

## BIOCHEMICAL MARKERS OF CALCIUM AND BONE METABOLISM IN THE MONITORING OF OSTEOPOROSIS TREATMENT

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### Abstract

Biochemical markers of bone turnover are extremely useful in monitoring the treatment in osteoporosis. Due to the fact that in postmenopausal osteoporosis, bone resorption is increased and biochemical markers are found to be significantly increased, a possible utility in evaluating the treatment effect emerged. It was proved that antiresorbptive treatment is decreasing the bone resorption markers serum level, which is a relevant aspect for treatment monitoring.

The aim of the present study was to evaluate the predictive value of the bone turnover markers changes for bone mineral density response in women treated for postmenopausal osteoporosis, for one year with antiresorbptive therapy.

The obtained results were conclusive: antiresorbptive treatment decreased significantly serum osteocalcin level and increased significantly serum parathyroid hormone (PTH) concentration after 6 months. The decrease in osteocalcin levels after 6 months was significantly correlated to the increase in lumbar spine bone mineral density (BMD) after one year of treatment. 25-OH vitamin D levels did not change significantly in 6 months of oral supplementation, probably due to the short time but also to insufficient doses in a deficient population.

In clinical practice, monitoring treatment with bone markers is useful mostly to identify the pure responder or non-responder to the treatment. This aspect could indicate and sustain an early change in treatment, before the first evaluation of the bone mineral density by DXA.

### Rezumat

Markerii biochimici ai *turnover*-ului osos sunt extrem de utili în monitorizarea tratamentului osteoporozei. Datorită faptului că în osteoporoza postmenopauză, resorbția osoasă este crescută și markerii biochimici au nivele semnificativ crescute, s-a evidențiat o posibilă utilitate a acestora în evaluarea efectului tratamentului. S-a demonstrat că tratamentul antiresorbtiv scade nivelul seric al markerilor resorbției osoase, care este un aspect relevant pentru monitorizarea tratamentului.

Scopul acestui studiu a fost de a evalua capacitatea predictivă a markerilor *turnover*-ului osos la femei tratate pentru osteoporoza post-menopauză, timp de un an, cu terapie antiresorbtivă. Rezultatele obținute au fost concludente: tratamentul antiresorbtiv a scăzut semnificativ nivelul osteocalcinei serice și a crescut concentrația serică a parathormonului, după 6 luni. Scăderea nivelurilor de osteocalcină după 6 luni a fost corelată în mod semnificativ cu creșterea densității minerale osoase a coloanei vertebrale lombare, după un an de tratament. Concentrația serică a 25 HO vitaminei D nu s-a modificat semnificativ în cele 6 luni de suplimentarea orală, probabil din cauza timpului scurt, și a dozelor insuficiente administrate unei populații deficitare.

În practica clinică, monitorizarea tratamentului cu ajutorul markerilor osoși este util mai ales pentru identificarea precoce a pacienților care răspund, sau nu, la tratament. Acest aspect ar putea indica și susține o schimbare a abordării terapeutice la începutul tratamentului, chiar înainte de evaluarea densității minerale osoase.

**Keywords:** osteoporosis, osteocalcin, osteoprotegerin, bone metabolism.

### Introduction

Biochemical markers of bone turnover are extremely useful in monitoring the treatment in osteoporosis. Due to the fact that in postmenopausal osteoporosis, bone resorption is increased and biochemical markers are found to be significantly increased, a possible utility in evaluating the treatment effect emerged. It was proved that antiresorbptive treatment is decreasing the bone resorption markers serum level; this aspect was found relevant for treatment monitoring [1, 7, 16].

Consequently, decreasing the bone resorption became the target of the antiresorbptive treatment.

In clinical studies, the decrease of the bone resorption occurs earlier than the increasing bone mineral density, so it might be a reliable marker for predicting the treatment response [10, 11].

The decrease in bone resorption markers in the first months of treatment, with more than 30% compared to the base line, it has a good predictive value for a good response in terms of bone mineral density and also decrease in fracture risk. Thus, monitoring the bone turnover specific resorption or formation specific biochemical markers is a mandatory aspect in research and a useful tool in clinical practice. The use of such markers in clinical practice is still limited due to the lack of specificity: in individuals, we still do not have reliable cut off levels for rigorous diagnostic differentiation [12, 14, 17].

