

Serum osteocalcin and carboxyterminal propeptide of type I procollagen in rheumatoid arthritis

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Abstract

Objectives—Previous reports indicate that serum osteocalcin (serum bone GLA protein (S-BGP)) and carboxyterminal propeptide of type I procollagen (PICP) can be used as indicators of bone formation and turnover. The purpose of this study was to assess the activity of bone formation in patients with rheumatoid arthritis (RA) using S-BGP and S-PICP. The biochemical data were compared with data obtained from bone histomorphometry.

Methods—Concentrations of S-BGP and S-PICP were measured in 119 women with RA aged 30–66 years and 47 healthy female controls matched for age. Bone histomorphometry of iliac crest samples was performed in 107 patients with RA.

Results—Weak to moderate correlations between the serum markers and histological bone formation parameters were found. Concentrations of S-BGP and S-PICP were significantly decreased in patients with RA compared with the controls (S-BGP 7.2 (2.3) v 8.7 (2.1) µg/l; S-PICP 105 (32) v 117 (38) µg/l. The lowest values were found in patients with recent onset RA.

Conclusions—These findings suggest that bone formation and bone remodelling are generally reduced in patients with RA.

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Generalised bone loss has been suggested to be an extra-articular manifestation of rheumatoid arthritis (RA), occurring in a subset of patients.¹ Inflammatory factors, functional impairment and concomitant corticosteroid treatment may contribute to decreased bone mass. The cellular mechanism of this bone loss has not been established.

Bone GLA protein or osteocalcin is a vitamin K dependent, non-collagenous protein of bone matrix, synthesised by osteoblasts.² Numerous reports indicate that serum osteocalcin (serum bone GLA protein (S-BGP)) can be used as an indicator of bone formation and turnover.^{3–4} Studies of S-BGP in RA have given discrepant findings.^{5–8}

Carboxyterminal propeptide of type I procollagen (PICP) is cleaved from type I procollagen during collagen synthesis and liberated into the blood,⁹ where it can be measured by radioimmunoassay.¹⁰ It has been

shown that serum PICP correlates with bone formation rate, assessed with double tetracycline labelling.^{11–12}

The purpose of this study was to assess the activity of bone formation in patients with RA using two different serum markers, S-BGP and S-PICP. These were compared with the data obtained from bone histomorphometry.

Subjects and methods

SUBJECTS

One hundred and nineteen women (mean (SD) age 51.1 (9.8) years, range 30–66 years) with classical or definite RA were studied. All patients fulfilled the American Rheumatism Association criteria for classic or definite RA.¹³ The patients for the study were selected from patients admitted for medical or surgical treatment to Kuopio University Hospital and the Rheumatism Foundation Hospital in Heinola from 1985 to 1990.

Informed consent was obtained from each patient and the study was approved by the local ethic committees. None of the patients had any other disease or were taking drugs known to affect bone mineral metabolism. Functional class was estimated according to the criteria of Steinbrocker *et al.*¹⁴

The patients were subdivided into three groups according to their treatment and disease duration. Group I comprised 20 patients who had not been treated with specific anti-inflammatory drugs (second line drug treatment) and whose disease had not lasted longer than 12 months. Non-steroidal anti-inflammatory drugs (NSAIDs) were allowed, however. Group II comprised 71 patients, who were treated with gold compounds, penicillamine, anti-malarial drugs, sulphasalazine, or NSAIDs. The duration of the treatment was at least one year. Patients who had received glucocorticoid treatment by mouth during the preceding six months were excluded. Most of the patients had not received systemic glucocorticoid treatment at any time. Group III included 28 patients who were receiving low dose glucocorticoid treatment. The duration of the treatment was at least one year (mean (SD) duration 6.6 (4.2) years, range 1–19 years), and the mean (SD) daily dose of prednisolone was 5.5 (1.8) mg. Additional drug treatment (see group II) was also allowed.

