

## Serum osteocalcin and vitamin D metabolites in patients with ankylosing spondylitis

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### Abstract

**Objectives**—Osteocalcin is the major non-collagenous protein of bone and is regarded as a specific index of bone formation. The aim of this study was to examine the rate of bone formation measured by osteocalcin in 38 patients with ankylosing spondylitis (AS) and its dependence on various parameters of calcium and phosphate metabolism.

**Methods**—Serum osteocalcin, alkaline phosphatase, parathyroid hormone, and 1,25-dihydroxyvitamin D were measured in 38 patients with ankylosing spondylitis and in 52 controls.

**Results**—Mean serum osteocalcin was significantly reduced in patients with AS (men 1.7 (1.1) ng/ml; women 1.2 (1.1) ng/ml) compared with the corresponding control groups (men 3.2 (1.3) ng/ml; women 4.1 (1.7) ng/ml). In contrast, alkaline phosphatase was only slightly but significantly higher (135 (44) U/l) in patients with AS than in the corresponding controls (114 (35) U/l). Serum parathyroid hormone (AS 3.1 (0.7) v 2.7 (0.6) mE/ml) and 1,25-dihydroxyvitamin D (AS 64.0 (34.5) v 52.4 (6.7) pg/ml) were slightly but not significantly higher in patients with AS. Consequently, as both hormones are known to stimulate osteocalcin synthesis, they are not responsible for low osteocalcin levels in patients with AS. No significant correlation between alkaline phosphatase and osteocalcin was found. Low serum levels of osteocalcin in patients with AS reflect lower osteoblastic activity in AS.

**Conclusions**—Bone turnover in patients with AS is characterised by low bone formation in the presence of normal levels of calcium regulating hormones.

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Patients with ankylosing spondylitis (AS) are known to have a certain degree of osteopenia.<sup>1-3</sup> Clinically, vertebral osteoporosis is a well recognised radiological feature. Reid *et al* showed a reduction in mean total body calcium by 5.3% in 20 patients with AS.<sup>4</sup> The pathogenesis of this osteopenia already seen in early AS<sup>5</sup> is controversial<sup>3,5</sup> and few data exist on bone turnover.<sup>6</sup> Alkaline phosphatase, hydroxyproline, and other collagen related substances have been widely used as biochemical markers for bone metabolism.

Although they have been useful in measuring bone turnover, they also have significant limitations.<sup>7,8</sup>  $\gamma$ -Carboxyglutamic acid containing protein of bone, the most abundant protein of bone derived from osteoblasts,<sup>9</sup> is a specific marker of bone formation. The synthesis of this protein, usually called osteocalcin, is regulated by calciotropic hormones.<sup>10,11</sup> In particular, 1,25-dihydroxyvitamin D stimulates the production of osteocalcin in osteoblasts.<sup>10</sup> The aim of this study was to examine the rate of bone formation (measured by the amount of osteocalcin) in patients with AS and its dependence on various parameters of calcium phosphate metabolism, especially parathyroid hormone and 1,25-dihydroxyvitamin D.

### Patients and methods

The study group consisted of an unselected group of 38 consecutive patients with mild to moderate AS (13 women and 25 men, mean ages 37 and 42 years respectively) attending an outpatient clinic for rheumatic disease. The control group (23 women and 29 men aged between 20 and 64 with a mean (SD) age of 37 (14.6) and 41.6 (17) years respectively) had no evidence of calcium or skeletal abnormalities by routine history, physical, and biochemical evaluation. Patients with AS had characteristic physical signs and radiographic features according to New York clinical criteria.<sup>12</sup> None of the patients received glucocorticoids and only two received non-steroidal anti-inflammatory drugs (NSAIDs).

For all subjects and patients blood samples were collected in the morning (8 am) after an overnight fast. Serum samples were separated by centrifugation and then frozen at -40°C.

Osteocalcin was measured in duplicate by a commercial radioimmunoassay (Immuno-Nuclear Corporation, Stillwater, MN, USA) by the method of Price and Nishimoto using purified calf bone GLA protein.<sup>13</sup> The sensitivity of the assay was 0.2 ng/ml and the concentration could be determined in all patients. In all cases the intraassay variation was less than 8% and the interassay variation was less than 12%. Parathyroid hormone was determined by a commercial radioimmunoassay (Fleurus, Belgium) using chicken antibody raised against human parathyroid hormone (c terminal). The interassay variation was less than 13%. 25-Hydroxyvitamin D, 24,25-dihydroxyvitamin D, and 1,25-dihydroxyvitamin D were determined as described elsewhere.<sup>14</sup>

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Serum calcium was determined by atomic absorption spectrometry; phosphate, creatinine, and alkaline phosphatase were measured using an autoanalyser. For the erythrocyte sedimentation rate (ESR) measurement, Westergren's method was used.

