Osteoporos Int (1999) 10:20–25

Osteoporosis International

Original Article

Bone Mass Change During Methotrexate Treatment in Patients with Juvenile Rheumatoid Arthritis

M. L. Bianchi[1], R. Cimaz[2], E. Galbiati[1], F. Corona[2], R. Cherubini[1] and M. Bardare[2]

[1]Centro Metabolismo Minerale e Osseo – Divisione Endocrinologia, Istituto Auxologico Italiano IRCCS, Milan; and [2]Clinica Pediatrica I dell'Università di Milano, Milan, Italy

Abstract. Thirty-two children affected by juvenile rheumatoid arthritis (JRA) were studied with serial measurements of bone mass for an average of 18 months, to evaluate the effects of long-term methotrexate (MTX) treatment on bone. Bone mineral density (BMD) was measured on lumbar spine and total body. During MTX therapy some increase in BMD was observed, though this was smaller than in a control group of healthy children. Axial (spine and trunk) and appendicular (upper and lower limbs) BMD showed similar increases. BMD, either as an absolute value or as a percent variation from baseline, did not correlate with either MTX dose or length of therapy. In children treated also with corticosteroids, these drugs negatively influenced bone mass increase. The main determinant of absolute spine BMD value appeared to be weight, while height and lean mass seemed to be the determinants of total body BMD. Pubertal stage and disease activity significantly influenced the yearly change in BMD. In conclusion, our data suggest that long-term, low-dose therapy with MTX does not induce osteopenia in children with JRA.

Keywords: Bone mass; Bone mineral density; Children; Methotrexate; Rheumatoid arthritis

Introduction

Methotrexate (MTX), a folic acid antagonist widely used in chemotherapeutic regimens, was introduced about a decade ago for the treatment of rheumatoid arthritis (RA), at a low dose administered weekly. Double-masked, placebo-controlled trials have established the efficacy of MTX in the treatment of adult and juvenile RA [1–4]. Toxicity is the main reason for discontinuing MTX in RA patients; the most important side-effects are hepatotoxicity and gastrointestinal complaints, while pulmonary and hematologic toxicity are uncommon [5,6].

Recent studies have focused on the possible effects of MTX on bone. MTX osteopathy was recognized in children with leukemia treated with high doses of MTX [7]. Moreover, two studies on patients with malignancies suggested an effect of MTX on bone mineral density (BMD) that could be dose-dependent [8,9]. Animal and in vitro cellular studies showed that even low doses of MTX can be toxic for bone [10,11], and one of these studies showed that the drug can accumulate in the cortical and trabecular bone of RA patients [11]. We could locate only a few studies on the effect of MTX on bone in patients affected by RA, all of them performed on adult subjects [12–15]. We believe it is of the greatest importance to examine the effects of MTX treatment for RA in the young, as their skeletal growth is already impaired by the disease. We therefore carried out a longitudinal study to assess the changes in BMD during low-dose weekly MTX treatment in children with juvenile rheumatoid arthritis (JRA).

Materials and Methods

Patients

Thirty-two patients (25 females, 7 males) fulfilling the American College of Rheumatology criteria for the classification of JRA [16] were studied. The age at onset

Correspondence and offprint requests to: Maria Luisa Bianchi, MD, Bone Metabolic Unit, Istituto Auxologico Italiano, IRCCS, via L. Ariosto, 13, I-20145 Milan, Italy. Tel: +39 02 5821 1483. Fax: +39 02 5821 1519. e-mail: ml.bianchi@auxologico.it.

of disease ranged from 6 months to 13 years (mean 5.9 ± 4.6 years). The onset of disease was oligoarticular in 13 cases, systemic in 10 and polyarticular in 9. During the study (i.e., between the first and the last BMD measurement) 22 patients had not yet entered puberty, 5 had already entered puberty and 5 had reached pubertal maturity. All patients were taking non-steroidal anti-inflammatory drugs (NSAIDs) and 16 were also taking corticosteroids (prednisone), and continued this treatment throughout the study. In addition, MTX was given as a second-line drug for arthritis. The age at the start of MTX treatment ranged from 2.6 to 15.4 years (mean 9.8 ± 5.3 years). Sixteen children were admitted to the study just before starting MTX therapy (Group 1). The other 16 children were admitted during established MTX therapy (Group 2). A control group of 45 healthy children matched for age and sex, who were not taking any drug, was also studied.