The groups were comparable with respect to age and menopausal status. There were no significant differences between groups II and

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Table 1 Age, weight, height, duration of disease, menopausal status, functional class, and disease activity of the patients with RA

	Group I (n=20)	Group II (n=71)	Group III (n=28)
Mean (SD) age (years)	48.3 (12.0)	51.9 (9.5)	51.4 (8.7)
Mean (SD) weight (kg)	64 (11)	66 (11)	67 (12)
Mean (SD) height (cm)	162 (5)	161 (6)	162 (6)
Mean (SEM) duration of disease (years)	0.4 (0.1)	16.4 (0.9)	18.0 (1.4)
Menopausal status (premenopausal/postmenopausal)	11/9	32/39	13/15
Functional class (1/2/3)	9/11/0	3/37/31	0/12/16
ESR (mm/hour)*	50.2 (27.6)	42.3 (24.8)	44.3 (21.1)
S-CRP (mg/l)*	30.4 (23.5)	25.0 (29.3)	34.3 (26.7)

*ESR=erythrocyte sedimentation rate; S-CRP=serum C reactive protein.

III with respect to duration of disease and functional class. Table 1 gives the clinical characteristics of the patients.

The control group consisted of 47 healthy female volunteers (mean (SD) age 48 (10) years, range 30–66 years) who had no disease and were not taking any drugs known to affect bone metabolism.

BIOCHEMISTRY

Blood samples were drawn in the morning after overnight fasting. Levels of S-BGP were determined by radioimmunoassay using a commercial kit from the Compagnie Oris Industrie SA (France). Each determination was carried out in duplicate. The intra-assay variation of the method was 6.9% for a sample of 6.7 µg/l and the interassay variation over 12 consecutive working days was 9.1% for a sample with a mean value of 8.0 µg/l. Values of S-BGP are available for 109 patients with RA and 47 control women.

Concentrations of S-PICP were measured in duplicate 100 µl aliquots by radioimmunoassay (commercially available from Orion Diagnostica, Finland).¹⁰ The intra-assay and interassay coefficients of variation were 3 and 5% respectively. Values of S-PICP are available for 119 patients with RA and 47 controls.

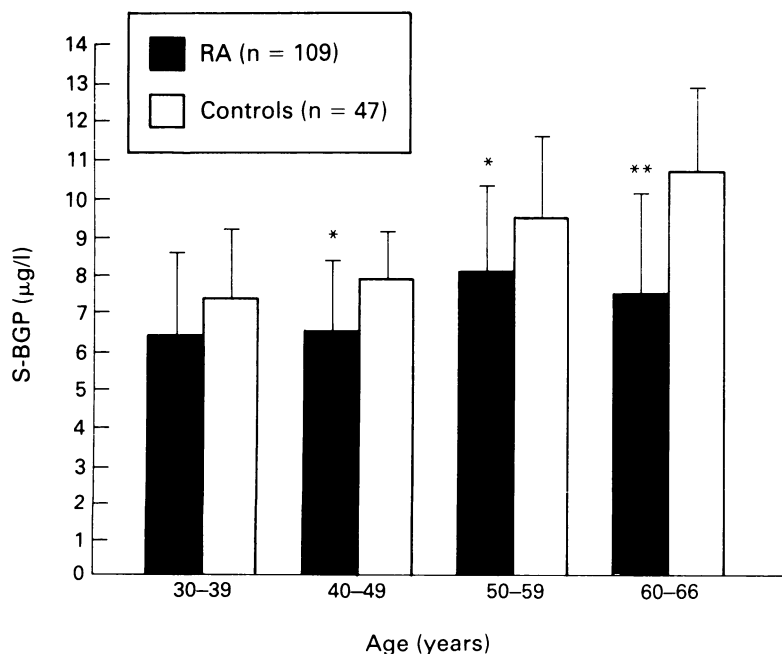


Figure 1 Mean (SD) serum osteocalcin (S-BGP) concentrations in patients with RA and controls in different age groups. * $p < 0.05$ difference between patients with RA and controls; ** $p < 0.01$ difference between patients with RA and controls.

Serum alkaline phosphatase (S-ALP), erythrocyte sedimentation rate (ESR), and C reactive protein (S-CRP) were determined using standard laboratory methods of Kuopio University Hospital.

BONE HISTOMORPHOMETRY

Bone histomorphometry of iliac crest samples was performed in 107 patients with RA. Tetracycline double labelling was performed in 93 subjects. The labelling method consisted of giving oxytetracycline 250 mg four times a day by mouth for one day, no drug for five days, and oxytetracycline again 250 mg four times a day for one day, followed by taking a biopsy sample five days later.¹⁵ Sections which had not been decalcified (5 µm) were stained with Masson Goldner trichrome stain. Thirty or more fields for each sample were measured in two to six consecutive sections with a Mertz graticule using a magnification of $\times 100$. Trabecular bone volume (BV/TV), osteoid volume (OV/BV), osteoid surface (OS/BS), and eroded surface (ES/BS) were measured. The mineral apposition rate was measured from unstained sections according to the method of Frost¹⁶ using a magnification of $\times 625$.

