EXTENDED REPORT

Evaluation of bone mineral density, bone metabolism, osteoprotegerin and receptor activator of the NF κ B ligand serum levels during treatment with infliximab in patients with rheumatoid arthritis

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Objectives: To examine whether treatment with anti-tumour necrosis factor (TNF) α prevents loss of bone mineral density (BMD) at the spine and hip (generalised) and in the hands (local) of patients with rheumatoid arthritis, and to study the changes in markers of bone metabolism, including receptor activator of the NF κ B ligand (RANKL) and osteoprotegerin (OPG), during anti-TNF treatment.

Patients and methods: 102 patients with active rheumatoid arthritis, who were treated with infliximab during 1 year, were included in this open cohort study. The BMD of the spine and hip (dual x ray absorptiometry) and hands dual x ray radiogrammetry was measured before the start of treatment and after 1 year. Changes in osteocalcin formation, β -isomerised carboxy terminal telopeptide of type 1 collagen (β -CTx, resorption), RANKL and OPG were determined at 0, 14, 30 and 46 weeks.

Results: The BMD of the spine and hip was unchanged during treatment with infliximab, whereas BMD of the hand decreased significantly by 0.8% ($p < 0.01$). The BMD of the hip in patients with a good European League Against Rheumatism response showed a favourable change compared with patients not achieving such a response. Serum β -CTx and RANKL were both considerably decreased compared with baseline at all time points. The decrease in β -CTx was associated with the decrease in Disease Activity Score of 28 joints and C reactive protein during the 0–14 weeks interval.

Conclusion: In patients with rheumatoid arthritis treated with infliximab, spine and hip bone loss is arrested, whereas metacarpal cortical hand bone loss is not stopped. The outcome of the study also supports a relationship between clinical response, in terms of reduced inflammatory activity, and changes in bone loss of the spine, hip and hands.

Bone loss in the joints is one of the hallmarks of rheumatoid arthritis. This local bone loss can be divided into erosions of the joint and periarticular osteoporosis. Apart from this localised process, generalised bone loss (osteoporosis) is increased in patients with rheumatoid arthritis.^{1–3} Recent literature suggests that these two types of bone loss are at least partly mediated through the same mechanism. A previous study from the Oslo–Truro–Amsterdam group showed that the Larsen Score for joint damage was associated with bone mineral density (BMD) of the hip and the occurrence of vertebral deformities in patients with rheumatoid arthritis.^{4–5} Other clinical studies in patients with rheumatoid arthritis found similar associations between osteoporosis and radiographic joint damage.^{6–10}

The receptor activator of the NF κ B ligand (RANKL) and osteoprotegerin (OPG) system provides a theoretical background for such a common mechanism. RANKL is a membrane-bound protein and a key factor in the activation and differentiation of osteoclasts by binding to its receptor RANK on premature osteoclasts. OPG is the antagonist of RANKL and suppresses the differentiation and activation of osteoclasts.^{11–12} RANKL expression is one among the factors up regulated by several proinflammatory cytokines—that is, tumour necrosis factor (TNF) α and interleukin 1 (IL1).^{13–14}

Currently, the most effective drugs for the treatment of rheumatoid arthritis are the TNF α blocking agents (infliximab, etanercept and adalimumab). Randomised controlled trials have shown that these drugs retard radiographic joint

destruction.^{15–16} This finding raises the question whether these agents also have the ability to prevent generalised bone loss in the spine and hip, and local bone loss in the hands in patients with rheumatoid arthritis. We aimed to address this question and to study changes in markers of bone metabolism, including RANKL and OPG, during such treatment.

