ELSEVIER

Clinica Chimica Acta 348 (2004) 147–154

www.elsevier.com/locate/clinchim

Markers of bone remodeling predict rate of bone loss in multiple sclerosis patients treated with low dose glucocorticoids

Jan J. Stepan[a,*], Eva Havrdová[b], Michaela Týblová[b], Dana Horáková[b], Veronika Tichá[b], Iveta Nováková[b], Vít Zikán[a]

[a] *Department of Internal Medicine 3, Charles University Faculty of Medicine, U Nemocnice 1, CZ-128 00, Prague, Czech Republic*
[b] *Department of Neurology, Charles University Faculty of Medicine, U Nemocnice 1, CZ-128 00 Prague, Czech Republic*

Received 24 February 2004; received in revised form 5 May 2004; accepted 7 May 2004

Abstract

Background: The aim of this study was to evaluate the clinical value of markers of bone remodeling in assessment of rate of bone loss in patients with multiple sclerosis (MS) long term treated with low dose glucocorticoids. *Methods:* The study involved 70 patients with MS. Motor function of the patients was evaluated using the Kurtzke Expanded Disability Status Scale (KEDSS). Bone mineral density (BMD) was determined at the lumbar spine and proximal femur at baseline and after 1.8 ± 0.8 years. Bone remodeling was assessed using circulating concentrations of type 1 collagen cross-linked C-telopeptide (beta CTX), aminoterminal propeptide of type I procollagen, and N-MID osteocalcin (OC). A control group of 140 age-matched healthy subjects was used to compare bone-turnover markers. *Results:* The plasma CTX concentration was the most significant parameter of bone remodeling which correlated with the rate of bone loss and with the KEDSS. The rate of bone loss at the proximal femur was not significantly different between tertiles of plasma OC concentrations. *Conclusion:* In physically active patients with MS treated with low-dose GC, the bone-turnover markers were not different from controls. Patients having plasma CTX but markers of bone formation higher as compared to controls were confirmed 2 years later as bone losers.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Biochemical markers; Collagen; Glucocorticoids; Multiple sclerosis; Osteoporosis

1. Introduction

Prevention of bone fractures is an appropriate way to reduce osteoporosis-related health expenditures (health care costs, morbidity and mortality) and to improve quality of life of the patients. The risk of osteoporosis during the following years depends on the current bone mass as well as on the rate of bone loss and microarchitectural deterioration predisposing to bone fragility. Rates of loss of bone mineral are difficult to measure precisely over a period of less than 2 years because bone mineral density (BMD) measurement errors for most of the dual-energy X-ray absorptiometry instruments are similar in magnitude to the average clinically relevant rate of loss of 1–3% per year [1]. Therefore, alternative ways to assess the rate of bone loss are needed.

Using radiotracers, high correlations have been found between the biochemical markers of the bone turnover (namely urinary deoxypyridinoline DPD) and

* Corresponding author. Tel.: +420-224962913; fax: +420-224922370.
E-mail address: jstepan@vol.cz (J.J. Stepan).

0009-8981/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.cccn.2004.05.012

PLAINTIFF'S DEPOSITION EXHIBIT

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

the calcium kinetics [2], indicating that the biochemical markers of the bone turnover have a potential to assess the rate of the bone loss and to improve the prediction of the risk of osteoporosis in postmenopausal women. Biochemical markers reflect the whole body rates of bone resorption and bone formation and may provide a more representative index of the overall skeletal bone loss than it would be obtained by measuring the rates of change in bone mineral density at specific skeletal sites containing different ratios of a trabecular to cortical component with different metabolic rates. In postmenopausal women, a sustained increase in bone turnover induces a faster bone loss and therefore an increased risk of osteoporosis, and biochemical markers are associated with bone loss measured at the forearm, calcaneus and hip, with the progressively greater risk of a rapid bone loss with increasing levels of markers [3].