STATISTICAL METHODS

Statistical analyses were carried out with the SPSS/PC program. The results are expressed as mean (SD) values. Comparability of the patient groups was tested by analysis of variance and the χ^2 test. Linear regression analysis was used to determine the relations between continuous variables. Spearman's correlation coefficients were calculated for non-continuous variables. Analysis of variance was used to test differences between the groups. The differences were located by the Newman-Keuls test. When the variances in the groups were unequal or the distribution was not normal, the Kruskal-Wallis test was used. Adjustment for age was made by analysis of covariance. For all statistical tests, significance was defined as $p < 0.05$.

Results

The levels of S-BGP showed a weak linear dependence on age in patients with RA ($r = 0.230$; $p = 0.016$). In controls a moderate linear dependence was found ($r = 0.520$; $p < 0.001$). Levels of S-BGP were significantly higher in postmenopausal women than in premenopausal women, both for patients with RA and controls (7.9 (2.5) v 6.5 (1.9) µg/l, $p = 0.002$; 9.8 (2.0) v 8.0 (1.9) µg/l, $p = 0.003$ respectively). There was a highly significant difference between patients with RA and controls in the mean S-BGP levels (7.2 (2.3) µg/l v 8.7 (2.1) µg/l; $p < 0.001$). When the values were adjusted for age the difference increased (7.2 v 8.9 µg/l; $p < 0.001$). The S-BGP values were lower in all age groups of patients with RA than in the controls; the difference was statistically significant in the age groups 40–49, 50–59, and 60–66 years (fig 1).

Table 2 Mean (SD) serum bone GLA protein (S-BGP) and carboxyterminal propeptide of type I procollagen (S-PICP) concentrations in patients with RA and controls

	Group I	Group II	Group III	Controls
Premenopausal women				
S-BGP ($\mu\text{g/l}$)	5.6 (2.1)*	7.0 (1.7)*	6.1 (2.0)*	8.0 (1.9)
S-PICP ($\mu\text{g/l}$)	94.2 (26.3)	98.5 (24.8)	106.9 (33.8)	113.9 (32.3)
Postmenopausal women				
S-BGP ($\mu\text{g/l}$)	7.1 (2.3)	8.3 (2.6)*	7.1 (2.1)*	9.8 (2.0)
S-PICP ($\mu\text{g/l}$)	80.6 (16.8)‡	115.1 (34.6)	112.6 (40.5)	122.8 (45.8)
All women				
S-BGP ($\mu\text{g/l}$)	6.2 (2.2)†	7.7 (2.3)*	6.6 (2.1)*	8.7 (2.1)
S-PICP ($\mu\text{g/l}$)	88.1 (23.1)†	107.6 (31.5)	110.0 (37.0)	117.3 (37.8)

*Significantly different from controls ($p < 0.05$).

†Significantly different from controls and group II ($p < 0.05$).

‡Significantly different from controls and groups II and III ($p < 0.05$).

Among the patients with RA the lowest values were found in those with a recent onset disease (table 2).

There was no significant correlation between age and S-PICP. Values of S-PICP showed a wide variation in the two study groups. There was a trend for higher S-PICP values in postmenopausal women with RA and in postmenopausal controls, but the difference did not reach statistical significance. The mean S-PICP level was significantly reduced in patients with RA compared with the controls (105 (32) $\mu\text{g/l}$ v 117 (38) $\mu\text{g/l}$; $p = 0.03$). Adjustment for age slightly increased the statistical significance of this difference ($p = 0.02$). Levels of S-PICP were lower in all age groups, but the difference was statistically significant only in subjects aged 50–59 years. In the oldest age group no difference between patients with RA and controls was found (fig 2). Consistent with S-BGP, the lowest S-PICP concentrations were found in patients with recent onset RA (table 2).

A significant correlation between S-PICP and S-BGP was found in the patients with RA ($r = 0.332$; $p < 0.001$); the relationship was weaker in the controls ($r = 0.278$; $p = 0.058$, NS). Levels of S-ALP correlated weakly with S-BGP ($r = 0.253$), but not with S-PICP.

