

Ankylosing Spondylitis, Psoriatic Arthritis, and Reactive Arthritis Show Increased Bone Resorption, But Differ with Regard to Bone Formation

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ABSTRACT. Objective. To test if markers of bone metabolism are altered in patients with seronegative spondyloarthropathies (SSpA).

Methods. We studied biochemical markers of bone resorption and bone formation, osteoprotegerin (OPG), and bone mineral density (BMD) in patients with psoriatic arthritis (PsA), ankylosing spondylitis (AS), and reactive arthritis (ReA) and healthy volunteers.

Results. The bone resorption markers urinary deoxypyridinoline and crosslinked telopeptide of collagen-I were significantly increased in patients with AS, PsA, and ReA; in PsA they correlated with the acute phase response (C-reactive protein and erythrocyte sedimentation rate). The bone formation markers were divergent: bone-specific alkaline phosphatase was increased in PsA, but not in AS or ReA. Osteocalcin levels were only elevated in AS. Serum levels of OPG were significantly increased in both AS and PsA. Dual energy x-ray absorptiometry (DEXA) measurements of lumbar spine and femoral neck revealed osteopenia in patients with AS, whereas the DEXA distribution was within normal range in PsA.

Conclusion. Our data indicate high and, particularly in AS, unbalanced bone turnover in SSpA, consistent with the decrease in BMD found in patients with AS. (J Rheumatol 2002;29:1430–6)

Key Indexing Terms:

SERONEGATIVE SPONDYLOARTHROPATHIES
PSORIATIC ARTHRITIS
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Rheumatic diseases are known to influence bone metabolism. In rheumatoid arthritis (RA) in particular, bone loss

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and the development of osteoporosis are well established¹⁻⁴. Mediators of inflammation, such as prostaglandins and interleukin 1 (IL-1) and IL-6, are known to influence bone metabolism⁵⁻⁷. In addition, the production of metalloproteinases by macrophages and bone cells leads to enhanced bone resorption and a high bone turnover status⁸. Moreover, inactivity and drugs such as corticosteroids or cyclosporin A may induce bone loss^{9,10}.

The consequences of seronegative spondyloarthropathies (SSpA) on bone metabolism are not yet as clear. These diseases, which among others comprise psoriatic arthritis (PsA), ankylosing spondylitis (AS), and reactive arthritis (ReA), differ from RA in their genetic associations, in the involvement of the sacroiliac joints, and the absence of rheumatoid factor. Moreover, their main focus of inflammation often appears to be the entheses rather than the synovium¹¹⁻¹⁴. In addition they are characterized by new bone formation^{15,16}.

Vertebral fractures are a recognized complication of advanced AS^{17,18}, with roughly a 7-fold increase of risk¹⁹. Quantitative computed tomography is reported to reveal osteopenia of vertebral bodies in advanced AS^{20,21}. Osteopenia and loss of bone mass in the axial skeleton and the hip have also been observed in early stages of AS using dual energy x-ray absorptiometry (DEXA)^{22,23}. In spite of

these findings, studies on markers of bone metabolism are scarce and conflicting^{24,25}. Therefore, both the status of skeletal remodeling and the relation with disease activity remain unclear in AS. In patients with peripheral PsA, no significant bone loss was found in clinical studies²⁶, whereas bone biopsies suggested a latent high turnover osteopathy²⁷. For ReA, no data on potential bone loss are available.

We investigated variables of bone resorption and bone formation in patients with AS, PsA, and ReA, compared to healthy controls. For bone formation, the bone matrix protein osteocalcin (OC) and the bone-specific isoenzyme of alkaline phosphatase (bone ALP) were measured²⁸⁻³⁰. For bone resorption, the crosslinked telopeptide of collagen-I (CTX-I) and urinary deoxypyridinoline (DPD) were measured³¹⁻³³. All 4 markers have been found to correlate with measures of bone formation or resorption, as also assessed by bone histomorphometry^{34,35}.