Bone loss due to glucocorticoids is steep during the first 12 months, and more gradual but continuous in subsequent years [4]. The mechanism of bone loss in patients on glucocorticoid treatment is complex [5]. Unlike estrogen deficiency in which both bone resorption and formation increased, a major effect of glucocorticoids is on osteoblast function. In addition to a decrease in osteoblast differentiation, glucocorticoids increase osteoblast and osteocyte apoptosis [6]. Depression of bone formation by glucocorticoid excess results in disruption of the fine balance among rate of supply of new osteoblasts and osteoclasts and the timing of the death of osteoclasts, osteoblasts, and osteocytes by apoptosis [7]. Clinically, decreased bone formation can be documented by decrease in circulating concentration of markers of osteoblast function such as osteocalcin and propeptides of type I procollagen [8,9].

The role of bone resorption in bone loss characteristic of glucocorticoid-induced osteoporosis is unexplained. The carly loss of bone with glucocorticoid excess is caused by extension of the life span of preexisting osteoclasts, an effect not preventable by bisphosphonates [10]. However, the effect of long-term low dose glucocorticoid administration remains controversial. Long-term effects of glucocorticoids on osteoclast function are inhibitory rather than stimulatory [11–13]. Clinically, an accelerated bone resorption can be documented by increase in serum or urinary concentration of collagen breakdown products such as the deoxypyridinoline cross-links and the markers involving the cross-linking site, the cross-linked N-terminal telopeptide of type I collagen (NTX), and the C telopeptide of type I collagen alpha1 (CTX) [14].

The contribution of the underlying disease, for which glucocorticoids are used, confounds the assessment of glucocorticoid effects on bone. Multiple sclerosis (MS) is a gait disorder characterized by acute episodes of neurological defect leading to progressive immobilization [15]. Long-term glucocorticoid use and progressive immobilization, along with vitamin D deficiency, are likely to be determinants for osteoporosis and skeletal muscle atrophy, and the increased risk of fracture in patients with multiple sclerosis [16–18].

The aim of this study was to evaluate the clinical value of markers of bone remodeling in assessment of rate of bone loss in patients with multiple sclerosis long term treated with low dose glucocorticoids, and to test the relative contribution of glucocorticoid dose and physical inactivity to rate of bone loss in patients with multiple sclerosis.

2. Patients

The study population involved 47 women and 23 men with multiple sclerosis (Table 1). Thirty-one

Table 1
Baseline characteristics of patients with multiple sclerosis (mean ± S.D.)

	Women	Men	All patients
No	47	23	70
Age (years)	40.3 ± 10.2	42.4 ± 12.1	41.0 ± 10.9
Weight (kg)	60.4 ± 8.0	76.7 ± 11.6	65.8 ± 12.0
Height (cm)	165.1 ± 6.4	180.3 ± 6.9	170.1 ± 9.7
Duration of MS (years)	10.0 ± 7.4	14.0 ± 6.0	11.3 ± 7.2
Glucocorticoids (years)	6.0 ± 5.4	6.7 ± 5.1	6.2 ± 5.3
Mean dose (mg/day)	7.4 ± 2.9	7.1 ± 2.7	7.3 ± 2.8
Cumulative dose (g)	29.9 ± 22.5	42.1 ± 25.8	33.9 ± 24.2
KEDSS	4.1 ± 1.8	5.0 ± 1.9	4.4 ± 1.9
BMD lumbar spine (*T*-score)	− 1.29 ± 1.27	− 1.81 ± 1.33	− 1.46 ± 1.30
BMD total femur (*T*-score)	− 1.25 ± 1.34	− 1.74 ± 1.17	− 1.41 ± 1.30
BMD femoral neck (*T*-score)	− 1.67 ± 1.33	− 2.01 ± 1.36	− 1.78 ± 1.34
Smokers (%)	44.7	52.2	47.1

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

women were premenopausal (mean age, 35.1 ± 8.1 years), 16 postmenopausal women (mean age, 50.3 ± 5.0 years) were on regular hormone replacement therapy. The patients received the daily recommended dose of calcium (500 mg) and vitamin D (400 IU). Excluded were patients with diseases, disorders or therapy with other drugs known to influence bone metabolism.

