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ORIGINAL ARTICLE

Shigeki Momohara · Hiroshi Okamoto · Toru Yago
Takefumi Furuya · Yuki Nanke · Shigeru Kotake
Makoto Soejima · Tamao Mizumura · Katsunori Ikari
Taisuke Tomatsu

The study of bone mineral density and bone turnover markers in postmenopausal women with active rheumatoid arthritis

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Abstract The aim of this study was to investigate determinants of reduced bone mineral density (BMD) in postmenopausal women with active rheumatoid arthritis (RA) and to evaluate whether there are common markers of bone loss. We evaluated BMD of the femoral neck using dual-energy X-ray absorptiometry, and the measured biochemical markers included serum bone-specific alkaline phosphatase (BALP), serum osteocalcin (OC), and serum cross-linked *N*-telopeptidases of type I collagen (NTx). Serum BALP and NTx concentrations were measured by enzyme-linked immunsorbent assay, and OC was measured using an immunoradiometric assay. One hundred and forty postmenopausal Japanese women who had not received treatment with bisphosphonates or hormone replacement therapy were entered into the study. Thirty-four patients (41.0%) had femoral osteopenia (T score –1 to –2.5) and 23 patients (27.7%) had osteoporosis ($T < -2.5$). The body mass index of patients with normal BMD (T score ≥ –1.0) was significantly higher ($P < 0.01$) than in patients with osteoporosis at the femoral neck. The T score exhibited a significant negative correlation with age and the duration of RA disease. Serum BALP and serum OC, markers of osteoblast function, were negatively related to erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and matrix metalloproteinase-3 (MMP-3). However, serum NTx, a marker of resorptive function, exhibited a positive correlation with ESR, CRP, and MMP-3. From these results, this study suggests that generalized bone loss occurs in active RA and is characterized by evidence of bone resorption that is correlated with the high levels of inflammation. Body mass index, disease duration, and high serum NTx level were common risk factors in osteoporosis of postmenopausal women with RA.

Key words Body mass index (BMI) · Bone mineral density (BMD) · Bone turnover markers · Osteoporosis · Rheumatoid arthritis (RA)

S. Momohara (✉) · T. Mizumura · K. Ikari · T. Tomatsu
Department of Orthopedic Surgery, Institute of Rheumatology, Tokyo Women's Medical University, 10-22 Kawada-cho, Shinjuku-ku, Tokyo 162-0054, Japan
Tel. +81-3-5269-1711; Fax +81-3-5269-1726
e-mail: smomohara@ior.twmu.ac.jp

H. Okamoto · T. Yago · T. Furuya · Y. Nanke · S. Kotake · M. Soejima
Department of Internal Medicine, Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan

Introduction

Osteoporosis is a frequent and serious complication of rheumatoid arthritis (RA). Systemic osteoporosis is a well-recognized complication of RA and decreased bone mineral density (BMD) has been demonstrated in several studies.[1,2] Consequently, affected individuals suffer an increased risk of fractures, with fracture of the proximal and distal femur being increased in patients with RA.[2,3] Insufficiency fractures in RA may also produce severe problems and may be underdiagnosed. In addition, it is likely that they are more frequent than commonly thought, especially in the lower limbs.[4] Various pathogenetic mechanisms contribute to systemic bone loss associated with RA.[1] The chronic inflammation associated with RA has been shown to be an important risk factor in the development of systemic osteoporosis.[5] However, the mediator mechanisms and the link between the local arthritic process and systemic bone loss are still unclear.

Moreover, RA sometimes requires long-term treatment with glucocorticoids that may lead to many well-known adverse events. Of all of the potentially serious side effects, glucocorticoid-induced osteoporosis is one of the most devastating complications of protracted glucocorticoids therapy in RA.[6] The quantitative assessment of periarticular and generalized bone loss in RA may be a reliable indicator of the future disease course and potential response in intervention studies.[7] Therefore, some studies have already shown the correlation of urinary pyridinoline (PYD) and deoxypyridinoline (DPD) levels with RA-related bone loss.[8]

Consequent to the clinical situation addressed above, the first aim of this study was to evaluate the frequency of osteoporosis in postmenopausal women with active RA. The second aim was to investigate whether there are relationships between inflammation and bone-turnover markers in postmenopausal patients with RA that might be of particular importance regarding the pathogenesis of osteoporosis in RA. Markers of bone formation include osteocalcin (OC), bone-specific alkaline phosphatase (BALP), and procollagen peptides. Bone resorption markers include tartrate-resistant acid phosphatase (TRAP) and collagen breakdown products, such as pyridinium cross-links, galactosyl hydroxylysine, and cross-linked telopeptides, such as type I collagen cross-linked *N*-telopeptides (NTx). Of these markers, DPD shows diurnal variation and the within-individual biological variation is large. Thus, serum BALP, OC, and NTx are the current choice of bone markers in this study.