Oral consent to be included in the study was obtained from the children's parents, and the study was approved by the ethics committees of both the authors' institutions.

Methods

The study has been in progress for 18 months on average and is continuing, with periodical measurement of BMD in all patients. MTX was administered intramuscularly, once a week, at an initial dose of 10 mg/m^2 body surface. The dose was progressively increased up to 25 mg/m^2 per week in the case of absent or insufficient clinical response.

The patients in group 1 underwent their first BMD measurement before starting MTX therapy, and the second measurement after at least 6 months of MTX therapy. Those in group 2 underwent baseline BMD evaluation upon admittance to the study, after about 3 years of MTX therapy (mean duration 27 ± 7.6 months), and the second measurement at least 6 months later. All the patients have had at least two BMD measurements, many of them three, and some even four, always at intervals of at least 6 months. In the control group children, BMD was measured twice: immediately upon recruitment, and after an interval of 12–24 months.

At each BMD measurement, weight, height, number of active joints and erythrocyte sedimentation rate (ESR) were recorded. The total dose of MTX per square meter taken up to the last densitometric study, as well as that of corticosteroids taken in the meantime, was also recorded. BMD was measured on lumbar spine and total body, using dual-energy X-ray absorptiometry (DXA). For all but the first 6 measurements (in 6 children of group 2), a Hologic QDR 2000 device (Hologic, Waltham, MA) was used. In those first 6 cases from group 2, the baseline measurement of BMD was performed using a Hologic QDR 1000W. The QDR 1000W scans were later reanalyzed with the software of the Hologic QDR 2000, and these recalculated results were used in the study. In addition, in these 6 cases the second measurement was performed twice on the same day with both the old QDR 1000W and the new QDR 2000, in order to verify that the different devices did not give significantly different results.

All BMD measurements were performed according to the following protocol. Lumbar spine scans were made with the patient lying supine on the imaging table, the legs raised by a support to reduce the physiologic lordosis and to align the disk spaces with the X-ray beam. In the lumbar spine the selected region of interest (ROI) included L2–4, with the spine well centered in the analysis box. Total body scans were made with the patient lying supine, the legs straight on the table, the feet stretched, and the arms moved away from the body and turned with palms toward the table. Total body measurement included BMD, fat and lean mass. Different subregions of interest were also analyzed, i.e., head, arms, legs, spine (thoracic and lumbar spine), ribs, pelvis. For BMD and both fat and lean masses, we considered the following ROIs: limbs (arms + legs), trunk (spine + ribs), pelvis. Bone mass was expressed as BMD (mg/cm^2). In our laboratory the coefficients of variation (CV) were <1% and <1.3% respectively for spine and total body.

Statistical analysis was performed using Excel 7 (Microsoft 1995) for descriptive statistics and Statgraphics 2.1 (Statistical Graphics) for regression analysis. The mean and standard deviation are reported for each variable. Paired Student's *t*-tests were used to compare baseline and final data for normally distributed variables; nonparametric tests were used for variables not normally distributed. ANOVA was used to compare changes between groups and controls. Simple and stepwise regression analyses were performed. Stepwise regression was chosen in order to identify the best subset of variables predictive of bone density in our RA children.

To calculate the difference between two successive measurements (T_n and T_{n-1}), we calculated the percent variation, i.e.:

$$\text{Variation } (\%) = \frac{T_n - T_{n-1}}{T_{n-1}} \times 100$$

Results

The data obtained from the two groups of children were analyzed both separately and together. In both groups, age, sex distribution, anthropometric data and disease severity (as assessed by the ESR value at the start of MTX treatment) were comparable (Tables 1, 2). In both groups, an increase in bone mass was observed during MTX therapy. Such increases were not significantly different between the two groups (Table 3), but the increase observed in each group was significantly different with respect to the control group of healthy children matched for age and sex (Fig. 1). Analyzing