PATIENTS AND METHODS

Patients

Consecutive patients with rheumatoid arthritis from four rheumatology departments, VU University Medical Centre (VUMC), Jan van Breemen Institute (JBI), Slotervaart Hospital, Amsterdam, The Netherlands, and Diakonhjemmet Hospital, Oslo, Norway, who were treated with infliximab for at least 1 year and had dual x ray absorptiometry (DEXA) measurements at baseline and at follow-up were included. All patients fulfilled the American College of Rheumatology criteria for rheumatoid arthritis, had active disease (Disease Activity Score of 28 joints (DAS-28) ≥ 3.2), and had previously failed at least two

Abbreviations: BMD, bone mineral density; β -CTx, β -isomerised carboxy terminal telopeptide of type 1 collagen; DAS-28, Disease Activity Score of 28 joints; DEXA, dual x ray absorptiometry; DMARD, disease-modifying antirheumatic drug; DXR, digital x ray radiogrammetry; EULAR, European League Against Rheumatism; IL1, interleukin 1; JBI, Jan van Breemen Institute; OPG, osteoprotegerin; RANKL, receptor activator of the NF κ B ligand; TNF, tumour necrosis factor; VUMC, VU University Medical Centre

disease-modifying antirheumatic drugs (DMARDs), including methotrexate.^{17, 18} In total, 138 patients were included in the cohort, but 36 patients dropped out in the first year of treatment: 28 because of non-response (78%), 6 because of side effects (16%) and 2 patients (6%) who died owing to infliximab-unrelated events. Thus, the data from 102 patients were eligible for analyses (table 1). The baseline characteristics of the dropout patients were comparable to those of the patients included in the study (data not shown).

Methods

Infusions with infliximab were given at 0, 2, 6 and 14 weeks, and after that at an interval of 8 weeks. Infliximab was given intravenously at a starting dose of 3 mg/kg. The dose of infliximab could be increased to 7.5 mg/kg in patients who showed inadequate response, according to clinical judgement. A dose increment occurred in 21 patients.

Demographics and disease-related data

The demographic data collected at baseline were recorded from medical history and patients' medical records. The following variables were collected: age, sex, disease duration, presence or absence of bony erosions, serum rheumatoid factor status (positive if immunoglobulin-rheumatoid factor >30 U/l), current and previous use of DMARDs, anti-osteoporotic drugs and corticosteroids.

Disease activity

The patients were assessed at each visit with core measures of disease activity, and the DAS-28 score was computed. The European League Against Rheumatism (EULAR) response criteria were applied to define response.¹⁷

BMD of hip and spine

BMD was measured before and after 1 year of infliximab treatment using (DEXA). A total of 102 patients had a DEXA measurement of the spine, and in 89 patients DEXA measurements of the hip were available. The DEXA machines

used were Hologic 4500 (Hologic, Waltham, Massachusetts, USA) in the Slotervaart hospital and the VUMC, a Lunar Expert (Lunar, Madison, Wisconsin, USA) at the Diakonhjemmet Hospital, and a Lunar DPX (Lunar) at the JBI. The same DEXA machine used at baseline was used for follow-up measurement for each patient. The coefficients of variation, measured with a local spine phantom for the different centres, were acceptable and comparable: the VUMC 0.56%, Slotervaart Hospital 0.48%, JBI 0.4% and Diakonhjemmet Hospital 0.8%. The BMD data of spine and hip (g/cm²) and t and z scores were determined using the local reference population provided by the manufacturer of the DEXA machine.

BMD of the hand

BMDs of the hand at baseline and after 1 year were measured on plain radiographs of the left and right hand (anteroposterior view) using digital x ray radiogrammetry (DXR; Pronosco X posure system, system 2.0, Sectra, Linköping, Sweden). This method measures cortical thickness from regions of interest at the centre of the second, third and fourth metacarpals, and a mean BMD surrogate is calculated. The theoretical background for this method has been fully described.^{19, 20} The coefficient of variation for this method was 0.25%.²¹ The Pronosco DXR system we used is validated only for evaluation of conventional radiographs. Fifty three patients had conventional radiographs available for evaluation. The baseline characteristics and change in disease activity of the subgroup of patients with hand radiographs were comparable to those of the whole group. The patient data for BMD of the hand (g/cm²) are given as the mean of the right and left hand.