All results are expressed as mean (SD) values. Comparisons for significance were made using Student's *t* test. The correlation was calculated according to the method of Dixon and Massey.<sup>15</sup>

### Results

Patients with AS presented with significantly ( $p < 0.01$ ) lower mean serum osteocalcin levels (men 1.7 (1.1) ng/ml; women 1.2 (1.1) ng/ml) than the corresponding control group (men 3.2 (1.3) ng/ml; women 4.1 (1.7); fig 1). In contrast, alkaline phosphatase was only slightly, but significantly, higher (135 (44) U/l;  $p < 0.025$ ) in patients with AS than in the control group (114 (35) U/l) (fig 2). Consequently, no significant correlation ( $r = 0.47$ ;  $p > 0.05$ ) between alkaline phosphatase and osteocalcin was found. No increased liver enzymes could be found in the five patients with increased alkaline phosphatase levels. Serum parathyroid hormone values were slightly but not significantly ( $p > 0.05$ ) higher than the controls (AS 3.1 (0.7) mE/ml; control 2.7 (0.6) mE/ml) (fig 3). Mean vitamin D metabolite concentrations (table, fig 4) and all serum calcium, phosphate and creatinine values were in the normal range. The concentration of 1,25-dihydroxyvitamin D<sub>3</sub> was in the upper limit of the normal range, but significantly different from the controls. The ESR was not increased except for two of the patients receiving NSAIDs. Their laboratory parameters did not differ significantly from the rest of the studied group.

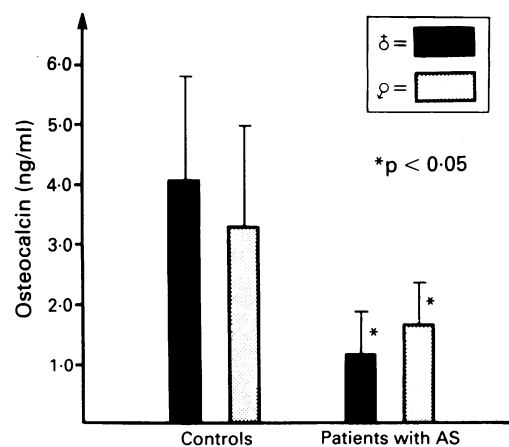


Figure 1 Serum osteocalcin in patients with ankylosing spondylitis (AS).

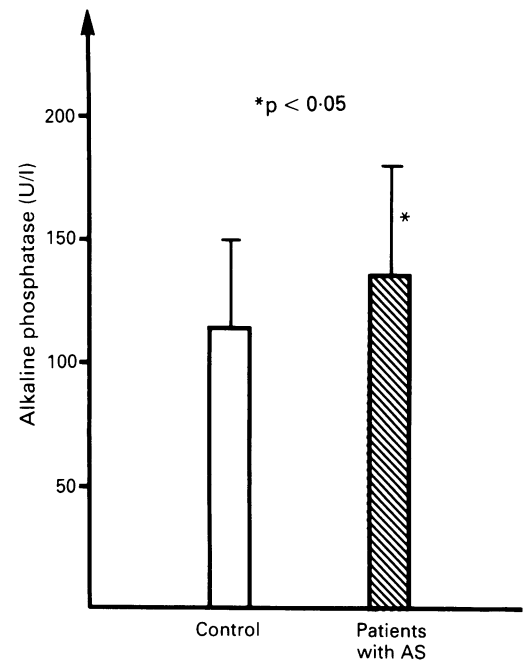


Figure 2 Alkaline phosphatase in patients with ankylosing spondylitis (AS).

### Discussion

Our results reflect a significant reduction of bone formation in patients with AS measured by low serum osteocalcin levels. Serum osteocalcin concentrations are decreased in clinical situations characterised by decreased bone turnover as low turnover osteoporosis.<sup>16</sup> Patients receiving glucocorticoids<sup>17 18</sup> in

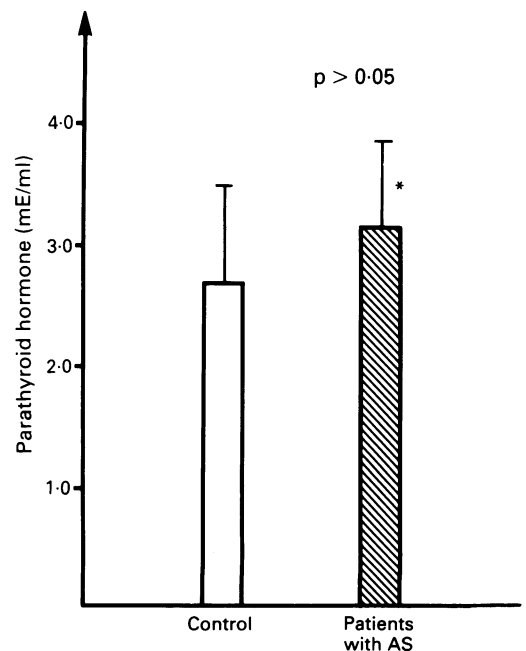


Figure 3 Parathyroid hormone in patients with ankylosing spondylitis (AS).

