Bone turnover in non-steroid treated rheumatoid arthritis

J E Compston, S Vedi, P I Croucher, N J Garrahan, M M O'Sullivan

Abstract
Objective—To examine whether changes in cancellous bone turnover and resorption cavity depth contribute to bone loss in patients with non-steroid treated rheumatoid arthritis.

Methods—Iliac crest biopsies were obtained from 37 patients with non-steroid treated rheumatoid arthritis, 13 male and 24 female, aged 37–71 years. Bone turnover and resorption cavity characteristics were quantitatively assessed using semi-automated computerised techniques.

Results—When compared with age- and sex-matched control values, there was a significant reduction in bone formation rate at tissue level and activation frequency (P < 0.001) in the patient group. The eroded perimeter, mean and maximum eroded depth and cavity area were also significantly reduced (P < 0.01, <0.005, <0.001 and <0.005 respectively).

Conclusion—These results demonstrate low bone turnover in non-steroid treated rheumatoid arthritis and indicate that the reduced bone mass in these patients is due mainly to a negative remodelling balance.


Both localised and generalised bone loss have been described in association with rheumatoid arthritis.1–3 Generalised osteopenia has been reported in both steroid treated and non-steroid treated patients and there is some evidence that fracture risk may also be increased, particularly in the former group.4–6 The pathogenesis of bone loss in non-steroid treated patients has not been clearly defined; possible pathogenetic factors include reduced physical activity,10–12 systemic effects of the disease itself and drugs used in its treatment.4–9,15–17 Previous studies have demonstrated a reduced cancellous bone area in iliac crest biopsies obtained from patients with rheumatoid arthritis16–20 and significant trabecular thinning has been reported in non-steroid treated patients.20 However, the mechanisms by which bone loss occurs have not been clearly defined. In an earlier study we demonstrated that the mean wall width, which reflects osteoblastic function, was reduced in non-steroid treated patients and a non-significant reduction in calculated resorption depth was also shown.21 These data indicate that remodelling imbalance contributes to the observed bone loss; however, it is uncertain whether increased bone turnover also plays a role. In this study we have assessed bone turnover in 37 patients with non-steroid treated rheumatoid arthritis. In addition, resorption cavity depth has been directly measured using a computerised technique.

Methods
PATIENTS AND CONTROL SUBJECTS
Thirty seven patients with definite or classic rheumatoid arthritis who had enrolled into a trial of second-line therapy were studied, 13 men aged 37–62 years (mean 50.1) and 24 women aged 35–71 years (mean 55.3). Nineteen of the women were postmenopausal. The median duration of disease was four years (range 1–25) and none had received systemic steroid therapy at any time. Most patients were taking non-steroidal anti-inflammatory drugs and all were receiving second-line therapy (penicillamine, hydroxychloroquine, auranofin or intramuscular sodium aurothiomalate); none was receiving methotrexate. No patient was housebound or restricted to a wheelchair and patients with a history of gastrointestinal disease or surgery, liver disease, chronic renal disease or endocrine disease were excluded from the study. All patients gave informed written consent for the bone biopsy and permission to carry out the study was granted by the local ethical committee. The modified Health Assessment Questionnaire (HAQ)22 ranged between 0 and 3 (mean 1.10); Steinbrocker Class23 was I in four patients, 2 in 26 patients and 3 in seven patients.

Control values were obtained from a subset of a group of 57 healthy subjects, details of whom have been described previously.24 These subjects gave informed written consent to undergo bone biopsy at the same time as a minor surgical procedure requiring general anaesthetic and the study was approved by the local ethical committee. Indices of bone formation were assessed in 37 patients and resorption cavity characteristics in 36; control data for the former were available in 40 subjects, 17 men aged 38–74 years (mean 56.7) and 23 women aged 32–80 years (mean 53.8). Fourteen of these women were postmenopausal. Because of the limited availability of new sections for quantitation, control data for resorption cavity measurements could only be obtained from 34 subjects, 16 male, aged 32–80 years (mean 54.6).
BONE HISTOMORPHOMETRY

Trans-iliac biopsies were obtained with a 6 or 8 mm Border trephine one inch below and behind the anterior superior iliac spine. Specimens were fixed in 10% phosphate buffered formalin and embedded in methylmethacrylate (BDH Ltd, UK) or LR White Resin (London Resin Company, UK). Eight µm thick undecalcified sections were obtained and stained with the von Kossa technique, using a van Gieson counterstain, or 1% toluidine blue (pH 4.2). Unstained sections, 15 µm thick, were also prepared for fluorescence microscopy. All patients received two, time-spaced doses of demethylchlortetracycline before the biopsy (300 mg twice daily on days 1, 2, 13 and 14), the biopsy being performed 3–5 days after the last dose.

