(19)	(22)	N vs. P
BV/TV (%)	14.38±3.92	0.1>p>0.05	12.06±5.03	10.93±3.92	ns
W. Th (μm)	34.01±3.58	p<0.01	30.76±3.54	29.12±4.95	ns
O. Th (μm)	8.43±2.02	p<0.05	6.97±2.04	6.76±1.79	ns
Tb. Th (μm)	140.7±22.3	0.1>p>0.05	124.4±34.3	107.4±27.4	0.1>p>0.05

there was no statistical significance. The FP value

tion of globally thinned trabeculae, rather than the disappearance of the trabelular elements. The resorption cavities of an abnormally shallow depth may be inadequately filled during the formation phase or cannot be filled because they remain in the reversal phase. This speculation is supported by the surface data in patients without corticosteroid therapy, where ES/BS was constant, but Oc. S/BS and Ob. S/BS were lower compared with the controls. On the other hand, the 5 cellular events at the site of bone remodeling can be detailed as follows: activation, resorption, reversal, formation, and quiescence. In the present study of patients without corticosteroid therapy, FP and Rs. P (including the resorption and reversal phases) tended to be more prolonged than our controls. The Rs. P value of group N was significantly longer compared with Recker's controls[20] (0.14 ± 0.14 years). The reversal period (Rv. P) was also calculated as FP $\times$ (ES-Oc. S)/OS. The Rv. P value of group N (0.26 ± 0.25 years) tended to be more prolonged than our controls (0.17 ± 0.08 years). We conclude that one of the mechanisms of generalized osteoporosis in RA patients without corticosteroids is reduced bone formation, which in addition causes an uncoupling of the formation phase from the bone resorption phase.

The effects of low-dose corticosteroids

Dempster et al.[26] reported a decline of W. Th in steroid-treated patients, but few studies have addressed the relationship between W. Th and other parameters of corticosteroid therapy. Correlation between age and the structural parameter in group N was not statistically significant, but the Tb. N in group P correlated negatively with age and the Tb. Sp in group P correlated positively with age. The W. Th in group P correlated negatively with the cumulative dose of prednisolone, and Tb. Th in group P correlated negatively with the mean daily dose of prednisolone. These findings suggest that declining W. Th, followed by reduced Tb. Th, may contribute to bone loss in patients receiving corticosteroids. Because Tb. Th declined, we believe that resorption cavities of either normal or deeper depth were inadequately filled during the formation phase. This speculation is supported by the surface data in group P, with no change in ES/BS and a decrease in Ob. S/BS, in spite of the maintained increase of Oc. S/BS compared with group N.

In the present study, MAR and Aj. AR in group P were significantly lower compared with group N. The decrease in Aj. AR caused a prolongation of FP. Ob. S/BS in group P was higher than that of group N. Although the population of osteoblasts was rich, a declining Aj. AR followed by a prolonged FP in group P was shown. These results suggest that individual osteoblast activity is suppressed by corticosteroids, even at low doses, when administered for a long period.

Corticosteroids reduce calcium absorption from the intestine and increase renal excretion. Ultimately, renal and intestinal losses of calcium result in secondary hyperparathyroidism, which bone resorption enhances. Bone histomorphometric studies have shown significant increases in the resorption surface[26-28]. In the current study, there was no significant difference in ES/BS between RA patients and controls, but Oc. S/BS in group P was significantly higher than in group N. However, fibrous tissue in the fields, as evidence of secondary hyperparathyroidism, was found in only 2 of our cases (data not shown).

In the present study, the value of N. Oc/TV ($0.31 \pm 0.40/mm^2$) in group P was significantly higher than that of group N ($0.10 \pm 0.15/mm^2$) and not significant compared with the age-matched controls ($0.19 \pm 0.17/mm^2$). Hahn et al.[29] demonstrated an increased N. Oc/TV ($0.34 \pm 0.12/mm^2$, mean $\pm$ SE) in patients who were taking prednisolone (mean daily dose: 17.3 mg). These results suggest that low-dose corticosteroids in RA lead to osteoclast recruitment, but the osteoclast count is not much higher than the normal range.

In RA and non-RA patients taking high-dose prednisolone (>20 mg/day), significant bone loss occurs, especially in the spine[30], and BFR/BV is reduced significantly in the iliac trabecular bone[31]. Assessing the net effects of low-dose corticosteroids on bone is difficult in RA because of the beneficial effects of therapy on disease activity[30] and physical activity. In conclusion, although low-dose corticosteroid therapy was not associated with decreased bone turnover, the negative balance caused by the combination of depressed osteoblast activity, stimulated osteoclast activity, and declining W. Th may cause a progression of osteoporosis in patients with RA. Corticosteroids have a direct effect on bone, inhibiting bone formation and enhancing bone resorption.

Finally, generalized osteoporosis was observed in RA patients with and without corticosteroid therapy; its characteristic finding was a decrease in the number of osteoblasts and/or suppression of osteoblast activity. In a prevention study of corticosteroid induced bone loss, the use of intermittent cyclical bisphosphonate therapy was effective[32-34]. However, for the treatment in osteoporosis with RA, the use of agents that enhance bone formation may lead to the development of new strategies.

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