### Concentrations of vitamin D metabolites in patients with ankylosing spondylitis

	25-Hydroxyvitamin D (ng/ml)	24,25-Dihydroxyvitamin D <sub>3</sub> (ng/ml)	1,25-Dihydroxyvitamin D (pg/ml)
Normal adults	20.1 (3.2)	2.5 (0.6)	52.4 (6.7)
Patients with spondylosing arthritis	21.6 (13.5)	2.3 (1.7)	64.0 (34.5)

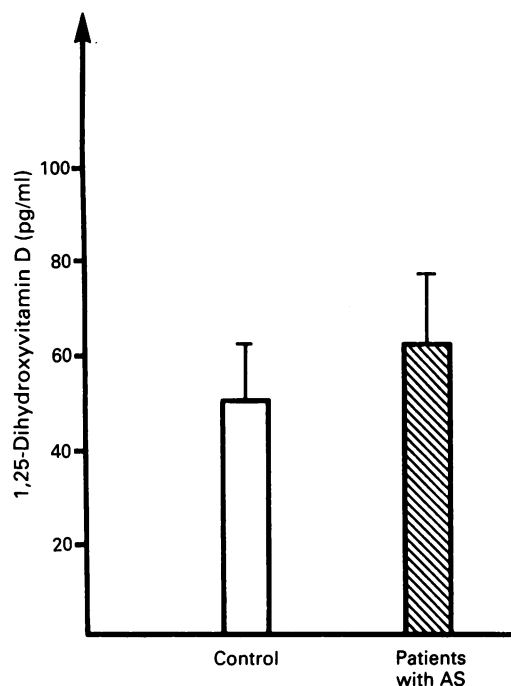


Figure 4 1,25-Dihydroxyvitamin D in patients with ankylosing spondylitis (AS).

particular show low levels of osteocalcin. None of our patients received glucocorticoids, however.

Reduced synthesis or increased degradation of osteocalcin does not seem to result from the effect of chronic disease on osteoblastic activity as normal circulating osteocalcin levels have been reported in a variety of chronic diseases of non-inflammatory origin.<sup>19</sup> Reduced circulating levels of osteocalcin have also been measured in other inflammatory arthritides, however.<sup>20 21</sup> Ekenstam *et al* showed a significant reduction in circulating levels of serum osteocalcin in patients with seronegative spondyloarthropathy, but only nine of 23 patients had AS.<sup>20</sup>

Alkaline phosphatase is reported to be increased in some patients (17%) with AS,<sup>22</sup> but was not related to increased bone fractions of alkaline phosphatase isoenzyme. A dissociation of alkaline phosphatase and osteocalcin as found in our patients with AS has also been reported by others.<sup>19 23-25</sup> It can be the result of a major hepatic fraction of alkaline phosphatase isoenzyme as reported by Sheehan *et al.*<sup>22</sup> We could not find increased  $\gamma$  glutamyl transpeptidases in the five patients with increased alkaline phosphatase, however. Laboratory values of the two patients receiving NSAIDs did not differ significantly from the rest of the studied group, except for an increased ESR. In contrast with osteocalcin, alkaline phosphatase does not seem to be a sensitive parameter of bone turnover in patients with AS.

According to our data changes in calcium phosphate metabolism in patients with AS are due to decreased bone formation (measured by low serum osteocalcin) as found in other diseases with osteopenia such as low turnover osteoporosis,<sup>16</sup> rheumatoid arthritis, and other inflammatory arthritides.<sup>20 21</sup> The synthesis of osteocalcin can be altered by different states of

parathyroid hormone<sup>23</sup> secretion. Price *et al*<sup>26</sup> showed low osteocalcin levels in patients with hypoparathyroidism. In contrast, levels of parathyroid hormone in our patients were at the upper limit of normal values. Consequently, serum 1,25-dihydroxyvitamin D<sub>3</sub>, the synthesis of which is stimulated by parathyroid hormone, was slightly but not significantly ( $p > 0.05$ ) increased. Although serum calcium and phosphate levels were in the normal range, it is conceivable that low bone formation leads to a net loss of calcium, being compensated for by a slight increase of parathyroid hormone (as discussed in osteoporosis type II) and 1,25-dihydroxyvitamin D in some patients with AS. Our studies show, however, that these two calcitropic hormones, which have a stimulating influence on osteocalcin synthesis, are not responsible for low levels of osteocalcin as they are in the upper limit of the normal range in patients with AS.

Our results indicate that bone turnover in patients with AS is characterised by low bone formation in the presence of normal levels of calcium regulating hormones.

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