Tetracycline-based measurements

Histomorphometric measurements in patients with rheumatoid arthritis were made using a 'Digitrad' digitising tablet and cursor with an LED point light source (Kontron Ltd) and an Olympus BHS-BH2 binocular transmitted light microscope with a BH2-DA drawing attachment (Olympus Optical Co, London). Mineralised bone perimeter was measured on unstained sections viewed by fluorescence microscopy at ×156 magnification, the labelled perimeter being traced with the cursor LED. The mineralising perimeter (M.Pm/B.Pm; %) was calculated as the double-labelled surface plus half the single-labelled surface. The mean distance between tetracycline labels was measured directly at ×312 magnification using the digitising tablet and cursor. Measurements were made at approximately four equidistant points along double labels; 3–4 sections from each biopsy were used for these measurements.

Because of fading of tetracycline fluorescence on the control sections, which had been cut 10 or more years previously and the unavailability of sufficient embedded material in most biopsies to enable preparation of new sections, the original control data which had been obtained using an eye-piece graticule and micrometer, were used. To evaluate possible intermethod differences, a comparison of the two techniques was performed by one observer in six biopsies obtained from postmenopausal women who formed part of a separate study.

The mean difference between the two methods was 0.36% for single-labelled and 0.02% for double-labelled surface (P = NS). For measurement of interlabel distance, a systematic difference was found between the two methods, the eye-piece micrometer producing a significantly higher value than the semi-automated method (P < 0.01). The mean (SD) difference between the two methods, assessed on six biopsies, was 0.882 (0.054) µm, corresponding to 0.074 (0.0451) µm/day mineral appositional rate; the values for mineral appositional rate obtained in patients with rheumatoid arthritis were therefore adjusted accordingly.

The following indices were calculated from the primary measurements:25

Mineral appositional rate (MAR; µm/day):

\[ \text{MAR} = \text{Interlabel width/period between the two labels (12 days).} \]

Tissue-based bone formation rate (BFR/B.Pm, µm2/µm/day);

\[ \text{BFR/B.Pm} = \text{MAR} \times \text{M.Pm/B.Pm}. \]

Activation frequency (Ac.f, year⁻¹):

\[ \text{Ac.f} = (\text{BFR/B.Pm})/\text{WW} \]

where WW is the mean width of completed bone remodelling units, measured on toluidine blue-stained sections viewed under polarised light at a magnification of ×156. Measurement of wall width in the patient and control group has been described previously.21

Measurement of resorption cavity characteristics

The method described by Garrahan et al20 was adapted for use with the digitising tablet and cursor for measurement in both the patient and control group. Cavities were identified on toluidine blue-stained sections viewed under polarised light at ×156 magnification and measured at ×375 or 750 magnification depending on the size of the cavity. Criteria for identification of resorption cavities included interruption of lamellae at an angle to the bone perimeter, absence of osteoid tissue and depth greater than 3 µm. A minimum of 20 cavities was assessed for each biopsy. The following indices were obtained:

Mean eroded depth (E.De, µm)

Maximum eroded depth (E.De Max, µm)

Eroded area (µm²)

Eroded perimeter (%)

Number of cavities/bone perimeter (N.Cv/B.Pm/mm)

STATISTICAL ANALYSIS

Differences between patients and controls were examined using a Mann Whitney test.

Results

Indices of bone turnover are shown in table 1.

Comparison of patients with control subjects revealed a significant reduction in mineralising perimeter (P < 0.001), bone formation rate at tissue level (P < 0.001) and activation frequency (P < 0.001). Eroded surface was significantly reduced in the patient group (P < 0.01), with a non-significant reduction in the number of

Table 1: Formative indices of remodelling in patients with rheumatoid arthritis and normal subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Rheumatoid arthritis</th>
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<tbody>
<tr>
<td></td>
<td>Mean (confidence interval)</td>
<td>Significance</td>
</tr>
<tr>
<td>Mineralising perimeter (%)</td>
<td>10.8 (9.0–12.5)</td>
<td>5.5 (4.2–6.7)</td>
</tr>
<tr>
<td>Mineral apposition rate (µm/day)</td>
<td>0.726 (0.689–0.762)</td>
<td>0.719 (0.659–0.778)</td>
</tr>
<tr>
<td>Bone formation rate µm²/µm/day</td>
<td>0.082 (0.068–0.096)</td>
<td>0.038 (0.030–0.046)</td>
</tr>
<tr>
<td>Activation frequency (year⁻¹)</td>
<td>0.0016 (0.0013–0.020)</td>
<td>0.0010 (0.0008–0.0012)</td>
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</table>
Bone turnover in rheumatoid arthritis

The mean eroded depth, maximum eroded depth and eroded area were all significantly reduced in patients with rheumatoid arthritis compared with the control group (P < 0.005, P < 0.001, P < 0.005 respectively; table 2). Results obtained in male and female patients were similar and values did not differ according to the second-line treatment given.