3. Materials and methods

3.1. Clinical assessment

Motor function of the patients was evaluated using the Kurtzke Expanded Disability Status Scale (KEDSS), a scale useful in measuring the ability to walk that is decisive for normal remodeling of bone [19]. Scoring agreement for trained examining physicians has been repeatedly confirmed [20,21]. Cut-off KEDSS 6 represents reasonable end of motor performance of the patient. 6.5 means only several meters with bilateral support, mostly within the flat, and 7 is only the ability of transfer to wheelchair from the bed. Glucocorticoid use was determined from an interviewer-administered questionnaire

3.2. Bone densitometry

BMD (g/cm^2) was determined using Hologic 4500A (Waltham, MA) densitometer. Normative values provided by Hologic (NHANES III normative values for the proximal femur) were used for the determination of *T*-scores (comparison with a gender-matched young normal reference population). The short-term precision in vivo error for BMD at the lumbar spine (L1–L4), total femur, and femoral neck was 0.7%, 0.9%, and 1.9%, respectively; the long-term precision in vitro error was 0.31%. BMD was measured at baseline and after 1.8 ± 0.8 years.

3.3. Biochemical analysis

Blood specimens were collected in the morning, after an overnight fast. Biochemical markers of bone turnover were measured at the baseline. Normative values for this laboratory were determined in age-matched healthy subject (94 premenopausal women, and 46 men).

The serum concentrations of intact aminoterminal propeptide of type I procollagen (PINP) was assessed by radioimmunoassay (Procollagen Intact PINP, Orion Diagnostica, Finland). The assay is not sensitive to the small molecular weight degradation products of the propeptide. The within run imprecision was below 5%, and between run imprecision was below 7% at concentrations between 20 and 90 ug/l. Normal values (mean and 1 S.D. range) determined in the control group were 33.1 ug/l (25.3–43.1 ug/l) and 35.2 ug/l (27.0–45.9 ug/l) in women and men, respectively.

The concentration of type 1 collagen cross-linked C-telopeptide (beta CTX) and N-MID osteocalcin (OC) in plasma was assessed using electrochemiluminesce-based immunoanalysis (the Elecsys 1010 Analyzer, Roche Diagnostics, Germany). The within run imprecision of the CTX was below 5% for samples >500 pg/ml, below 7% for samples between 200 and 500 pg/ml and below 10% for very low CTX concentrations samples. The between run imprecision results were below 7% for samples >500 ng/l and below 9% for samples between 200 and 500 ng/l. The detection limit was <10 ng/l. Normal values (mean and 1 S.D. range) determined in the control group were 267 ng/l (204–350 ng/l) and 294 ng/l (220–393 ng/l), in women and men, respectively. The within run imprecision for the OC was <5%, and between run imprecision was <6% at concentrations between 11 and 40 ug/l. Normal values (mean and 1 S.D. range) determined in the control group were 20.6 ug/l (15.5–27.4 ug/l) and 21.2 ug/l (15.6–28.9 ug/l), in women and men, respectively.

3.4. Statistical analysis

Data were expressed as mean value ± standard deviation if not otherwise stated. Logarithmic transformation was used to normalize non-normal distributions. The original skewed data were tested to confirm the validity of the transformation. The differences in clinical and biochemical characteristics between groups were compared using *t*-test. The differences in biochemical markers between groups of patients with MS and controls were compared using one-way ANOVA. Subsequent Bonferroni *t*-test

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

Table 2
Kurtzke Expanded Disability Status Scale (KEDSS), biochemical markers of bone remodeling and rate of bone loss (RBL, % per year) in patients with multiple sclerosis

	Women	Men	All patients
No	47	23	70
KEDSS ≥ 6 (%)	29.8	56.5	38.6
RBL lumbar spine	− 0.69 ± 2.53	− 1.29 ± 3.50	− 0.89 ± 2.87
RBL total femur	− 1.69 ± 2.37	− 2.96 ± 3.46	− 2.10 ± 2.82
RBL femoral neck	− 2.31 ± 2.79	− 2.84 ± 3.19	− 2.49 ± 2.90
Osteocalcin (ug/l)	19.2 (10.0–36.9)	20.6 (13.4–31.8)	19.7 (10.9–35.4)
PINP (ug/l)	33.3 (19.1–58.4)	38.5 (24.6–60.3)	35.0 (20.6–59.2)
beta CTX (ng/l)	318 (188–538)*	387 (234–640)*	349 (213–570)

*$p < 0.05$ as compared with the normal values for this laboratory, in healthy subjects (t-test).

was used to determine differences between the groups. The correlation was analyzed using least square linear regression analysis. A $p < 0.05$ was considered statistically significant. Analyses were made using the SigmaStat statistical software version 3.0 (Jandel, San Rafael, USA).