In addition, we analyzed osteoprotegerin (OPG). OPG is a member of the tumor necrosis factor (TNF) receptor family, which, as a decoy receptor for RANK ligand (RANKL = OPGL), inhibits osteoclast differentiation and activation^{36,37}. OPG is mainly secreted by osteoblasts³⁸, whereas RANKL is a surface receptor, primarily expressed by T cells and bone marrow cells and prominently involved in osteoclastogenesis³⁹.

We will show that increased bone resorption is a constant finding in AS as well as in PsA and ReA. In contrast, SSpA differ with regard to markers of bone formation. In addition, we report on OPG in all 3 diseases.

MATERIALS AND METHODS

Patient characteristics. We investigated 30 patients (22 men/8 women, age range 24–65 yrs) with AS as defined by the New York criteria⁴⁰; 23 patients (17 men/6 women, age range 18–68 yrs) with PsA as defined by the European Spondylarthropathy Study Group⁴¹; and 10 patients (5 men/5 women, age range 31–70 yrs) with ReA as defined by the Amor criteria⁴². Disease duration was 1.3 ± 0.9 years for ReA, 10.4 ± 9.3 years for PsA, and 9.2 ± 8.5 years for patients with AS. The control group consisted of 41 healthy volunteers (25 men/16 women, mean age 41.7 ± 11.1 yrs, range 20–66 yrs). Plasma and serum samples were prepared according to standard protocols and immediately analyzed or frozen and stored at -70°C until they were analyzed by ELISA or radioimmunoassay (RIA).

Assessment of C-reactive protein (CRP) showed significant elevations in all patient groups (ReA 1.1 ± 0.5 mg/dl, PsA 1.3 ± 1.2 mg/dl, AS 2.8 ± 2.5 mg/dl vs < 0.50 mg/dl in the control group), reflecting mild to moderate disease activity. In one patient with PsA mild elevations of ALAT and γ -glutamyl transferase were detected. In all other patients and controls routine blood measures were within the normal range. No differences in serum levels of calcium, phosphorus, parathyroid hormone, or 25-OH vitamin D could be found for any patient cohort compared to sex and age matched controls. Levels of thyroid stimulating hormone, luteinizing hormone, follicle stimulating hormone, progesterone, and testosterone were within normal limits for the respective ages.

One patient with ReA, 2 with AS, and 3 with PsA were receiving low dose (≤ 10 mg/day) glucocorticoid therapy. These patients were not significantly different in any variable measured.

Markers of bone formation. Serum levels of OC and bone ALP were determined by immunoradiometric assay (IRMA) (CIS Bio International, Gif-sur-Yvette, France; Hybritech, San Diego, CA, USA) according to the

manufacturers' recommendations. Intraassay variations were 3.8% for OC and 6.7% for bone ALP, interassay variations 5.2% for OC and 8.1% for bone ALP.

Markers of bone resorption. Second morning urine was immediately frozen at -70°C . Samples were tested for DPD excretion by enzyme ELISA using the Pylinks®-D kit (Metra Biosystems Inc., Mountain View, CA, USA; within-run precision 4.8%, between-run precision 8.4%). Serum levels of CTX-I were measured by RIA (Orion Diagnostica, Espoo, Finland; within-run precision 6.2%, between-run precision 7.9%).

Osteoprotegerin. OPG serum levels were detected in random subgroups of 13 AS, 20 PsA, and 10 ReA patients as well as 32 controls using a commercial ELISA (Biomedica, Vienna, Austria) that detects both free and bound OPG. The detection limit was 2.7 pg/ml and recombinant human OPG was used as the standard. The relative specificity of monomeric vs dimeric forms of OPG has not been determined. The identical assay sold by another company was recently described⁴³. Inter- and intraassay variations were $\leq 10.0\%$.

Statistical evaluation. Statistical calculations were performed using SPSS® for Windows v 8.0 (SPSS Inc.). Data were evaluated by normality test, equal variance test, and Student t test (with and without variance correction). Data not normally distributed were analyzed using nonparametric methods. All data are given as mean \pm SD, unless otherwise indicated.