Patients and methods

All patients were postmenopausal Japanese women and fulfilled the American College of Rheumatology criteria for RA[9] with RA disease activity that met at least two of the following criteria: more than six painful joints; more than three swollen joints; erythrocyte sedimentation rate (ESR) >20.0 mm/1st h; and/or C reactive protein (CRP) >1.0 mg/dl. None of the patients had used drugs affecting bone metabolism (bisphosphonates or HRT) in the past 2 years, although the use of calcium, vitamin D3, and vitamin K2 was allowed. Only patients who were not receiving glucocorticoids or who were on a stable dose of prednisolone (<10 mg daily) were included in the study. There were 140 patients in the study with a mean age of 64.3 ± 8.7 years (range, 48–89 years) and a mean duration of RA disease of 14.4 ± 8.0 years (range, 2.3–38.5 years).

We evaluated bone mineral density (BMD) using dual-energy X-ray absorptiometry (DEXA) with EXPERT-XL DEXA Images device (GE_LUNAR, Madison, WI, USA). Bone mineral density at the femoral neck was measured in 83 patients because the lumber spine of many of these patients might have severe spondylotic changes. Bone mineral density of all patients could not be measured in this study. Osteoporosis was defined according to the WHO (World Health Organization, 1994) as a value for BMD.[10] The BMD of a patient is expressed as a *T* score (the difference in standard deviation [SD] compared with peak bone mass in a young adult of the same race and sex) or a *Z* score (the difference in SD compared with healthy age-matched controls of the same race and sex). Persons with a BMD > 1 SD below the mean in young adults (i.e., *T* score < –1) are considered to have low BMD whilst those with a BMD > 2.5 SD below the mean in young adults (i.e., *T* score < –2.5) are considered to have osteoporosis.[11]

The measured biochemical markers included serum BALP, OC, and serum NTx. Serum BALP and NTx concentrations were measured by enzyme-linked immunosorbent assay (ELISA), and OC was measured using an immunoradiometric assay (IRMA). At the same time, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), and matrix metalloproteinase-3 (MMP-3) levels were also measured. Erythrocyte sedimentation rate and CRP were measured by standard laboratory techniques. Rheumatoid factor was detected by latex fixation test whilst IgG-RF and MMP-3 were measured by ELISA. The levels of BALP, OC, and NTx were compared with the CRP, ESR, RF, and MMP-3 levels.

Statistical analysis

Data were analyzed using the StatView for Windows Statistical Program. For correlation analysis, we used the Spearman correlation coefficient. The Mann–Whitney *U*-test was used to compare the parameters of the various patient groups. *P* values of less than 0.05 were considered statistically significant.

Results

Bone mineral density

A reduced bone mass (*Z* score ≤1.0 SD) was found in at least one site of the hip joint in 31.3% of these patients (n = 26). Thirty-four patients (41.0%) had femoral osteopenia (*T* score –1 to –2.5) and 23 patients (27.7%) had osteoporosis (*T* < –2.5). We also analyzed the relationship between BMD and body mass index (BMI). The BMI of patients with a normal BMD (*T* score ≥ –1.0) was significantly higher ($P < 0.01$) than in patients with osteoporosis at the femoral neck (Fig. 1).

The *T* score exhibited a significant correlation with age and RA disease duration whilst the *Z* score exhibited a significant correlation with RA disease duration, and no correlation was found between those scores and the markers of bone turnover (Table 1).