Markers of bone metabolism

In a total of 72 patients serum samples were available for evaluation. These patients also had baseline characteristics and change in disease activity similar to that of the entire patient group. Serum was collected in the morning (non-fasting) before each infusion and stored immediately at -20°C or lower until analyses. Markers of bone metabolism were measured at 0, 14, 28 and 46 weeks. Bone formation was measured by osteocalcin, and bone resorption was determined by β -isomerised carboxy terminal telopeptide of type 1 collagen (β -CTX) using commercial assays according to the instructions of the manufacturer (Roche Diagnostics, Elecsys 2010, Mannheim, Germany). Levels of osteoclast-regulating proteins, including the soluble RANKL and OPG, were determined in serum using an ELISA from Immunodiagnostik (Bensheim, Germany). All assays had an intra-assay and interassay coefficient of variation of <5%.

Statistical analysis

Paired t tests and Wilcoxon rank tests were used to examine longitudinal changes in BMD and markers of bone metabolism, where appropriate. Subgroup analyses were carried out to investigate differences in change in BMD of the spine, the hip and the hand in different groups. Patients were categorised according to achieved EULAR response, use of prednisolone and bisphosphonate, and presence of radiographic erosions. Changes in BMD between these subgroups were analysed using an independent Student's t test or Mann-Whitney U test as necessary.

Associations between changes in BMD and changes in disease activity and bone markers were also examined in a multivariate linear regression model adjusted for age, sex, disease duration, erosive disease, prednisolone and bisphosphonate use.

Similar regression analyses were carried out with bone markers as the dependent variable and disease activity as

Table 1 Baseline characteristics of the patients with rheumatoid arthritis (n = 102)

Demography	
Age (years), mean (SD)	53 (13)
Sex, female, n (%)	84 (82)
Disease duration years, median (range)	8 (1–49)
Erosive disease, n (%)	84 (82)
IgM-RF positive, n (%)	75 (74)
Disease activity	
DAS-28, mean (SD)	5.4 (1.2)
ESR (mm/h), mean (SD)	32 (23)
CRP (mg/l), median (range)	14 (1–174)
Drugs	
Methotrexate, n (%)	98 (96)
Prednisone, n (%)	46 (45)
Anti-osteoporotic drugs, n (%)	26 (26)
BMD	
Vertebral spine (g/cm ²), mean (SD), n = 102	0.993 (0.19)
t Score, mean (SD)	-0.82 (1.6)
z Score, mean (SD)	-0.09 (1.5)
Total hip (g/cm ²), mean (SD), n = 89	0.830 (0.14)
t Score, mean (SD)	-1.02 (1.2)
z Score, mean (SD)	-0.51 (1.2)
Hand, mean (SD), n = 53	0.497 (0.98)

BMD, bone mineral; CRP, C reactive protein; DAS-28, Disease Activity Score of 28 joints; ESR, erythrocyte sedimentation rate; IgM-RF, immunoglobulin G-rheumatoid factor.

independent variable. The changes in markers of bone metabolism and the association with disease activity markers were investigated separately between the different time points: 0–14, 0–30 and 0–46 weeks.

All data were analysed using the SPSS V.11.1 software package. A value of $p < 0.05$ was considered to be significant.

RESULTS

Disease activity

The mean (standard deviation (SD)) disease activity measured by DAS-28 decreased from 5.5 (1.2) at baseline to 3.8 (1.4) after 14 weeks, 3.6 (1.3) after 30 weeks and 3.5 (1.4) after 46 weeks ($p < 0.001$ at all time points compared with baseline). In all, 40 patients had a good response, 43 patients a moderate response and 19 patients a non-response at 46 weeks as defined by the EULAR response criteria.

Bone mineral density

One fifth of the 102 patients had osteoporosis (t score < -2.5 at either spine or hip), and osteopenia (t score < -1.5 but > -2.5 at either spine or hip) was present in almost half (47%) of the patients. A considerably lower BMD of the spine and hip was observed at baseline in patients with erosive disease than in patients with non-erosive disease (data not shown).