Bone mineral measurements. DEXA measurements for the lumbar spine and the nondominant hip were performed in 17 AS and 19 PsA patients on a Hologic QDR 4500 densitometer. Values reported are z scores (standard deviations for age and sex matched normal individuals).

RESULTS

As shown in Table 1, the biochemical markers of bone resorption were significantly increased in patients with AS, PsA, and ReA compared to controls. This was true for serum CTX-I levels as well as the urinary excretion of DPD (Table 1). Moreover, unlike in AS, both markers were significantly correlated with inflammation measures in PsA [erythrocyte sedimentation rate (ESR) for CTX-I and CRP; Figure 1] and ReA (ESR only; Figure 2), suggesting an association of increased bone turnover with the inflammatory activity of joint disease. CTX-I was positively correlated with disease duration in patients with PsA ($r = 0.670$, $p = 0.009$), but not in patients with other diseases (data not shown). No other biochemical marker was significantly associated with disease duration (data not shown).

With regard to bone formation, the situation was not as clear. In AS, OC but not bone ALP levels were elevated (Table 1). In the PsA group, a significant increase in bone ALP but not OC was noted. Neither measure was abnormal in patients with ReA. In contrast to markers of bone resorption (see above), no correlations between CRP or ESR and bone formation markers were observed.

Interestingly, as shown in Table 1, the serum levels of OPG were increased in patients with AS (44.3 ± 19.7 ng/ml; $p = 0.046$), PsA (47.4 ± 22.6 ng/ml; $p = 0.011$), and ReA (45.3 ± 23.2 ng/ml; $p = 0.056$) compared to controls (35.2 ± 10.0 ng/ml). No significant correlation between OPG and markers of inflammation was observed (data not shown).

DEXA measurements were in accord with previously published data^{44,45}: patients with AS had reduced bone

Table 1. Biochemical markers of bone metabolism in healthy controls and patients with AS, PsA, and ReA.

	HC, n = 41	AS, n = 30	PsA, n = 23	ReA, n = 10
Age, yrs	41.7 ± 11.1	44.2 ± 12.7	45.2 ± 12.2	47.8 ± 11.7
ESR, mg/dl	ND	34 ± 25	21 ± 26 [†]	20 ± 15
CRP, mg/dl	< 0.5	2.8 ± 2.5	1.3 ± 1.2 [†]	1.1 ± 0.5
Bone resorption				
CTX-I, ng/ml	3.3 ± 0.7	4.7 ± 1.6***	4.8 ± 1.8***	4.8 ± 4.1**
DPD, nmol/mmol creatinine	4.3 ± 1.5	6.4 ± 2.7**	6.2 ± 2.2**	8.0 ± 3.4*
Bone formation				
Bone ALP, ng/ml	10.1 ± 3.5	11.0 ± 4.5	12.5 ± 4.6*	9.1 ± 3.3
OC, ng/ml	17.8 ± 5.5	22.0 ± 4.5*	20.7 ± 7.3	16.8 ± 3.4
OPG ^{††} , ng/ml	35.2 ± 10.0	44.3 ± 19.7*	47.4 ± 22.6*	45.3 ± 23.2

Bone ALP: bone-specific isoenzyme of alkaline phosphatase, OC: osteocalcin, CTX-I: crosslinked telopeptide of collagen-I, DPD: urinary deoxypyridinoline, OPG: osteoprotegerin, ND: not determined.

* p < 0.05 patients vs controls, ** p < 0.003 patients vs controls, *** p < 0.0001 patients vs controls. † Not normally distributed values, Kruskal-Wallis test used for comparisons. †† For OPG n = 32, 13, 20, and 10 for controls, AS, PsA, and ReA, respectively.

mineral density (BMD) values at the femoral neck compared to age matched controls (defining the z score) (Figure 3). The respective t scores indicated a high prevalence of osteopenia (lumbar spine -0.99 ± 1.33 , femoral neck -1.95 ± 1.33): 47% were osteoporotic (t score less than -2.5). However, the lumbar spine BMD values were not significantly diminished, which may partly be due to concomitant ankylosis of the lumbar spine. Patients with PsA had normal bone densities at both sites, which is also reflected by a significant difference between the left hip z scores of patients with AS and PsA ($p = 0.0012$, Student t test).