Table 1. Correlation coefficients (r) obtained by regression analyses of *Z* and *T* scores and disease-related factors and bone turnover markers (n = 83)

	T score		*Z* score	
	r	*P*	*r*	*P*
Age (years)	–0.505	<0.0001	–0.142	0.239
Disease duration (years)	–0.296	0.012	–0.266	0.024
BALP (U/l)	–0.223	0.101	–0.173	0.207
OC (ng/ml)	–0.106	0.48	–0.043	0.773
NTx (U/l)	–0.115	0.34	–0.031	0.796

The *T* score exhibited a significant correlation with age and rheumatoid arthritis (RA) disease duration whilst the *Z* score exhibited a significant correlation with RA disease duration. No correlation was found between those scores and the markers of bone turnover

BALP, bone-specific alkaline phosphatase; OC, osteocalcin; NTx, cross-linked *N*-telopeptidases of type 1 collagen

Table 2. Age distribution of serum BALP, serum OC, and serum NTx

Age (years)	BALP (U/l)	OC (ng/ml)	NTx (U/l)		*P* value[a]
<50	23.03 ± 9.60	4.03 ± 1.96	14.29 ± 3.40	*n* = 13	
50–60	26.29 ± 9.48	4.13 ± 1.95	13.76 ± 4.17	*n* = 42	n.s.
60–70	25.53 ± 7.60	4.73 ± 2.34	16.57 ± 6.84	*n* = 51	n.s.
70–80	28.53 ± 8.72	6.18 ± 2.74	18.06 ± 7.11	*n* = 28	n.s.
>80	26.52 ± 10.48	5.25 ± 0.95	13.17 ± 2.34	*n* = 6	n.s.

n.s., not significant
[a] With respect to each bone marker level in patients younger than 50 years of age

Table 3. Regression analyses of bone turnover markers and disease-related markers (*n* = 140)

	BALP	OC	NTx
ESR	0.029	0.138	0.286**
CRP	0.122	–0.027	0.284**
MMP-3	0.006	0.022	0.214*
RF	–0.058	0.023	–0.072
IgG-RF	–0.105	0.008	0.058

The markers of osteoblastic function OC and BALP exhibited a negative relationship with ESR, CRP, RF, IgG-RF, and MMP-3, whereas the marker of resorptive function NTx exhibited a positive correlation with ESR, CRP, and MMP-3. Assessment of the relationship between RF and IgG-RF and all bone markers including NTx indicated no significant correlation
ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; RF, rheumatoid factor; MMP-3, matrix metalloproteinase-3
* $P < 0.05$, ** $P < 0.01$

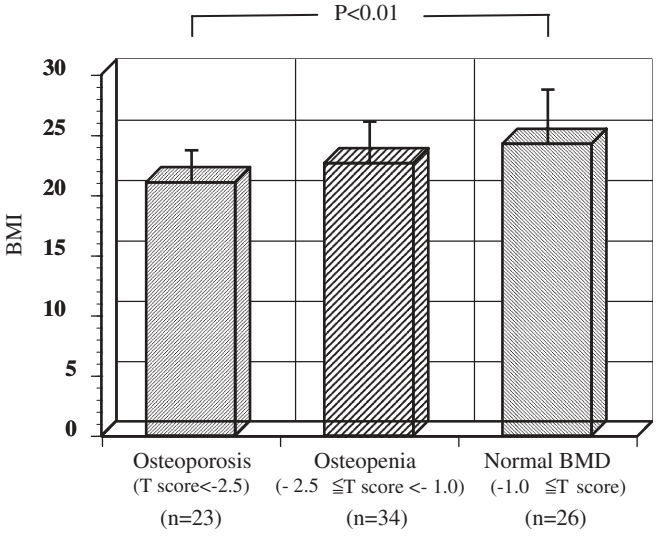

Fig. 1. The relationship between bone mineral density (*BMD*) and body mass index (*BMI*). The BMI of patients with a normal BMD (*T* score > –1.0) was significantly higher ($P < 0.01$) than in patients with osteoporosis at the femoral neck

Bone turnover markers

The levels of all the various bone markers measured did not correlate with age or the duration of RA disease ($P > 0.05$). The relationship between age and each bone turnover marker is shown in Table 2. There was no difference in serum BALP, OC, or NTx levels according to age distribution (Table 2). The markers of osteoblast function BALP and OC exhibited no relationship to ESR, CRP, RF, IgG-RF, and MMP-3 whereas the marker of resorptive function NTx exhibited a positive correlation with ESR, CRP, and MMP-3 (Table 3). Specifically, NTx levels correlated with ESR ($r = 0.286$, $P = 0.0006$) (Fig. 2), CRP ($r = 0.284$, $P = 0.0006$) (Fig. 3), and MMP-3 levels ($r = 0.214$, $P = 0.0144$) (Fig. 4). However, assessment of the relationship between RF and IgG-RF and all bone markers including NTx indicated no significant correlation (Table 3). We then analyzed the correlation between daily prednisolone (PSL) dosage and bone turnover markers. Although there was no correlation between BALP, NTx, and daily PSL dosage, there was a negative correlation between OC and the daily PSL dosage ($r = –0.228$, $P = 0.0366$).