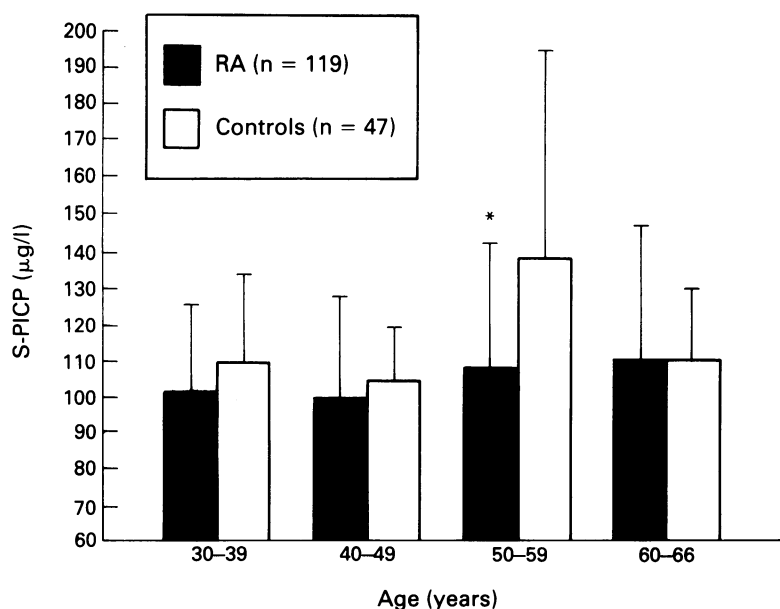


Figure 2 Mean (SD) serum carboxyterminal propeptide of type I procollagen (PICP) in patients with RA and controls in different age groups. * $p < 0.05$ difference between patients with RA and controls.

We did not find any significant relationship between S-BGP or S-PICP and disease activity as assessed by ESR or S-CRP value in the whole patient group. The ESR was insignificantly higher in patients with recent onset RA, however, and there was also a weak negative correlation between ESR and S-PICP in these patients ($r = -0.365$; $p = 0.114$). Neither was there any significant difference in S-BGP or S-PICP between the different functional classes when the treatment group was considered.

In patients with RA a significant correlation was detected between S-BGP and osteoid volume (OV/BV) and OS/BS as assessed by histomorphometry ($r = 0.341$ and $r = 0.263$ respectively). Levels of S-PICP showed a weak to moderate linear correlation with OV/BV ($r = 0.326$), OS/BS ($r = 0.264$) and mineral apposition rate ($r = 0.240$). There was no significant correlations between BV/TV or ES/BS and the serum markers (table 3). For closer analysis we divided the data into premenopausal and postmenopausal subgroups. In premenopausal patients with RA the correlation coefficients between S-BGP and osteoid parameters were higher than in the whole group (OV/BV; $r = 0.415$, $p = 0.005$; OS/BS; $r = 0.350$, $p = 0.018$). Similarly, S-PICP showed higher correlations with OV/BV ($r = 0.464$; $p = 0.001$) and OS/BS ($r = 0.439$; $p = 0.002$) in premenopausal patients. In contrast, in postmenopausal women the correlations between the serum markers and osteoid parameters were mostly insignificant (r values from 0.14 to 0.31). No significant correlations of osteoid parameters with S-ALP were found.

Discussion

Generalised osteoporosis is a common abnormality in patients with RA; the cellular mechanism of this bone loss is not known, however.¹⁷ We studied bone metabolism in patients using two serum markers suggested to reflect bone formation (osteoblastic activity or bone matrix formation).

Previous studies of the circulating concentration of osteocalcin in RA have found this to be increased,^{18, 19} decreased,^{5, 20} or normal.^{7, 8} Marhoffer *et al*⁸ showed increased levels of S-BGP in older patients with recent onset RA

Table 3 Correlations between serum bone GLA protein (S-BGP), serum carboxyterminal propeptide of type I procollagen (S-PICP), and bone histomorphometric data in 107 patients with RA

Mean (SD) bone histomorphometric data*	S-BGP	S-PICP
BV/TV (%) (14.8 (3.7))	-0.135	-0.090
OV/BV (%) (2.2 (1.4))	0.341‡	0.326‡
OS/BS (%) (19.8 (9.3))	0.263§	0.264§
ES/BS (%) (3.4 (1.3))	-0.037	0.065
MAR ($\mu\text{m/day}$) (0.47 (0.11))†	-0.030	0.240¶

*BV/TV=trabecular bone volume; OV/BV=osteoid volume; OS/BS=osteoid surface; ES/BS=eroded surface; MAR=mineral apposition rate.

