BIOCHEMICAL MARKERS OF BONE TURNOVER IN SERONEGATIVE SPONDYLARTHROPATHY: RELATIONSHIP TO DISEASE ACTIVITY

A. G. MACDONALD, G. BIRKINSHAW,* B. DURHAM,* R. C. BUCKNALL and W. D. FRASER*

Departments of Rheumatology and *Clinical Chemistry, Royal Liverpool University Hospital, Prescot Street, Liverpool L7 8XP

SUMMARY

To investigate bone turnover in patients with seronegative spondylarthropathy, a bone formation marker, type 1 procollagen carboxy-terminal propeptide (P1CP), and resorption markers, the pyridinium cross-links of collagen [urinary free (f) PYR and DPYR], were measured. The median f-PYR, f-DPYR and P1CP (± interquartile range) were 15.8 (6.00) nmol/mmol creatinine, 3.8 (2.2) nmol/mmol creatinine and 101.5 (38) µg/l, respectively. There was a positive correlation between resorption markers and acute-phase reactants such as C-reactive protein (r = 0.4 for PYR, r = 0.42 for DPYR, P < 0.05), and a negative correlation observed between P1CP and the erythrocyte sedimentation rate (r = −0.64, P < 0.05). In the subgroup of patients with an elevated CRP concentration, the concentration of PYR and DPYR was significantly increased (f-PYR 25.7 vs 15.8 and f-DPYR 6.6 vs 3.8, P < 0.01 for f-PYR, P < 0.05 for f-DPYR). This study suggests than an elevation in acute-phase response in patients with seronegative spondylarthropathy is associated with increased concentration of bone resorption markers with a tendency for reduction in bone formation markers. This may represent uncoupling of bone formation and resorption, leading to bone loss in such patients.

Key words: Seronegative spondylarthropathy, Pyridinium cross-links, Type 1 procollagen carboxy-terminal propeptide, Acute-phase response.

OSTEOPOROSIS is a prominent feature of a number of rheumatic diseases, either as a result of the disease process itself or as a consequence of drug therapy, particularly long-term steroid use. In ankylosing spondylitis, there has been increasing interest in the importance of bone loss as well as bone formation. Ralston et al. [1] demonstrated that patients with established ankylosing spondylitis had a higher incidence of vertebral crush fracture, suggesting that this complication may often contribute to the overall morbidity of the condition. At the other end of the spectrum, Will et al. [2] demonstrated increased bone turnover in patients with early disease before syndesmophyte formation, suggesting that bone loss in early disease may be a primary initiating event in producing the typical kyphosis. Attempts to measure bone mineral density at the spine by dual-energy X-ray absorptiometry have met with technical difficulties, but it has recently been shown that osteoporosis, as defined by reduced bone density at the femoral neck, is present in patients with advanced ankylosing spondylitis (AS) [3].

The pyridinium cross-links, pyridinoline and deoxypyridinoline (PYR and DPYR), have been shown to be useful measures of bone resorption in conditions such as osteoporosis, Paget’s disease and hyperparathyroidism [4–6], and have also been demonstrated to be elevated in various rheumatic diseases, including rheumatoid arthritis (RA) and osteoarthritis (OA) [7, 8]. To increase understanding of the relevance of these findings requires consideration of bone formation rates. The C-terminal propeptide of type 1 procollagen (P1CP) is recognized as a marker of type 1 collagen synthesis and has been shown to correlate with bone formation histomorphometrically [9, 10]. The aim of this study was to investigate bone turnover by using biochemical markers of bone formation and resorption, and to study any relationship between disease extent and activity and indices of bone turnover.

PATIENTS AND METHODS

Patients

Twenty-seven patients with seronegative spondylarthropathy were identified for study. Details of the study group are summarized in Table I. Five patients had radiological evidence of sacroilitis without AS and the remainder had radiological changes varying from erosion of the vertebral bodies to advanced ankylosis. All patients in whom HLA status was known were B27 positive. Patients were assessed on one occasion at the rheumatology research clinic by one observer (AGM). Clinical assessment of spinal movement was by modified Schober’s test [11]. Laboratory activity was assessed by erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) at the same visit. Patients on corticosteroids and long-term anticonvulsants were excluded, as were those on thyroxine where the dose had required alteration in the preceding 3 months. None of the female patients were current hormone replacement therapy (HRT) users. Only two patients were not receiving a non-steroidal anti-inflammatory drug (NSAID), which has not been shown to alter