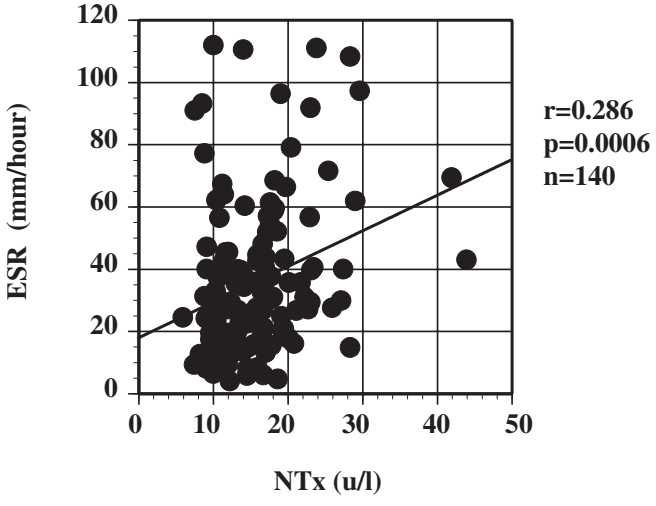

Fig. 2. Correlations between serum cross-linked *N*-telopeptidases of type 1 collagen (*NTx*) and erythrocyte sedimentation rate (*ESR*) in postmenopausal women with rheumatoid arthritis (RA)

Discussion

Osteoporosis is common in postmenopausal women with RA. Recently it was shown that the presence of joint erosions in patients with RA was associated with generalized

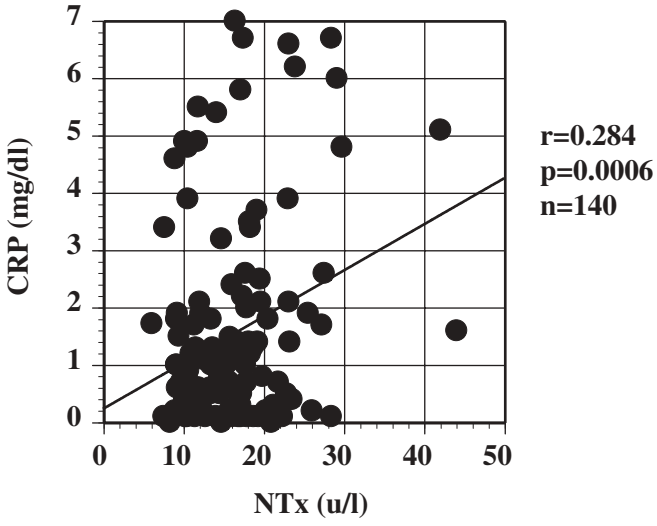

Fig. 3. Correlations between serum NTx and C-reactive protein (*CRP*) in postmenopausal women with RA

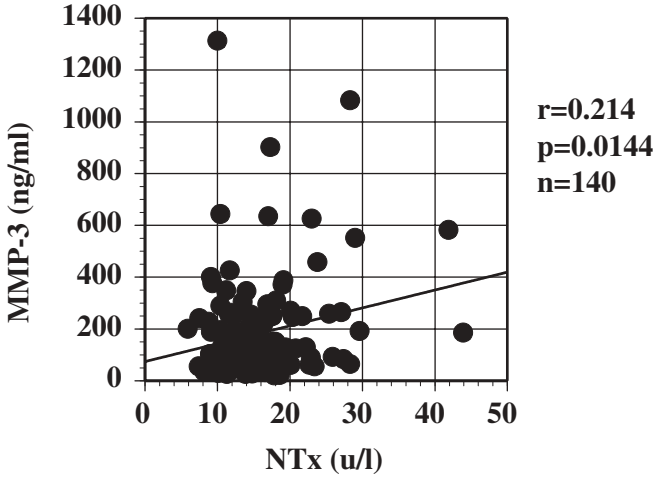

Fig. 4. Correlation between serum NTx and matrix metalloproteinase-3 (*MMP-3*) in postmenopausal women with RA

osteoporosis.[12] Concerning postmenopausal Japanese women with active RA in our study, 34 patients (41.0%) had femoral osteopenia and 23 (27.7%) had osteoporosis. Thus, about 70% of postmenopausal women with RA who had received no previous treatment with HRT or bisphosphonates exhibited osteopenia or osteoporosis. However, age increased the risk for osteoporosis whilst BMI demonstrated a beneficial effect upon bone density. A low body weight is a potent risk factor for osteoporosis,[13,14] and is associated with a reduced BMD[12,15] and fractures in RA.[16] According to our data, the BMI of patients with a normal BMD was significantly higher ($P < 0.01$) than in patients with osteoporosis at the femoral neck. Therefore, we are of the opinion that low body weight is related to reduced bone mineral density in RA.