Discussion

Our results show significantly reduced bone formation at the tissue level in patients with non-steroid treated rheumatoid arthritis and indicate that bone loss in these patients is due to a negative remodelling balance rather than increased bone turnover. This conclusion is consistent with our previous demonstration that the mean wall width, reflecting the amount of bone formed in individual remodelling units, is reduced in non-steroid treated rheumatoid arthritis and that the observed reduction in iliac crest cancellous bone area is associated with trabecular thinning. Thus bone loss due to increased bone turnover results in an increased risk of trabecular penetration and erosion and thus reduced connectedness of the trabecular microstructure, whereas reduced bone formation favours trabecular thinning, with greater preservation of bone architecture.

The significant reduction in bone formation rate demonstrated in this study is consistent with data reported by Ramser et al in rib cortical bone obtained from seven patients with salicylate treated rheumatoid arthritis. In that study reduced tetracycline uptake was shown in three of five patients, and reduced or normal osteoid surface has been reported in several histomorphometric studies. Although an increase in the surface extent of resorption has been reported in two studies, this may reflect decreased formation rather than increased resorption. In contrast, in our study and that of Ng et al, normal osteoid surface was found although osteoclastic surface was not assessed. Thus the few histomorphometric data available indicate reduced bone turnover in patients with non-steroid treated rheumatoid arthritis.

Assessment of biochemical indices of bone turnover in rheumatoid arthritis has produced conflicting results. Low, normal and increased levels of serum osteocalcin, a marker of bone formation, have been reported and increased excretion of the collagen cross-links, deoxypyridinoline and pyridinoline, which reflect bone resorption, has also been reported in one study of patients with active disease. Several factors may contribute to the reported differences: inclusion in some studies of corticosteroid-treated patients; use of other disease modifying drugs and non-steroidal anti-inflammatory agents; variations in disease activity and severity between studies. Kinetic assessment of bone turnover has generally demonstrated normal or reduced turnover at sites distant from diseased joints although high accretion rates have been reported in bone adjacent to inflamed joints, a finding confirmed by histomorphometric studies. In patients with active disease, it is possible that increased bone turnover at juxta-articular sites may lead to elevated serum and/or urine levels of bone markers of formation and resorption in the absence of increased bone turnover at other skeletal sites.

Resorption cavity size has not previously been assessed in rheumatoid arthritis. In the present study, significant reductions in mean and maximum eroded depth were demonstrated. These data includes identifiable cavities for measurement, regardless of their stage of completion and thus does not enable accurate calculation of remodelling balance. However, the trabecular thinning observed in this patient group indicates that the reduction in wall width was quantitatively greater than that in the completed eroded depth, resulting in a negative remodelling balance. The cause of the reduction in resorption cavity size has not been established; osteoblast dysfunction might itself be indirectly responsible since many aspects of osteoclastic function are controlled by these cells.

This study clearly demonstrates that bone turnover is reduced in patients with non-steroid treated rheumatoid arthritis; although bone formation rates assessed by histomorphometry have not been previously reported in iliac crest bone from such patients, in the two other studies in which tetracycline labels were administered before biopsy, poor uptake was a feature. Low bone turnover does not result in bone loss and thus a negative remodelling balance appears to be primarily responsible, although we cannot exclude the possibility that increased bone turnover may have contributed to bone loss at an earlier and more active stage of the rheumatoid disease process, since disease activity was well controlled in the majority of our patients. The pathogenesis of reduced bone formation associated with rheumatoid arthritis is unclear; in some studies, disease duration and activity have been shown to correlate with bone loss and reduced mobility may also play a role. In addition, non-steroidal disease modifying and anti-inflammatory agents may affect osteoblast function; the

Table 2 Resorption cavity characteristics in patients with rheumatoid arthritis and normal subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (confidence interval)</th>
<th>Significance of difference</th>
<th>p value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Rheumatoid arthritis</td>
<td></td>
</tr>
<tr>
<td>Mean eroded depth (μm)</td>
<td>21.3 (19.8–22.8)</td>
<td>18.1 (16.5–19.8)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Maximum eroded depth (μm)</td>
<td>28.2 (25.9–30.9)</td>
<td>28.2 (33.1–36.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Eroded area (μm²)</td>
<td>3568 (3227–3910)</td>
<td>2810 (3361–3260)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Eroded surface (%)</td>
<td>2.31 (1.89–2.74)</td>
<td>1.72 (1.56–2.04)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Number cavities (mm²)</td>
<td>0.084 (0.071–0.097)</td>
<td>0.078 (0.066–0.090)</td>
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</table>
effects of these agents on bone remodelling have not been evaluated. Finally, although some data indicate that fracture risk may be increased in patients treated with cortico-
steroids, and there is less evidence in non-steroid treated patients and the clinical significance of bone loss associated with rheumatoid arthritis requires further studies.

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37 Siebel M J, Duncan A, Robins S P. Urinary hydroxy-
38 Dymon A R. Calcium kinetics in osteopenia and para-