4. Results

BMD T-score ≥ 1 S.D. at the lumbar spine and proximal femur was found in 20% patients (11 women and 3 men, KEDSS, 3.3 ± 2.0; bone loss at the total femur, −1.10 ± 2.06% per year). BMD T-score between −1 and −2.5 S.D. was found in 45.7% patients (23 women and 9 men, KEDSS, 4.0 ± 1.8; bone loss, −1.52± 1.97% per year). BMD T-score below −2.5 S.D. was found in 34.3% patients (13 women and 11 men, KEDSS, 5.6 ± 1.3; bone loss, −3.47± 3.63% per year).

Mean values for the variables are shown in Tables 1 and 2. The biochemical markers are compared with the corresponding values in the control group.

The correlation matrix for the variables is shown in Table 3. In patients with multiple sclerosis, a significant positive correlation was found between biochemical markers of bone remodeling (Fig. 1). However, only the marker of bone resorption (plas-

Table 3
The correlation matrix and the statistics for the "best" subset in the multiple linear regression analysis for rate of bone loss (RBL) at the proximal femur as dependent variable in patients with multiple sclerosis

Variable	1	2	3	4	5	6	7	8
1 RBL femur	–							
2 RBL neck	0.44*							
3 RBL spine	0.24	0.34*						
4 KEDSS	− 0.56*	− 0.39*	− 0.11					
5 CTX	− 0.45*	− 0.46*	− 0.12	0.46*				
6 PINP	− 0.21	− 0.22	− 0.08	0.26	0.55*			
7 Osteocalcin	− 0.12	− 0.11	− 0.03	0.24	0.46*	0.86*		
8 GC total dose	− 0.25	− 0.23	− 0.01	0.42*	0.02	− 0.05	− 0.07	
9 BMI	0.13	− 0.02	0.01	− 0.11	− 0.04	0.01	− 0.08	− 0.08
Multiple correlation				0.60				
Adjusted squared mult. correlation				0.34				
F-statistics (df 2.67)				19.12				
Significance				< 0.001				

Variable	Coefficient	Standard error	t	Significance
KEDSS	− 0.682	0.165	− 4.14	< 0.001
Beta-CTX	− 0.003	0.001	− 2.19	0.03
Constant	2.172	0.744	2.92	0.005

*Correlation coefficients at the 0.005 level of significance.

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

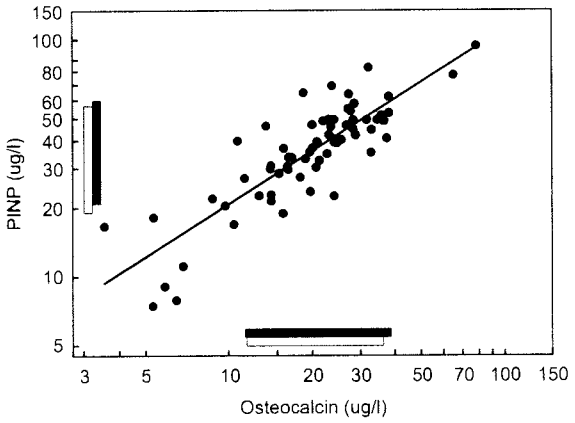

Fig. 1. Correlation between circulating osteocalcin and PINP ($y = -46.0 - 3.86x$, $r = 0.46$, $n = 120$, $p < 0.001$). ln $y = 0.768 \ln x + 1.269$, $r = 0.86$, $n = 70$, $p < 0.001$. The solid bars indicate normal range ($x \pm 2$ S.D.) for this laboratory (light: women, dark: men).