Forsblad D'Elia et al.[17] reported that increased CRP levels and a long duration of disease were determinants of erosive disease in postmenopausal women with RA. Orstavik et al.[18] described that the occurrence of vertebral deformities was independently associated with age and long-term glucocorticoid use as well as reduced BMD, and that there was a consistent relationship between BMD and vertebral deformities. In our study, the *T* score correlated significantly with age and RA disease duration. Therefore, age and disease duration appear to be important factors in the pathogenesis of osteoporosis in patients with RA.

The evaluation of levels of bone markers can provide valuable information regarding bone turnover in RA. We initially studied the serum levels of BALP, OC, and NTx according to the age distribution in postmenopausal women. As shown in Table 2, there was no significant difference among these patients.

Seriolo et al.[19] reported that serum OC levels were significantly lower ($P < 0.001$) in patients with active RA compared with patients without active RA and controls. Those data suggested that generalized bone loss occurred in active RA and was characterized by evident bone resorption that correlated with elevated levels of inflammatory markers.[19] Cortet et al.[20] also reported that glucocorticoid-treated elderly patients with RA with severe disease and high OC levels have marked osteoporosis at the hip. However, from our data there is no significant correlation between serum OC and BALP levels and markers of RA activity such as MMP-3 in postmenopausal women with RA. Therefore, we suggest that markers of osteoblastic bone formation cannot indicate RA disease activity.

On the other hand, Gough et al.[5] demonstrated that biochemical markers of bone resorption may be raised and correlated with the disease activity in RA.[21] Spot urine concentrations of NTx and DPD were significantly higher ($P < 0.01$) in active RA patients than in patients with nonactive RA and controls.[19] Correlation between the change in serum NTx and worsening of the erosion score provides biochemical evidence that osteoclast is the principal cell type responsible for focal bone resorption in RA.[22] In our study, we also found that high disease activity in postmenopausal women with RA was associated with an increase in bone resorption, as reflected by the positive significant correlation between parameters of disease activity (CRP and ESR) and NTx. Moreover, there was positive correlation between MMP-3 and NTx. Therefore, our data indicate that NTx is a critical determinant of increased bone resorption in postmenopausal RA women with high disease activity. In fact these results suggest that generalized bone loss occurs in active RA and is characterized by an evident bone resorption correlated with the high levels of inflammation.

The use of glucocorticoids and their influence on BMD is widely debated. Most cross-sectional studies agree that the cumulative dose of PSL is an important determinant of osteopenia in RA.[12,23,24] However, longitudinal studies have given inconclusive results.[25,26] Forsblad d'Elia et al.[17] demonstrated the importance of considering the intra-articular and intramuscular cumulative dose of glucocorticoids because there was a negative correlation between the total amount of injected glucocorticoids and bone mass of the forearm, total hip, and femoral neck. In our study, only

serum OC exhibited a negative weak correlation with the daily PSL dosage, suggesting that PSL may influence bone formation. However, there was no correlation between BALP, NTx, and the daily PSL dosage. Osteocalcin undergoes in vitro degradation, and assay results are sometimes variable. Therefore, further research is necessary on whether the daily PSL dosage may influence the serum level of OC.

In summary, in this study of 140 postmenopausal women with active RA, 34 patients (41.0%) had femoral osteopenia whilst 23 patients (27.7%) had osteoporosis. Common risk factors in RA osteoporosis included BMI, disease duration, the daily PSL dosage, laboratory indices of inflammation, and serum NTx. This study suggests that generalized bone loss occurs in active RA and is characterized by evidence of bone resorption that is correlated with the high levels of inflammation. We suggest that the more aggressive treatment of RA in the future will not only prevent joint destruction and inflammation but may well reduce the incidence of osteoporosis and its untoward consequences.

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