Table 1. Anthropometric data

	Group 1	Group 2
No. of patients	16	16
Sex	13F, 3M	12F, 4M
Age (years)	8.9 ±4.1	8.7 ±4.7
Weight (kg)	30 ±16.7	28.3 ±14.7
Height (cm)	127.4 ±22.7	125.5 ±23.5
Body surface (m^2)	1.02±0.09	0.97±0.09
Body mass index	17.6 ±4.4	17.5 ±3.1

Body mass index = Weight in Kg /(Height in m)^2

Table 2. Clinical data

	Group 1	Group 2
Disease duration (months)	56.2±46	181 ±106
Duration of MTX therapy (months)	16 ±9.5	53.6±19
MTX cumulative dose (mg)	728 ±623	2231 ±665
No. of patients treated with CS	6	10
CS cumulative dose (mg)	3980 ±2238	7287 ±6618
ESR (mm/h) at start of MTX	60.3±8.8	65.8±9.1

MTX, methotrexate; CS, corticosteroids; ESR, erythrocyte sedimentation rate.

Table 3. Bone mass data (absolute values)

	Spine (mg/cm^2)	Total body (mg/cm^2)
Group 1		
BMD before MTX	620±190	760±120
BMD after MTX	650±210*	800±140*
Group 2		
BMD baseline	620±170	740±100
BMD after MTX	660±200**	770±90**

*p<0.02 versus before value (paired Student's t-test).
**p<0.02 versus baseline value (paired Student's t-test).

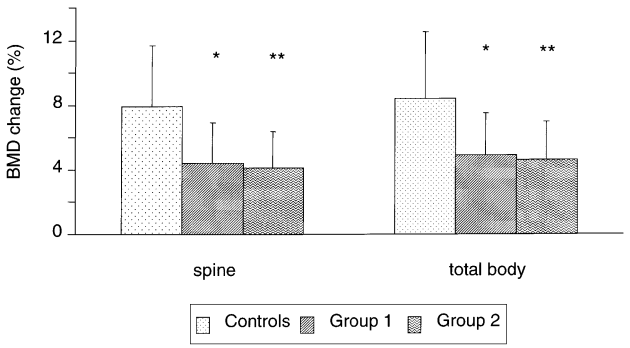

Fig. 1. Yearly increase in bone mineral density (BMD) in the two groups of children with juvenile rheumatoid arthritis (JRA) treated with methotrexate compared with a group of controls matched for age and sex. In both groups of children with JRA some increase in BMD was observed for both spine and total body. These increases were not significantly different between the two groups (* = NS). However, these increases were significantly lower (** = ANOVA, p<0.02) than those observed in controls.

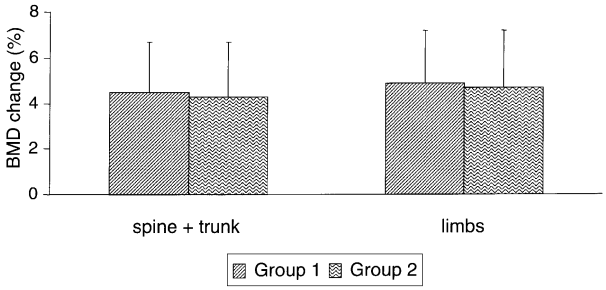

Fig. 2. On a total body scan of the axial (spine + trunk) and appendicular (lower and upper limbs) skeleton BMD showed similar rates of increase in both groups of children treated with methotrexate.

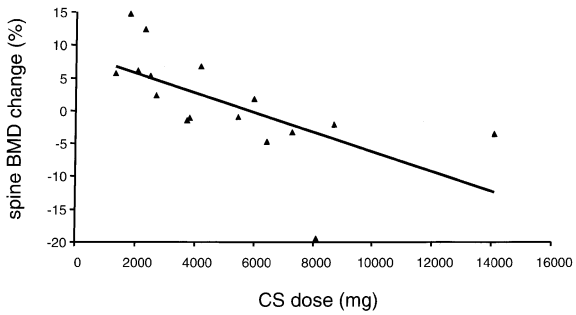

Fig. 3. The change in spine BMD (i.e., the difference between the first and last BMD values, calculated as a percentage) versus cumulative dose of corticosteroids (CS) showed a significant negative regression: $r = -0.63$, p<0.01.

