Role of bone markers in monitoring the therapeutic effect of bisphosphonates in postmenopausal osteoporosis

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ABSTRACT

Objective: Aim of the study was to detect values of bone markers as a way to reflect response to therapy in osteoporosis.

Materials and methods: The study involved data of 73 female patients suffering from postmenopausal osteoporosis that were treated with bisphosphonates in the period of twelve months. Values of osteocalcin, P1NP and β-Cross Laps were measured before starting therapy, six months and twelve months after the start of therapy. Bone mass density (BMD) was measured with densitometry before therapy and twelve months after the start of therapy. Values of bone markers were determined by in vitro quantitative determination in human serum and plasma. Data base was configured in Microsoft Office Excel 2010. Statistical analysis was done by IBM SPSS Statistics v. 21.0 for Windows programme. Data was represented in table and graph form with help of classic methods of descriptive statistics.

Results: Results in this study have showed significant reduction in serum values of bone formation markers (osteocalcin, P1NP) and also bone resorption markers (β-cross laps) after inducing bisphosphonate therapy in patients with postmenopausal osteoporosis during a period of 12 months. Results have also showed significant increase in bone mass density respectively increase in vertebral T-score values and also increase in femoral T-score values.

Conclusion: dynamics of bone markers significantly reflects therapeutic effect of bisphosphonate therapy in postmenopausal osteoporosis.

Keywords: osteoporosis, therapy, bisphosphonates, P1NP, osteocalcin, β-Cross Laps

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INTRODUCTION

Osteoporosis is defined as systemic bone disease with decrease in bone mass and destruction of bone architecture and consequential bone fragility and fractures. [1] It’s one of the main causes of morbidity and mortality in the elders. [2] According to World Health Organization it is estimated that around 200 million people with osteoporosis worldwide have caused about 2.5 million bone fractures per year. [3] The National Osteoporosis Foundation (NOF) estimates that 55% of all Americans aged 50 and older in the year 2002 had either osteoporosis or osteopenia (low bone mass) [4]. Treatments for osteoporosis have been shown to increase bone strength and reduce fracture risk. The drugs most commonly used to treat osteoporosis are bisphosphonates: stable analogs of naturally occurring inorganic pyrophosphate [5]. Bisphosphonate therapy (alendronate, risedronate or zoledronic acid) is recommended for reducing the risk of vertebral and non-vertebral fractures in postmenopausal women and men over 50 years of age at high risk of fracture (those with osteoporosis by bone mass density criteria or a prior minimal trauma fracture). [6]

Dual-energy X-ray absorptiometry or densitometry (DXA) is used to diagnose osteoporosis and assess fracture risk and also to monitor treatment effect. Many clinical practice guidelines, including those of the National Osteoporosis Foundation, recommend the use of DXA to monitor osteoporosis therapy. The suggested interval between baseline and follow-up bone mass density (BMD) testing after starting therapy is typically one to two years, with subsequent intervals determined according to clinical circumstances [7].

It is of great value to find a way for earlier insights in effects of therapy to encourage patients in properly taking therapy or to discuss reasons of treatment failure. [8] Bone markers are a group of bio molecules that are produced during the process of bone formation and...
resorption and are more and more acknowledged as crucial diagnostic help in management of osteoporosis. Bone markers do not replace bone densitometry but are significant complementary tool that allows us to have information of bone mass loss. Bone markers reflect different steps in bone formation and resorption process. They reflect metabolic activity of bone cells and products that are released during bone formation or fragments that are transported into blood during bone resorption process. Bone formation markers that are most commonly used are osteocalcin, PINP, PICP and ALP. Most commonly used bone resorption markers are TRAP and Tartrate Resistant Acid Phosphatase (TRAP) and degradative bone marrow products (C-terminal telopeptide type I-ICTP, β-CrossLaps, N-terminal telopeptide type I-NTX) [9,10,11,12,13].

Considering growing number of patients with osteoporosis in population and wider use of antiresorptive therapy, in the year 2000, International Osteoporosis Foundation (IOF) has released recommendations on using bone markers in monitoring therapy response and detecting early risks of osteoporotic fractures, as well as in choosing the best therapy for osteoporosis. The recommendation is to use at least one bone formation marker and one bone resorption marker, preferably markers that are measured in serum rather than ones measured in urine. [14]

Primary aim of this study was to detect values of bone markers as a way to detect response to therapy in osteoporosis.