The aim of the study was to evaluate the predictive value of the bone turnover markers changes for bone mineral density response in women treated for postmenopausal osteoporosis.

## Materials and Methods

*Subjects* were 72 women (mean age 63 years) with postmenopausal osteoporosis treated for one year with antiresorbative therapy in the Elias Hospital Endocrinology Department, Bucharest, Romania.

*Methods.* Osteoporosis diagnostic was based on the WHO (World Health Organization) criteria, the T-score expressed as standard deviations (SD), either lumbar spine or neck, below or equal to  $-2.5$ . Bone densitometry was evaluated using a GE Lunar DXA (dual X-ray absorptiometry) for lumbar (L1-L4) scan and femoral (neck) scans, based on the DXA evaluation standard deviations. Results were expressed as bone mineral density (BMD) at lumbar and femoral neck sites, T score (SD resulted from comparison to young adult database) and Z score (SD resulted from comparison to age and sex matched database). All these parameters were evaluated at baseline and after one year of antiresorbative treatment for further analyses.

For bone metabolism, total serum calcium, total alkaline phosphates (total ALP), the parathyroid hormone (PTH), 25-Hydroxy Vitamin D (25-OH vitamin D), serum osteocalcin, serum osteoprotegerin and serum beta-crosslabs were measured at baseline and after 6 months of treatment. Serum osteocalcin, osteoprotegerin and 25-OH vitamin D were assessed using immunologic ELISA methods, PTH was analysed through a chemiluminometric method, while calcium and ALP were measured using spectrophotometric methods.

*Ethics statement.* Our research involved human participants and has been approved by the Elias Hospital Ethics Committee in January 2009. All clinical investigations have been conducted according to the principles expressed in the Declaration of Helsinki and patients were included in the study after signing the informed consent.

*Statistical analysis.* Data analysis was performed using the Statistical Program for Social Sciences for Windows (SPSS, Version 20). Variables were tested for normal distribution across study groups using Shapiro-Wilk test and comparison between mean, median and skewed values. Descriptive summary measures of the study variables were computed as appropriate: mean and standard deviation (SD) in normal distributed variables and median and interquartile range (IQR) in other variables. Pearson Correlation's coefficients were computed for bivariate correlation and significant correlations were discussed based on the p value. Student's t test was used to compare variables across normal distributed variables. Non-parametric Mann Withney U test was used to compare the continuous variables between study groups and  $\chi^2$  test for categorical data when

variables were not normal distributed. For predictive value of different variable, the Discriminant function in SPSS was used.

A p value < 0.05 was considered statistically significant.

### Results and Discussion

From baseline characteristics, it may be seen from table I the mean age and BMI (body mass index) of the patients: all patients were postmenopausal and had a normal BMI in average. The T score at lumbar spine of -3.65 SD shows that most of the patients had very low BMD (bone mineral density) comparing to young adults database. This was expected since all of them had a T score less than -2.5 SD either at spine or femoral neck. As is commonly found in osteoporosis, femoral T score was higher in average comparing to lumbar spine. There were some differences between Z score and T score in baseline, aspect which may suggest some cases with early menopause or unknown secondary cases of osteoporosis.

*One year treatment effect on bone densitometric dynamics.* After one year of standard treatment with antiresorbative drugs associated with vitamin D supplementation, the lumbar spine increased significantly (Table I); all other bone densitometric values remained unchanged during the follow up period. The femoral neck density actually decreased during the follow up but without significance. This aspect is normal, in small sized study groups and also in individuals, after only one year of treatment, no significant change in femoral neck could be found due to more slowly bone metabolism in the hip bones compared to vertebral bones.