© 1997 British Society for Rheumatology
TABLE I

Clinical characteristics of patients in the study

<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>22:5</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>46</td>
<td>32–71</td>
</tr>
<tr>
<td>Disease duration (yr)</td>
<td>20</td>
<td>4–43</td>
</tr>
<tr>
<td>Schober’s (cm)</td>
<td>3</td>
<td>0–6</td>
</tr>
<tr>
<td>X-ray (sacroiliitis:AS)</td>
<td>5:20</td>
<td></td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>18.5</td>
<td>3–66</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>15.8</td>
<td>1–83.3</td>
</tr>
</tbody>
</table>

TABLE II

Correlation between clinical indices and markers of bone turnover (Spearman)

<table>
<thead>
<tr>
<th></th>
<th>f-PYR</th>
<th>f-DPYR</th>
<th>P1CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>−0.14</td>
<td>0.49</td>
<td>−0.05</td>
</tr>
<tr>
<td>Disease duration</td>
<td>−0.11</td>
<td>0.58</td>
<td>−0.33</td>
</tr>
<tr>
<td>Spinal mobility</td>
<td>−0.10</td>
<td>0.61</td>
<td>0.04</td>
</tr>
<tr>
<td>ESR</td>
<td>0.12</td>
<td>0.65</td>
<td>−0.64</td>
</tr>
<tr>
<td>CRP</td>
<td>0.40</td>
<td>0.05*</td>
<td>0.42</td>
</tr>
</tbody>
</table>

*Represents results of statistical significance.

cross-link concentrations. Three patients were receiving sulphasalazine, none were receiving any other ‘anti-rheumatic’ drugs.

Methods

Urinary free pyridinoline (f-PYR) and deoxy-pyridinoline (f-DPYR) were measured in 2 h fasted urine samples. The method used to measure free cross-links in urine is a modification of the high-performance liquid chromatography (HPLC) method described by Black et al. [12]. Acidified urine is applied to microgranular cellulose (CC31) in butanol (1/4) and washed before elution with heptafluorobutyric acid (0.1%). The eluent is then analysed by ion-pair reverse-phase HPLC using fluorescence detection. Acetylated PYR (Metra Biosystems, Oxford) is used as an internal standard. Creatinine (creat) was measured in urine by standard automated techniques (Boehringer Mannheim, Lewes) and results are expressed as f-PYR/creat and f-DPYR/creat (nmol/mmol). The interassay CV for both methods was <5.5% across the working concentration range for the assay [13].

P1CP was measured in serum samples by a commercially available radioimmunoassay (Orion Diagnostica, Espoo, Finland). For the concentration range in this study, the interassay CV was <6.6%.

ESR was measured by the Westergren method (mm in first hour) and CRP was measured on a Hitachi analyser (Boehringer).

Reference ranges for the markers of bone resorption were established using a local population of normal volunteers. A total of 90 volunteers, 38 male and 52 female, were sampled. The female age range was 18–52 and the male range 20–65. No subject was taking any medication known to interfere with calcium metabolism and all were consuming a normal diet. Comparison of free cross-links and total cross-links (following acid hydrolysis of an identical sample of urine with 4 M HCl for 24 h using the method described above [12]) confirmed the strong correlation between these measurements in normal volunteers (y = 0.52x + 13.6, r = 0.98, free PYR correlated against total PYR; and y = 0.49x + 2.7, r = 0.94, free DPYR correlated against total DPYR). The 95% reference interval was calculated for the values obtained in the normal subjects and established the reference ranges as f-PYR 5.0–21.8 nmol/mmol creatinine, f-DPYR 0.4–6.4 nmol/mmol creatinine, and the ratio of f-PYR/f-DPYR 3.3–5.2.

Serum P1CP reference ranges supplied by the kit manufacturer were established using samples from 74 blood donors (33 male and 41 female, age range 20–60). The distribution of concentrations was skewed and the reference ranges were calculated (mean ± 2 s.d.) to be 50–170 μg/l (female) and 38–202 μg/l (male).

All other reference ranges were established using samples obtained from the Mersey Blood Transfusion Service. A total of 470 samples from patients covering

![Fig. 1.—(a) f-PYR concentrations (nmol/mmol creat) in AS patients with reference range shown. (b) f-DPYR concentrations (nmol/mmol creat) in AS patients with reference range shown. (c) P1CP concentrations (μg/l) in AS patients with reference range shown.](image-url)
the age range 18–55 were used and the 95% reference interval calculated for normalized data. The CRP reference range was <5 mg/l and ESR < 20 mm/h.