BMD of the vertebral spine and hip remained unchanged during treatment with infliximab. In contrast, BMD of the hand decreased significantly by 0.004 g/cm^2 (-0.8%) during the year ($p < 0.05$; table 2). The mean z scores of the spine and hip showed an increasing trend: 0.039 and 0.023, respectively ($p = 0.334$ and 0.376).

Using the smallest detectable difference for each measurement, we calculated the number of patients who lost, gained and maintained BMD for each site (table 3).

There was no significant difference in change in BMD between the patients from different centres, patients with and without bone markers for evaluation, and patients with and without DXR measurements.

Markers of bone metabolism

The bone resorption marker, β -CTx (ng/ml), decreased considerably from 0 to 14 weeks and remained markedly decreased during the rest of the year (fig 1A). The marker for bone formation, osteocalcin (ng/ml), was appreciably increased at 14 weeks compared with baseline, but not at 30 and 46 weeks (fig 1B). RANKL (pmol/ml) decreased markedly during the year (fig 1C), whereas OPG (pmol/ml) did not change markedly (fig 1D). This resulted in a favourable change in the RANKL/OPG ratio (fig 1E).

Table 2 Mean percentage change (SD %) in the bone mineral density of the spine, hip and hand for all patients and for patients with (40%) and without (60%) good European League Against Rheumatism (EULAR) response

	All	Good EULAR response	
		No	Yes
Vertebral-spine (n = 102)	0.20 (5.1)	-0.57 (5.0)	0.74 (5.3)
Total hip (n = 89)	-0.20 (3.6)	-0.68 (3.8)	0.77 (3.4)*
Hand (n = 53)	-0.82 (2.4)	-1.2 (2.6)	-0.63 (2.3)

* $p < 0.001$ versus those without good response.

Table 3 Number of patients losing, maintaining and gaining bone mineral density during 1-year treatment with infliximab

	Gain n (%)	Maintain, n (%)	Lose, n (%)
Vertebral-spine (n = 102)	32 (31)	39 (38)	31 (30)
Total hip (n = 89)	14 (16)	61 (68)	15 (16)
Hand (n = 53)	6 (11)	22 (42)	25 (47)

Associations between changes in disease activity, BMD and bone markers

The change in the BMD of the spine, hip and hand was numerically larger in patients without good EULAR response than in patients achieving a good response (a decrease in $\text{DAS} > 1.2$ resulting in a $\text{DAS} < 3.2$; table 2). This difference was significant between these two groups at the hip.

The BMD changed non-significantly in both the prednisone users and the non-prednisone users, by $+0.2\%$ and -0.04% at the spine, by -0.2% and -0.28% at the hip, and by -0.9% and -0.7% at the hand, respectively. No significant difference was found in change in BMD between the prednisone and non-prednisone users.

Subgroup analysis for bisphosphonate use also did not show any difference in the change in BMD between the users and non-users.

In the multivariate linear regression model investigating the influence of disease activity on markers of bone metabolism, we found a relationship between the change in β -CTx and the change in DAS-28 and C reactive protein during the 0–14-week time interval ($C = 0.302$, $B = 0.015$, $p < 0.05$ and $C = 0.315$, $B = 0.002$, $p < 0.05$, respectively). At the other time intervals there was no significant relationship between β -CTx and markers of disease activity. OPG showed a weak inverse association with the change in DAS-28 at all the time intervals (data not shown). No significant association was found between changes in disease activity and levels of osteocalcin or RANKL at any of the time intervals.

DISCUSSION

The main findings of this study were that 1-year treatment with infliximab arrests generalised bone loss in patients with rheumatoid arthritis, without stopping the localised bone loss at the hands.

An increased generalised bone loss is usually found in observational studies of patients with rheumatoid arthritis owing to the negative effects of rheumatoid arthritis on bone loss (disease activity and immobilisation).^{2–3} We compared our results with those of historical control groups from earlier studies of patients with established rheumatoid arthritis, which have shown a bone loss of up to 0.7% at the spine and -1.1% at the hip during 1 year, when treated with conventional DMARDs.^{22–24} The generalised annual bone loss in patients with early rheumatoid arthritis is even larger: -4.2% at the spine and -2.1% at the hip.^{25–26} Our study population consisted of patients with early and established rheumatoid arthritis, and 27 patients had disease duration < 3 years, emphasising the favourable changes in BMD we found in our study.