Table 4. Correlations between bone mass data and anthropometric and clinical data

Versus	Spine BMD	Total body BMD
Age	r = 0.30 (NS)	r = 0.41 (p<0.05)
Weight	r = 0.92 (p<0.001)	r = 0.83 (p<0.01)
Height	r = 0.49 (p<0.05)	r = 0.92 (p<0.001)
Body mass index	r = 0.50 (p<0.05)	r = 0.62 (p<0.02)
Lean mass	r = 0.58 (p<0.02)	r = 0.93 (p<0.001)
Fat mass	r = 0.65 (p<0.01)	r = 0.50 (p<0.05)
	Yearly change in spine BMD	*Yearly change in total body BMD*
Pubertal stage	r = 0.63 (p<0.02)	r = 0.65 (p<0.02)
ESR decrease	r = 0.58 (p<0.02)	r = 0.52 (p<0.02)

Table 5. Stepwise regression between BMD data and anthropometric data

Independent variables (included in the model)	Spine BMD (dependent variable) Model R^2 = 0.52		Total body BMD (dependent variable) Model R^2 = 0.61	
	Partial R^2	(p value)	Partial R^2	(p value)
Weight	0.32	(0.001)	0.15	(NS)
Height	0.13	(NS)	0.31	(0.001)
Lean mass	0.16	(NS)	0.30	(0.001)
Fat mass	0.27	(0.01)	0.09	(NS)
Age	0.11	(NS)	0.13	(NS)

axial BMD (i.e., spine and trunk) and appendicular BMD (i.e., upper and lower limbs) separately, a similar increase was observed, thus indicating the presence of a uniform increase at the different skeletal sites (Fig. 2).

Bone mass, considered either as an absolute value or as a percent variation, did not correlate with MTX therapy: neither cumulative MTX dose nor treatment length appeared to influence bone mass (r = 0.17 and $r = 0.12$, respectively). This result did not change according to whether the two groups were considered separately or together. In contrast, the cumulative dose of corticosteroids clearly exerted a negative effect on bone mass: in particular, spine BMD showed a high negative regression with the cumulative corticosteroid dose (Fig. 3), while total body BMD appeared to be only slightly affected by steroid use ($r = -0.39$, $p = 0.05$). Different factors had a significant influence on the absolute value of bone density: age, weight, height and lean mass showed a significant correlation with both spine and total body BMD (Table 4). The same correlation was observed in both groups. In particular, the main determinant of spinal BMD appeared to be weight (stepwise regression, $R^2 = 0.32$), while height and lean mass seemed to be the most important factors influencing total body BMD (R^2 = 0.31 and 0.30, respectively) (Table 5). Pubertal stage and disease activity (as indicated by the decrease in the ESR value during therapy) seemed to have an influence on the yearly change in BMD (Table 4). The most consistent increase in bone mass (+12.7 ± 4.3%) was observed in the 5 children who completed puberty during our period of observation.

Discussion

In RA, juxta-articular osteopenia at the level of involved joints [17,18] is commonly observed. A generalized osteopenia/osteoporosis is often found, due to many concurrent factors, such as reduced mechanical load (poor mobility), long-term steroid treatment, and the humoral and local factors of the inflammatory process. The complexity of the disease, the multi-organ involvement, the degree of immobilization and the various drugs used for treatment have not yet allowed researchers to draw definitive conclusions on the main factors affecting bone.

The introduction of methotrexate in the treatment of JRA raises the question whether it could be a further risk factor for bone loss. Some studies [7–12] have pointed to a possible direct toxic action of this drug on bone tissue, especially in younger patients. MTX has been extensively used for the treatment of leukemia in children and some studies reported multiple fractures, osteopenia, bone pain [7,19,20] and delayed healing of fractures [7]. Two points must, however, be stressed. In malignancies, MTX must be taken in very high doses for long periods. Moreover, in these studies the patients affected by osteoporosis and/or fractures often suffered from multisystemic MTX toxicity. In a recent study on 23 young adults affected by osteosarcoma, MTX (either 7.5 g/m^2 or 750 mg/m^2 in 9 infusions over a period of 8 months) was used without inducing fractures or bone pain: but in the patients treated with the higher doses, a lower bone mineral content at ultradistal radius was observed [21]. Recently, it was reported that MTX for 18 months (no dosage specified) was associated with a significantly reduced BMD in the forearm of 11 postmenopausal women affected by primary biliary cirrhosis, compared with 11 women in a similar condition but not on MTX (no significant differences where observed in BMD at spinal and femoral sites) [22].