**Statistics**

The values obtained in this study for f-PYR, f-DPYR and P1CP did not clearly follow a Gaussian distribution, therefore non-parametric statistics were used throughout. Correlation between variables was by Spearman correlation coefficient. The significance of differences between groups was assessed by Mann–Whitney U-test.

**RESULTS**

The values obtained in the study group are shown in Fig. 1 and compared with the defined reference range for patients without bone or joint disease.

No correlation between the bone markers studied and age, disease duration and spinal mobility was evident. For urinary f-PYR and f-DPYR, a weak correlation was observed with CRP concentration \((r = 0.4, r = 0.42, \text{respectively}, P < 0.05 \text{ for both})\). For P1CP, a negative correlation with ESR was observed \((r = -0.64, P < 0.05)\), but not with CRP. These relationships are summarized in Table II.

To investigate this relationship further, patients with an elevated acute-phase response (CRP > 20 mg/l) were compared with those who did not. Both f-PYR and f-DPYR were significantly higher in patients who had an elevated CRP (PYR 25.7 vs 15.8, DPYR 6.6 vs 3.8, \(P < 0.01 \text{ for PYR}, P < 0.05 \text{ for DPYR, Mann–Whitney} \)) as shown in Fig. 2. P1CP values were not significantly different in these subgroups, but a trend toward lower concentrations was noted in patients with an elevated ESR. No clear differences were observed between males and females or between those with sacroiliitis and definite AS, although the female and sacroiliitis groups were too small to permit meaningful analysis.

**DISCUSSION**

This study is consistent with a recent similar study of biochemical markers of bone turnover in AS which used osteocalcin as a bone formation marker [14]. Both studies showed that disease activity was associated with increased resorption markers. In our study there was, in addition, a trend towards reduced formation markers in patients with an elevated acute-phase response which was not found in the previous study [14]. The elevation in urinary f-PYR and f-DPYR was less marked in our study, and appeared only to be present in those with active inflammatory disease. Both studies, however, support the hypothesis that uncontrolled inflammation is associated with bone loss as in other conditions such as RA [15–17].

The available data on bone formation are less consistent. Osteocalcin has been reported as being either normal or decreased in previous studies [14, 18]. This uncertainty again mirrors the position in RA, where osteocalcin has been reported as being decreased, normal or increased [19–21]. For these reasons, as well as for reasons of assay stability, we have preferred to investigate the utility of P1CP as a bone formation marker in patients with rheumatic disease. This study did suggest an inverse relationship between bone formation and acute-phase response, although all values did fall within the reference range. The wide range of values observed in the normal population may limit the usefulness of a single value, although marked changes may be observed in follow-up studies, e.g. following bed rest [22]. The available data, whichever marker is used, do clearly suggest that bone formation is ‘uncoupled’ from resorption in active inflammatory arthritis as assessed by ESR and CRP, with increased bone resorption without a corresponding increase in markers of bone formation.

We consider that the elevation in resorption markers observed in patients with active spondylarthritis reflects increased bone turnover and although PYR is widely distributed in a number of tissues, including cartilage, we think it unlikely that abnormal cartilage metabolism contributes significantly to these results. The correlation between f-PYR and f-DPYR was strong \((r = 0.62, P = 0.01)\), and the ratio of f-PYR to the more bone-specific f-DPYR was within the normal
range (median PYR/DPYR = 3.73). Given the slower rate of cartilage turnover, it seems likely that these results are specific for bone collagen turnover.

The patients who had evidence of elevated bone resorption did not appear to share any other characteristics than elevated acute-phase response. In particular, the results were not explained by early post-menopausal females in this group, nor was there any history of recent fracture. Advanced spinal disease was not, in itself, associated with elevated PYR and DPYR, although the relationship between acute-phase response and bone resorption was still observed in late disease, suggesting continuing bone resorption.

Acute-phase reactants are of limited clinical utility in the assessment and management of patients with seronegative spondylarthropathy as a normal ESR does not exclude active disease, but if the relationship between acute-phase response and bone loss were to be confirmed in such patients, this might allow strategies for targeted intervention to be developed. This study was of cross-sectional design in a relatively small number of patients, but in view of the similar findings in other published work, the results should stimulate further longitudinal studies to investigate these relationships further. New technological advancements in bone density measurement will allow changes in biochemical markers to be correlated with BMD changes.

Osteoporosis is widely regarded as a major public health priority in an ageing population, and patients with rheumatic disease, including AS, appear to be a specific at-risk group. Greater understanding of abnormalities of bone metabolism can be obtained by the study of biochemical markers and further research in this area should yield further valuable insights.

REFERENCES