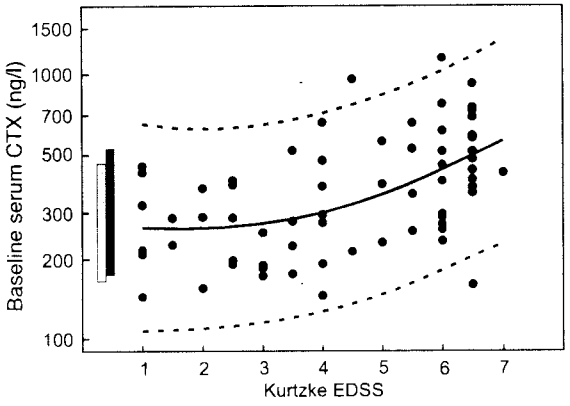

Fig. 3. Relationship between the Kurtzke EDSS and plasma CTX. Dotted line: prediction intervals. ln $y = -0.093x + 0.027x^2 + 5.64$, $r = 0.51$, $n = 70$, $p < 0.001$. The solid bars indicate normal range ($x \pm 2$ S.D.) for this laboratory (light: women, dark: men).

ma CTX) correlated significantly with the rate of bone loss at the proximal femur (Fig. 2) and with KEDSS (Fig. 3). The rate of bone loss at the proximal femur was not significantly different between tertiles of plasma OC concentrations (ANOVA).

The multiple regression analysis of the results in multiple sclerosis indicated that the plasma CTX concentration was the most significant parameter of bone remodeling which correlated with the rate of bone loss and with the motor function of the patients (KEDSS) (Table 3). None of the remaining variables (age, weight, height, duration of multiple sclerosis, duration of GC treatment and the average and total dose of GC) entered the regression model. After adjustment for BMI, the plasma CTX concentration remained significant predictor of the rate of bone loss ($p < 0.05$).

Mean values for the biochemical markers of bone remodeling and rate of bone loss by KEDSS are shown in Table 4.

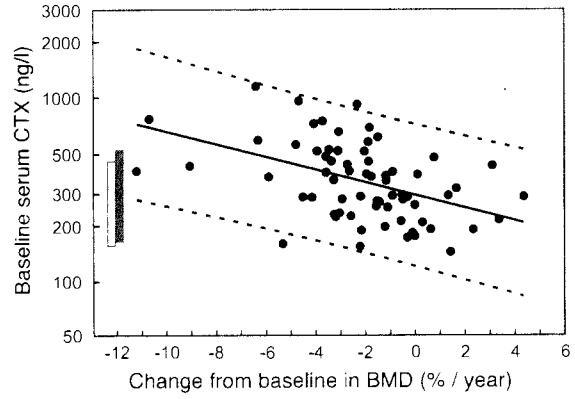

Fig. 2. Relationship between the rate of BMD change at the total proximal femur and plasma CTX. Dotted line: prediction intervals. ln $y = -0.079x + 5.867$, $r = 0.59$, $n = 70$, $p < 0.001$. The solid bars indicate normal range ($x \pm 2$ S.D.) for this laboratory (light: women, dark: men).

Table 4
Biochemical markers of bone remodeling and rate of bone loss (RBL, % per year) by Kurtzke Expanded Disability Status Scale (KEDSS) in patients with multiple sclerosis

	KEDSS 1–5.5	KEDSS>5.5
No	43	27
RBL femur	−1.02 ± 2.18	−3.83 ± 2.89*
RBL neck	−1.74 ± 2.85	−3.70 ± 2.58*
RBL spine	−0.82 ± 3.21	−1.00 ± 2.28
beta-CTX	295 (190–458)	455 (287–720)**
PINP	31.8 (19.6–51.7)	40.7 (23.2–71.4)***
Osteocalcin	17.9 (10.3–30.9)	23.0 (12.3–42.8)

*$p < 0.005$ as compared with KEDSS 1–5.5 (t-test).
**$p < 0.05$ as compared with KEDSS 1–5.5 and with control group (ANOVA).
***$p < 0.05$ as compared with KEDSS 1–5.5, not with control group (ANOVA).

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.