The use of MTX for the treatment of RA has two special features. First, the disease requires long-term therapy, so that the risk of bone toxicity must be specifically assessed. Second, relatively low doses are generally needed. A study reported localized osteopenia and bone pain in two elderly patients affected by RA and treated with MTX [13]: however, given the patients' age, we cannot exclude other pathogenetic mechanisms underlying skeletal alterations. A few studies on small animals, and one study on synovial and bone tissues of patients submitted to surgery for rheumatoid articular lesions, demonstrated the presence of MTX in both trabecular and cortical bone [12,23–27]. This was linked to the possibility of bone damage, on the basis of inhibition of osteoblastic activity and stimulation of osteoclastic recruitment, resulting in a net increase in bone resorption. In a brief report on 20 aged patients affected by RA and treated with MTX, a significant reduction in forearm BMD was observed compared with 20 subjects not receiving MTX [15]. In both groups BMD was lower than normal, and this may reflect only the frequently observed peri-articular osteopenia of the wrist (or elbow) in RA patients. Moreover, this study included postmenopausal women, who could be independently affected by postmenopausal osteoporosis. In a recent study on a large number of adult patients affected by RA, treatment with MTX for more than 3 years did not induce changes in spine or hip BMD different from those of patients not treated with MTX [14].

The lack of specific clinical studies on children with JRA induced us to summarize the results of our current research, even if it is not completely exhaustive yet. To our knowledge, this is the first longitudinal study on a relatively large number of children aimed at measuring bone mass changes during chronic therapy with MTX. We have not included a control group of children with the same degree of disease but not treated with MTX, as we considered it unethical to exclude children from such an effective therapy as MTX, during an active phase of their disease, for the long duration of the study. In order partially to obviate this problem, we studied a group of children who were about to start MTX therapy (group 1) and a group already on long-term MTX treatment (group 2). The choice of two such groups allowed us to verify whether the de novo introduction of the drug could highlight a different behavior of bone mass with respect to a course of therapy started about 3 years previously.

According to our results, MTX seems not to influence bone mass either at the beginning of treatment or after a long-term therapy. Both the length of MTX therapy and total administered dose were taken into account. As previously stated, the single MTX dose was low and not likely to induce adverse effects on bone tissue. However, considering the cumulative dose, a substantial amount of the drug could certainly be accumulated in bone cells. We only considered bone mass, an epiphenomenon of bone cellular processes. A direct evaluation of bone cells was not attempted for obvious reasons in a pediatric study. A uniform increase in bone mass at the different sites of the skeleton was observed, with both appendicular and axial sites showing similar increases. Thus, MTX seemed not to influence specifically trabecular or cortical bone even in the case of high total doses. Perhaps in the studies on leukemic patients bone toxicity was induced by very high single doses, while low single doses of MTX (even given over a relatively long time) never reach cellular levels toxic for bone.

In accordance with our previously published data [28,29] and other reports [30], corticosteroids showed a negative action on bone mass, and in particular on the predominantly trabecular sites, such as spine. In fact, lower increases in spinal bone mass were found in patients with higher cumulative doses of corticosteroids. Recent data confirm that low doses (5–9 mg/day) of prednisone for long-term therapy are also able to lower bone mass [31].

In our children the main determinants of bone mass were anthropometric parameters. Weight seemed to account for approximately 32% of the variability of bone mass at the spinal level, while height and lean mass were responsible for 31% and 30% of the total body mass respectively. Chronological age was not the main determinant of bone mass, contrary to the situation in normal children. This observation was not surprising, however, as we are considering chronically ill children. On the basis of the severity, evolution and episodes of recruitment of the underlying disease, children of the same age presented completely different physical conditions, different clinical pictures and different therapy regimens, and this might explain the secondary role of age also in bone mineralization. In fact, as happens in kidney transplanted children and children with other pathologic conditions, age was not correlated with bone density in the present study.