DISCUSSION

We examined bone formation and bone resorption data in patients with AS, PsA, and ReA by measuring biochemical markers. All the measurement variables have been shown to be representative of bone resorption or formation²⁸⁻³². Such markers are also clearly associated with bone loss in a variety of diseases^{46,47}.

Biomarkers are known to be reflective of both systemic⁴⁸ and local⁴⁹ alterations of bone metabolism. SSsPA are, however, systemic rather than local diseases. Moreover, in contrast to RA, evidence of significant local bone loss around afflicted joints is relatively uncommon. We would therefore argue that our findings are probably reflective of systemic bone metabolism.

For all 3 types of SSsPA investigated, there is clear biochemical evidence of increased bone resorption by virtue of the uniformity of elevated serum CTX-I and urine DPD levels. Given that our results indicate increased resorption in all 3 diseases, one might expect a significant loss in bone density. In addition, one should find some correlation with disease activity if the bone loss were to be associated with the disease itself. Indeed, bone loss has been found in

studies on AS⁴⁴ and was observed at the femoral neck in our patients with AS. In contrast, no bone loss was found at the lumbar spine, but this may constitute a false negative finding due to the new bone formation of ankylosis.

As indicated by the term CTX-MMP⁵⁰, metalloproteinases may efficiently generate CTX-I⁵¹. Since such metalloproteinases are overexpressed in chronic arthritis⁵², the serum CTX-I elevation we observed could be caused by increased bone resorption and/or increased disease induced metalloproteinase production. In this regard, it is interesting that CTX-I is increased in PsA, whereas evidence of bone loss in PsA cannot be found in the literature or in our data. Increased bone resorption in this disease is, however, also suggested by an increase in DPD, which is not known to be influenced by metalloproteinases.

Given the pattern of joint involvement in this disease, a false negative result with the DEXA measurements is also rather unlikely. Thus, one might assume that new bone formation occurs as a balance to bone resorption. Indeed, the increase of bone ALP in PsA seen in this study suggests such new bone formation, since other common reasons for the increase of bone ALP, such as hypovitaminosis D, could be excluded. In AS, where the data on osteoporosis²² and our DEXA data show significant diffuse bone loss, we found no significant increase in bone ALP. In contrast, the OC levels were clearly increased in this disease and one might be tempted to speculate that the increased OC levels we observed are the consequence of the continuing bone formation of ankylosis.

In ReA, there is only evidence of increased bone resorption, but not of increased bone formation. However, ReA is not known to be commonly associated with significant bone loss. Here, one might infer the short duration of this mostly self-limiting disease. Such difference due to a difference in disease duration could also influence bone metabolism as