**Table I**  
Densitometric parameters dynamics

	Baseline	One year follow up
Age (years)	63.00±14.6	64.10±14.9
BMI (kg/m <sup>2</sup> )	23.17±3.21	23.24±3.21
Spine BMD*(g/cm <sup>2</sup> )	0.793±0.162	0.796±0.150
Spine T score (SD)	-3.65±0.6	-3.45±0.5
Spine Z score (SD)	-2.17±0.79	-1.9±0.81
Femoral BMD (g/cm <sup>2</sup> )	0.759±0.54	0.752±0.62
Femoral T score (SD)	-1.72±0.32	-1.69±0.52
Femoral Z score (SD)	-0.37±0.7	-0.47±0.8

\*p<0.05 (Student t test; data are expressed as mean and SD)

*Biochemical parameters dynamics after 6 months of treatment.* In table II it can be seen the evolution of all the measured biochemical parameters at baseline and after 6 months of treatment. After 6 months of antiresorbative treatment associated with 1 g calcium and 800 UI vitamin D3,

daily, or 0.5 mcg alphacalcidol daily in selected cases, urinary calcium excretion, total ALP, osteocalcin, 25-OH vitamin D and PTH suffered significant changes. The increase in the urinary calcium is somehow in contradiction with the decrease of the bone resorption, but on the other hand, the calcium supplementation could have been the cause of increased calcium excretion, even more the 25-OH vitamin D decreased significantly. We may say that vitamin D supplementation was not sufficient in a population with low levels of vitamin D at the baseline. It is also known that it takes more than 6 months of supplementation to increase the vitamin D serum levels.

The osteocalcin serum levels decreased significantly with no change in the crosslaps levels (Table II). This aspect reveals an inhibitory effect of the treatment on the bone turnover even the specific resorption marker, crosslaps did not decrease significantly. The increase in the PTH levels during the treatment (Table II) is the common result when antiresorptive treatment is used due to the inhibition of the bone resorption. It can be said that it is an indirect marker of the bone resorption inhibition during treatment.

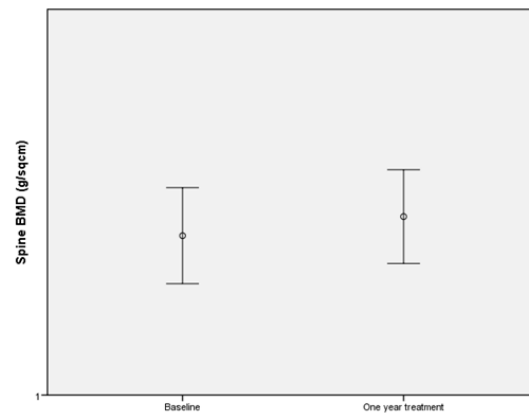
In a small number of patients (n=16), osteoprotegerin was measured in baseline and after 6 months of treatment. No significant change in this marker was registered (Table II).

**Table II**  
Bone markers dynamics

	Baseline	Six months follow up	p value*
Serum total Calcium (mg/dL)	9.4±0.12	9.6±0.16	NS
Calcium excretion (mg/24 hrs)	65±1.30	87±1.70	<0.03
Serum Phosphate (mEq/L)	2.9±0.16	3.2±0.2±	<0.05
Total ALP (UI/L)	78 ±14.4	65± 18	<0.05
Blood urea nitrogen (g/dL)	0.75±0.5	0.72±0.6	NS
Osteocalcin (ng/mL)	19±15.75	13.55±11.32	<0.01
Betacrosslaps (ng/mL)	0.53±1	0.356±0.627	NS
PTH (pg/mL)	66.8±10.7	79±8.2	<0.01
25-OH vitD (ng/mL)	18±16.41	17.5±16	<0.01
Osteoprotegerin (pmol/L)	5.15±0.54	5.16±1	NS

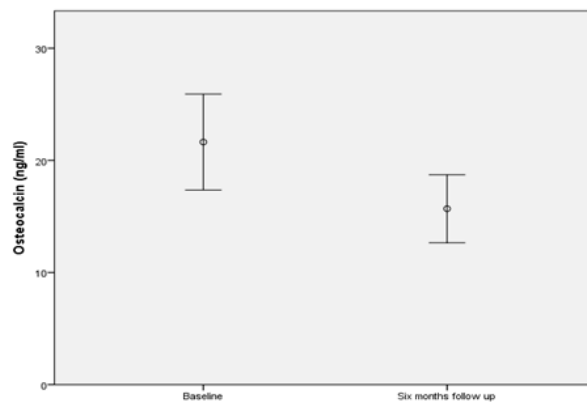
\*p<0.05 (Student t test; data are expressed as mean and SD)

After one year of treatment, lumbar spine BMD increased significantly (Fig. 1) suggesting the efficacy of the antiresorptive treatment.