Two factors played a role in the changes in bone mass during the period of observation: pubertal stage and disease activity (expressed as the ESR value). Puberty is a potent, normal physiologic stimulus for bone mineralization, and it is interesting that in JRA puberty maintains its dominant role. The maximum increase in bone mass was observed in the 5 children who completed puberty, and in each case puberty status (i.e., Tanner stage) was correlated with the rise in BMD.

Disease activity was a criterion for starting MTX therapy, so that all the children presented active disease. ESR was one of the indexes used to judge the response to MTX therapy: a decrease in ESR of at least 50% compared with the pre-treatment value was considered a positive response. During MTX, in almost all cases, a reduction in disease activity was observed. The reduction in ESR was correlated with the increase in bone mass, indicating that an improvement in the disease was accompanied by an improvement in bone status. The reduction in disease severity obtained by MTX and the reduction or withdrawal of corticosteroids (with the amelioration of their adverse effects such as puberty delay or osteopenia) might both have a positive effect on bone. The overall (spine and total body) increase in bone mass was obviously lower in children with JRA than in normal children. However, an increase in bone mass indicated the presence of a positive calcium balance in bone, even if the increase was less than expected on the basis of age. In conclusion, our data suggest that low-dose MTX treatment does not negatively influence bone mass by inducing osteopenia and that, if corticosteroids can be reduced or withdrawn, it could even aid in the protection of skeletal integrity in growing children affected by JRA.

References

1. Weinblatt ME, Coblyn JS, Fox DA. Efficacy of low-dose methotrexate in rheumatoid arthritis. N Engl J Med 1985;312:818–22.
2. Williams HJ, Willkens RF, Samuelson CO Jr, Alarcón GS, Guttadauria M, Jarboro C, et al. Comparison of low-dose oral pulse methotrexate and placebo in the treatment of rheumatoid arthritis: a controlled clinical trial. Arthritis Rheum 1985;28:721–30.
3. Weinblatt ME, Weissman BN, Holdsworth DE, Fraser PA, Maier AL, Falchuk KR, et al. Long-term prospective study of methotrexate in the treatment of rheumatoid arthritis: eighty-four-month update. Arthritis Rheum 1992;35:129–37.
4. Alarcón GS, Tracy IC, Blackburn WD Jr. Methotrexate in rheumatoid arthritis: toxic effects as the major factor in limiting long-term treatment. Arthritis Rheum 1989;32:671–6.
5. Nesbit M, Kribit W, Heyn R, Sharp H. Acute and chronic effects of methotrexate on hepatic, pulmonary and skeletal systems. Cancer 1976;37:1948–54.
6. Ragab AH, Fresh RS, Vietti TJ. Osteoporotic fractures secondary to methotrexate therapy of acute leukemia in remission. Cancer 1970;25:580–5.
7. Gnudi S, Butturini L, Ripamonti C, Avella M, Baci G. The effects of methotrexate on bone: a densitometric study conducted on 59 patients with methotrexate administered at different doses. Ital J Orthop Traumatol 1988;14:227–31.
8. Stanisavljevic S, Babcock AL. Fractures in children treated with methotrexate for leukemia. Clin Orthop Rel Res 1977;125:139–44.
9. May KP, Sterling GW, McDermott MT, Huffer WE. The effect of low-dose methotrexate on bone metabolism and histomorphometry in rats. Arthritis Rheum 1994;37:201–6.
10. Scheven BAA, van der Veen MJ, Damen CA, Lafeber FPJG, van Rijn HJM, Bijlsman JWJ, Duursman SA. Effects of methotrexate on human osteoblasts in vitro: modulation by 1,25-dihydroxyvitamin D_3. J Bone Miner Res 1995;10:874–80.
11. Bologna C, Edno L, Anaya J-M, Canovas F, Berghe MV, Jorgensen C, et al. Methotrexate concentrations in synovial membrane and trabecular and cortical bone in rheumatoid arthritis patients. Arthritis Rheum 1994;37:1770–3.
12. Preston SJ, Diamond T, Scott A, Laurent MR. Methotrexate osteopathy in rheumatic disease. BMJ 1993;52:582–5.
13. Katz JN, Leboff MS, Wade JP, Brown EM, Liang MH. Effect of