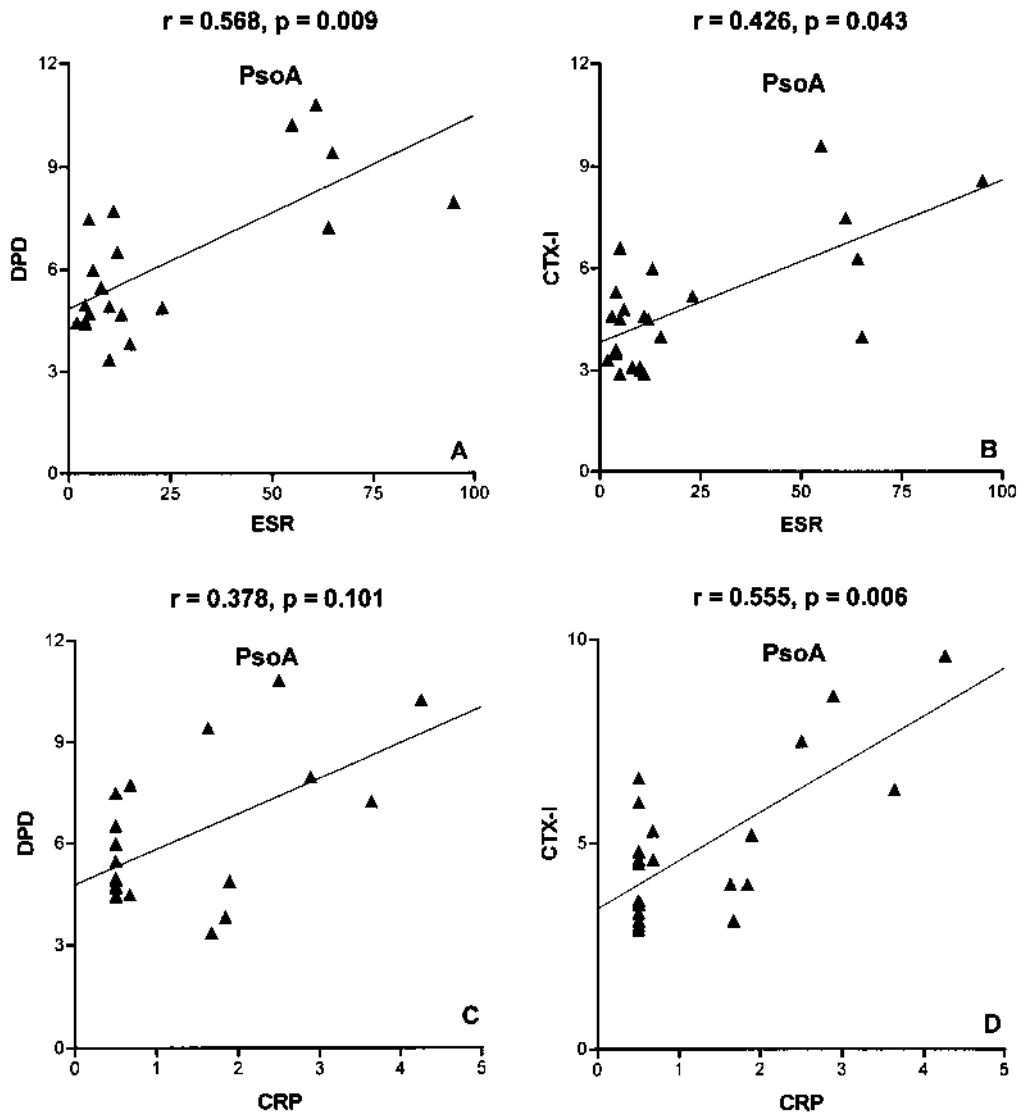


Figure 1. Markers of inflammation correlate with bone resorption measures in PsA. Each triangle represents a single patient with PsA. ESR (A, B) and CRP (C, D) are depicted on the x-axis, DPD (A, C) and CTX-I (B, D) on the y-axis. Trend lines are derived from Pearson correlation coefficients.

revealed by the markers used in this study. Indeed, CTX-I data were positively associated with disease duration, at least in PsA. In contrast, we could not detect such an association with any other marker in PsA, AS, or ReA. Moreover, the effect on CTX-I remained limited to PsA. Thus, in addition to disease duration, differences in the cytokine milieu might play a significant role.

It is also interesting that the acute phase response correlates with increased bone resorption in PsA, since this is similar to findings in RA^{4,53}. Unlike in PsA (and also RA), there is no such association in AS. However, this may be because the acute phase response does not seem to be a good marker of AS disease activity⁵⁴.

The elevations of OPG in patients with AS and PsA we observed have not been reported before. Such increase

might represent the reaction of the osteoblast-stromal cell to enhanced bone resorption. Alternatively, the OPG elevation could also be associated with the altered ossification of the spine reported in AS, but this does not explain its elevation in PsA^{55,56}. Thus it is likely that OPG is upregulated by TNF⁵⁷, which is involved in AS and PsA as seen in *in vitro* studies^{58,59}, and by the clinical success of TNF blocking therapies^{60,61}.