† $n = 93$.

‡ $p < 0.001$.

§ $p < 0.01$.

¶ $p < 0.05$.

and a significant correlation between S-BGP and ESR. In patients with early onset RA and in patients with advanced RA, however, S-BGP was normal.

In this study S-BGP was consistently lower in patients with RA than in healthy controls. The serum concentration was lowest in patients with recent onset RA and with no specific treatment for rheumatism. In spite of the recent onset, the activity of the disease may have been highest in these patients. The results suggest that S-BGP values increase during the treatment of RA. In agreement with our results, decreased S-BGP values were found to be normalised during remission inducing drug treatment in patients with RA by Ekenstam *et al.*²⁰ The patients treated with low dose glucocorticoids had lower S-BGP levels than patients in group II. More profound decreases in S-BGP levels in patients with RA treated with corticosteroids have been reported,^{21 22} most probably in association with larger doses of the drugs.

Among patients with RA the mean S-BGP was highest in patients treated with specific antirheumatic drugs (group II). In this study no relation between S-BGP and inflammatory activity of the disease defined by ESR or S-CRP could be found. This agrees with reports of Ekenstam *et al.*,²⁰ Gevers *et al.*,¹⁸ and Nolla *et al.*²³ In contrast, Magaro *et al.*¹⁹ and Marhoffer *et al.*⁸ reported high serum osteocalcin values in patients with RA with active arthritis. The discrepancies between the studies may be explained by the duration of the disease, the activity of RA, decreased kidney function of the study subjects, different drugs given in the treatment of RA, or different assays.

In contrast with the histomorphometric studies of Brown *et al.*³ and Garcia-Carrasco *et al.*,⁴ only weak correlations between osteoid parameters and S-BGP levels were found in this study. A suggestion for the differing results lies in different study subjects. Brown *et al.*³ studied women with postmenopausal osteoporosis and Garcia-Carrasco *et al.*⁴ studied healthy subjects. In this study premenopausal women showed higher correlations than postmenopausal women. Furthermore, the quality of osteoid is different in different metabolic bone diseases. Serum osteocalcin is known to correlate more weakly with lamellar than woven osteoid.²⁴

The carboxyterminal propeptide cleaved from type I collagen (PICP) can be measured in blood by radioimmunoassay.¹⁰ The PICP is produced by osteoblasts at a rate which is equimolar with the rate of bone collagen synthesis, and because it is not incorporated into the bone matrix, serum PICP might be a valuable marker of bone formation.^{25 26} Serum PICP is probably a better bone formation marker than serum osteocalcin because type I collagen comprises over 90% of the organic matrix of bone, whereas osteocalcin accounts for only 2% (or 10–20% of the non-collagenous proteins of bone).²⁷

In this study patients with RA had significantly reduced serum PICP levels

compared with healthy controls. Patients with recent onset RA had the lowest serum PICP concentrations. The results are thus comparable with serum levels of osteocalcin. In contrast with S-BGP, in patients treated with corticosteroids S-PICP was not further decreased. We suggest that low dose corticosteroid treatment does not decrease bone formation significantly. Only the moderate correlation between S-PICP and S-BGP indicates that the two biochemical parameters do not measure exactly the same thing. In contrast with S-BGP, a significant correlation between mineral apposition rate and S-PICP was found.

We could not find any significant relation between age and S-PICP. In healthy women, however, there was an increase in mean S-PICP in the age group 50–59 years, suggesting increased bone turnover just after the menopause (fig 1). In patients with RA the effect of the menopause was weaker, probably as a result of the disease.

In this study patients with RA showed significantly reduced levels of S-BGP and S-PICP. This suggests generally reduced bone formation and bone remodelling in patients with RA. For the first time decreased S-BGP and S-PICP levels were shown in patients with recent onset RA and with no specific anti-inflammatory treatment. We suggest that factors related to RA itself are responsible for decreased bone formation. The role of NSAIDs in this process could not be excluded, however.

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