methotrexate on bone density and calcium homeostasis in rheumatoid arthritis. Clin Res 1989;37:509A.
14. Buckley LM, Leib ES, Cartularo KS, Vacek PM, Cooper SM. Effects of low dose methotrexate on the bone mineral density of patients with rheumatoid arthritis. J Rheumatol 1997;24:1489–94.
15. Minaur NJ, Elvins D, Ring F, Beresford JN, Bhalla AK. Proximal forearm bone mineral density is reduced in rheumatoid arthritis on long-term methotrexate. Osteoporos Int 1996;6(Suppl 1):S295.
16. Cassidy JT, Levinson JE, Bass JC, Baum J, Brewer EJ Jr, Fink CW, et al. A study of classification criteria for a diagnosis of juvenile rheumatoid arthritis. Arthritis Rheum 1986;29:274–81.
17. Robison DR. Tashijan AH, Levine L. Prostaglandin-stimulated bone resorption by rheumatoid synovia. J Clin Invest 1985;56:1181–8.
18. Krane SM. Mechanisms of tissue destruction in rheumatoid arthritis. In: Mc Carthy DJ, editor. Arthritis and allied conditions. 10th ed. Philadelphia: Lea & Febiger, 1985:593–604.
19. O'Regan S, Melhorn DK, Newman AJ. Methotrexate-induced bone pain in childhood leukemia. Am J Dis Child 1973;126:489–90.
20. Schwartz AM, Leonidas JC. Methotrexate osteopathy. Skeletal Radiol 1984;11:13–6.
21. Ripamonti C, Avella M, Gudi S, Figus E. Effetti del methotrexate ad alte e basse dosi sulla massa ossea in soggetti trattati per osteosarcoma delle estremità. Minerva Med 1993;84:131–4.
22. Blum M, Wallestein S, Clark J, Luckey M. Effect of methotrexate treatment on bone in postmenopausal women with primary biliary cirrhosis. J Bone Miner Res 1996;11(Suppl 1):S436.
23. May KP, Mercill D, McDermott MT, West SG. The effect of methotrexate on mouse bone cells in culture. Arthritis Rheum 1996;39:489–94.
24. Friedlaender GE, Tross RB, Doganis AC, Kirkwood JM, Baron R. Effects of chemotherapeutic agents on bone. J Bone Joint Surg [Am] 1984;66:602–7.
25. Wheeler DL, Vander Griend RA, Wronski TJ, Miller GJ, Keith EE, Graves JE. The short- and long-term effects of methotrexate on the rat skeleton. Bone 1995;16:215–22.
26. Ward SB, Smith JB, Maccario D, Abruzzo JL. Serum osteocalcin in assessment of methotrexate induced osteoporosis. Arthritis Rheum 1992;35(Suppl 5):R5.
27. Tishler M, Caspi D, Graff E, Segal R, Peretz H, Yaron M. Synovial and serum levels of methotrexate therapy of rheumatoid arthritis. Br J Rheum 1989;28:422–3.
28. Bianchi ML, Bardare M, Caraceni MP, Cohen E, Falvella S, Borzani M, et al. Bone metabolism in juvenile rheumatoid arthritis. J Bone Miner Res 1990;9:153–62.
29. Bardare M, Bianchi ML, Furia M, Gandolini GG, Cohen E, Montesano A. Bone mineral metabolism in juvenile chronic arthritis: the influence of steroids. Clin Exp Rheumatol 1991;9:29–31.
30. Sambrook PN, Jones G. Corticosteroid osteoporosis. Br J Rheum 1995;34:8–12.
31. Cooper C, Coupland C, Mitchell M. Rheumatoid arthritis, corticosteroid therapy and hip fracture. Ann Rheum Dis 1994;54:49–52.

Received for publication 17 July 1998
Accepted in revised form 10 November 1998