It is evident that OPG is one partner within a complex system, which otherwise mainly involves membrane bound receptors, namely RANK and RANKL. Thus, interpretation of our findings has to be cautious. At present, however, OPG is the only soluble component within the RANK-RANKL-OPG system that can be determined from patient serum, and OPG may well reflect a consequence of a general overac-

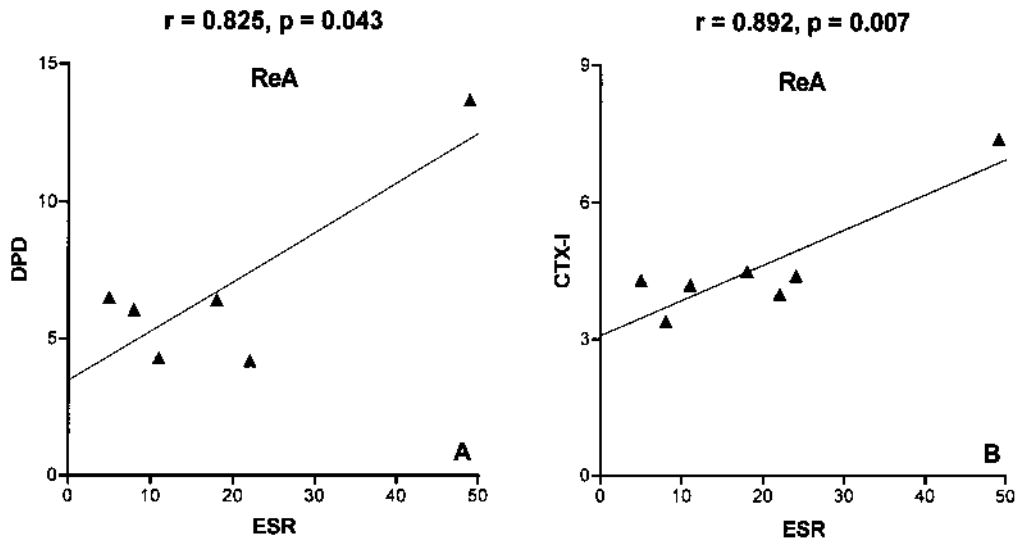


Figure 2. ESR correlates with CTX-I and DPD in ReA. Each triangle represents a single patient with ReA.

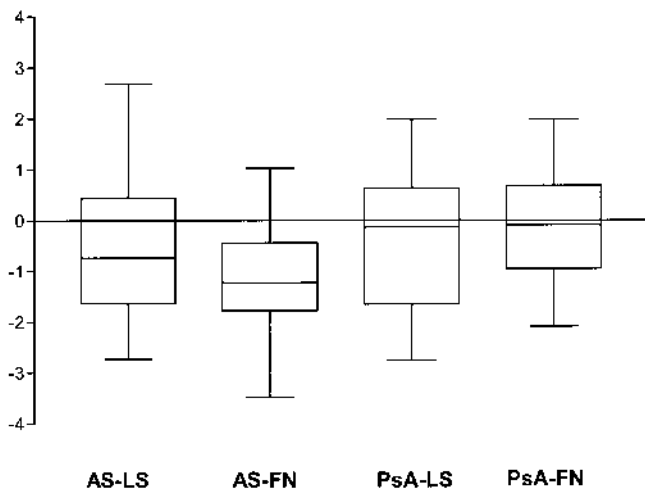


Figure 3. BMD measurements (z scores) at the lumbar spine (LS) and femoral neck (FN) from a cohort of patients with AS and PsA. Patients with AS show decreased BMD, mainly at the femoral neck. Data are means \pm SD.

tivity of this system. In line with this idea, it is interesting that OPG recently has been shown to regulate B lymphocyte maturation⁶².

Taken together, our findings indicate enhanced bone resorption in all 3 seronegative SpA studied, namely AS, PsA, and ReA. In addition, differences in markers of bone formation may represent significant differences between SSpA disease entities. In particular, there is no indication of severe osteoporosis in PsA, where elevated bone ALP values suggest ongoing bone formation. Finally, increased OPG levels in all 3 diseases may be associated with the

inflammation induced production of cytokines and might reflect overactivity within the RANK-RANKL-OPG system.

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