The z scores in our patients showed an increasing trend, supporting the observation that treatment with infliximab arrests loss of BMD in patients with rheumatoid arthritis. Comparison of our data with the data derived from historical control groups having methodological limitations clearly shows that bone loss is far less in patients treated with an active regimen of TNF α blockers.

Figure 1 (A–E) Changes in median β -isomerised carboxy terminal telopeptide of type 1 collagen (β -CTx, resorption), osteocalcin formation, receptor activator of the NF κ B ligand (RANKL), osteoprotegerin (OPG) and RANKL/OPG ratio during treatment with infliximab. * $p < 0.05$ versus baseline.

In contrast with the effects on BMD of the spine and the hip, BMD of the hand measured by DXR decreased considerably. There is a lack of data on hand BMD measured by DXR in patients with established rheumatoid arthritis treated with conventional DMARDs. Therefore we do not know how this bone loss at the hand compares with other cohorts of patients with rheumatoid arthritis. A comparable finding was observed in a small pilot study comparing patients with rheumatoid arthritis ($n = 10$ in each group) treated with infliximab or methotrexate. In this study, the bone loss was fully arrested in the infliximab group at the spine and hip but not at the hands.²⁷ In the present study, patients with a good EULAR response had increased BMD of the spine and hip. However, bone loss at the hand was also not fully arrested in the good responder group. The bone loss at the hands may suggest that the negative effects of patients with rheumatoid arthritis are not fully blocked. This hypothesis of a suboptimal anti-inflammatory control at the hands is supported by findings from a recently published double-blind randomised controlled trial showing that the rate of hand bone loss measured by DXR was considerably lower in patients with rheumatoid arthritis treated with prednisolone than in patients treated with placebo, but dropped markedly over time in both treatment groups.²¹ In the large randomised controlled trial of patients with rheumatoid arthritis treated with infliximab, radiological damage was also not fully arrested, especially in patients treated with infliximab 3 mg/kg. Radiological joint damage was, however, fully arrested in the high-dose infliximab group (10 mg/kg).^{16, 28} Thus, despite infliximab having a favourable

effect on the disease activity in most patients, it might not fully block the radiological damage and bone loss at the hands at a dose of 3 mg/kg.

Bone resorption, as depicted by β -CTx, decreased during the treatment with infliximab (as expected), whereas bone formation (assessed by osteocalcin) increased. We found that the change in disease activity (DAS-28 and C reactive protein) between 0 and 14 weeks was independently related to the change in bone resorption marker β -CTx between 0 and 14 weeks. This supports the view that infliximab reduces disease activity and thereby prevents bone loss.

The change in osteocalcin was not observed in patients not using prednisone, and was more pronounced in the patients with rheumatoid arthritis using prednisone (data not shown). Prednisone is a well-known suppressor of bone formation.²⁹ Hence, the increase in osteocalcin is probably induced by a decrease in prednisone dosage during the study, because of the good clinical response to infliximab.

The osteoclast-activating protein RANKL showed a linear decrease during the treatment with infliximab. This resulted in a favourable change in the RANKL/OPG ratio. In the Combinatie Therapie Bij Reumatoide Artritis study, an intervention study in patients with early rheumatoid arthritis, the RANKL/OPG ratio at baseline was a good predictor of radiological joint damage.³⁰ Overall, the changes in bone markers during 1 year with infliximab treatment support the bone-sparing effect of infliximab observed in our study.

In summary, our study shows that in patients with rheumatoid arthritis treated with infliximab, loss of BMD

at the hip and spine is arrested, but not that at the hands. The fact that bone loss was arrested at the hip and the spine seems to be to a large extent due to a decrease in disease activity.

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