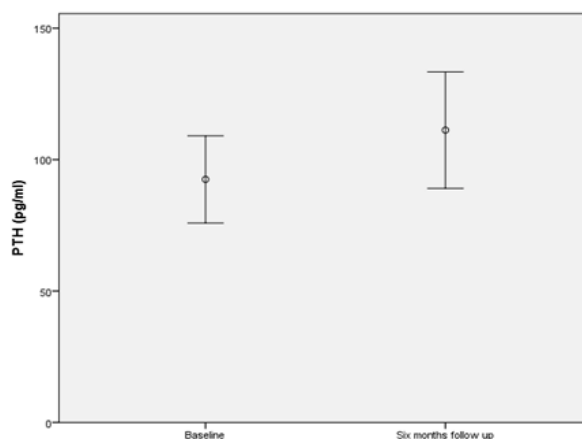
**Figure 1**

The evolution of lumbar spine BMD

In terms of biochemical parameters of the bone turnover, we may conclude that after 6 months of antiresorptive treatment associated with calcium and vitamin D supplementation, osteocalcin decreased significantly (Fig. 2) and PTH increased significantly (Fig. 3) as indicators for early inhibition of the bone turnover during treatment.

**Figure 2**

Serum osteocalcin after 6 months of antiresorptive treatment BMD



**Figure 3**

Serum PTH after 6 months of antiresorbptive treatment

In order to evaluate the predictive value of this early change in biochemical parameters for the later occurring increase in BMD correlation coefficients were tested for differences in lumbar spine BMD (sBMD), serum levels of osteocalcin (Oscdif) and serum levels of crosslaps (crossdif) respective.

When looking to the significant results, it can be seen that the BMD increase after one year of treatment can be predicted by the osteocalcin decrease measured after 6 months of treatment ( $r=-0.284$ ,  $p<0.001$ ). The decrease in osteocalcin serum levels after 6 months of antiresorbptive treatment might be an early indicator of successful or poor response therapy.

In our data, patients treated with antiresorbptive treatments showed a significantly decrease in the values of osteocalcin after 6 months of treatment, which was in concordance with the response of the bone mineral density. So, we found a significant difference between the values of bone turnover markers osteocalcin after 6 months of treatment, compared to the baseline.

After 6 months of treatment, osteocalcin level decreased by 29% compared to the base line, showing a constant decrease and almost a normalization of the bone turnover on treatment. The decrease in osteocalcin levels in the first months of treatment is correlated with the increase of the bone mineral density after one year of treatment.

Osteoprotegerin levels were not modified significantly after 6 months of treatment. Osteoprotegerin is not known as a bone marker for treatment efficiency but, being correlated with the osteoblast activity, we have observed the treatment induced changes.

In our data there were no significant differences during treatment in terms of osteoprotegerin serum levels. One limitation of our studies is the lack of RANK (Receptor activator of nuclear factor kappa-B) ligand values measurements, which has to be correlated and reported to the osteoprotegerin levels.

In clinical practice, monitoring treatment with bone markers is useful mostly to identify the pure responder or non-responder to the treatment [11]. Actually, in the case of the lack of decrease of bone turnover in the first 6 months after treatment, the patient may be among the poor responder subjects and it is a high risk for osteoporotic fractures. This aspect could indicate and sustain an early change in treatment, before the first evaluation of the bone mineral density by DXA.

### Conclusions

Antiresorptive treatment decreased significantly serum osteocalcin and increased significantly serum PTH after 6 months.

The decrease in osteocalcin levels after 6 months was significantly correlated to the increase in lumbar spine BMD after one year of treatment sustaining the predictive value of early measurement of biochemical bone turnover markers before the DXA BMD evaluation.

25-OH vitamin D levels did not change significantly in 6 months of oral supplementation, probably due to the short time but also to insufficient doses in a deficient population.

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