**MATERIALS AND METHODS**

This was a retrospective-prospective study conducted at Nuclear Medicine Clinic, Clinical Centre University of Sarajevo, from May, 2013, to January 2015. Approval was obtained from the Ethical committee of Medical Faculty University of Sarajevo. All patients provided written informed consent to participate in the study. Eligibility criteria were female gender, age from 50 and older, diagnosed osteoporosis with densitometry. Exclusion criteria were male patients and patients who had secondary osteoporosis. 73 patients were included in the study. Patients were treated with bisphosphonate- Alendronate, 70 mg per week. We measured values of osteocalcin, PINP and β-Cross Laps in serum, in the month before starting therapy, six months and twelve months after the start of therapy. BMD was measured with densitometry before therapy and twelve months after the start of therapy.

Values of bone markers were determined by in vitro quantitative determination in human serum and plasma. Main test for all three of bone markers was sandwich test principle. Blood was taken from patients and it then went into the process of two incubations. After this, the reaction mixture was aspirated into the measuring cell where the microparticles were magnetically captured onto the surface of electrode.

Application of a voltage to the electrode then induced chemiluminescent emission which was measured by a photomultiplier. Results were determined via a calibration curve which is instrument-specifically generated by 2-point calibration. Database was configured in Microsoft Office Excel 2010. After checking data integrity statistical analysis was done by IBM SPSS Statistics v. 21.0 for Windows programme. Data was represented in table and graph form with help of classic methods of descriptive statistics.

For the description of the sample depending on the nature of the data, adequate methods of classical descriptive statistics were used: arithmetic mean (M), standard deviation (SD), median (Me), interquartile range - IQR (Q1 or 25.Perc. and Q3 or 75.Perc., The absolute frequency (n), relative frequency (%), cumulative frequency (n), the normal frequency distribution of continuous numerical variables was performed by histogram, quantile diagrams and formal testing by Kolmogorov-Smirnov test. Differences between the first and second measurements - Δ1, first and third measurements - Δ2 and second and third measurements - Δ3 for osteocalcin, β-crosslaps and PINP were performed and the difference between the first (at the beginning) and the second measurement (After 12 months) for T-score vertebrae and T-score femur.

**RESULTS**

Mean value of osteocalcin in serum at the start of therapy (µg/L) was 33.7 (IQR=24.6 to 45.7), 6 months...
after therapy it was 24.1 (IQR=19.8 to 34.4) and 12 months after inducing therapy it was 18.5 (IQR=14.1 to 26.2), therefore there was significant decrease in serum values of osteocalcin after inducing anti-resorptive therapy. (Table 1)

Friedman’s test was done to determine difference in values of osteocalcin in serum in the 12/month research period. Comparison of couples was done with Bonferroni correction (SPSS, 2012). The significance level was (Adj.Sig) p < 0.0167. Values of osteocalcin in serum were significantly different in different points of research, $\chi^2=121.178$, p < 0.001. (Figure 1)

Mean value of $\beta$-crosslaps in serum at the start of therapy (µg/L) was 0.55 (IQR=0.38 to 0.74), 6 months after therapy it was 0.42 (IQR=0.28 to 0.58) and 12 months after inducing therapy it was 0.28 (IQR=0.16 to 0.44). (Table 2).

Friedman’s test was done to determine difference in values of $\beta$-crosslaps in serum in the 12/month research period. Comparison of couples was done with Bonferroni correction (SPSS, 2012). The significance level was (Adj.Sig) p < 0.0167. Values of osteocalcin in serum were significantly different in different points of research $\chi^2=81.986$, p < 0.001. (Figure 2).

Mean value of P1NP in serum at the start of therapy (mcg/L) was 56.6 ((IQR=39.5 do 74.6), 6 months after therapy it was 43.2 (IQR=30.1 do 55.7) and 12 months after inducing therapy it was 30.5 (IQR=21.3 do 43.1). (Table 3).

Friedman’s test was done to determine difference in values of P1NP in serum in the 12/month research period. Comparison of couples was done with Bonferroni correction (SPSS, 2012). The significance level was (Adj.Sig) p < 0.0167. Values of P1NP in serum were significantly different in different points of research $\chi^2=98.192$, p < 0.001. (Figure 3)

Mean value of vertebral T-score (SD) at the start of the research was -2.80 (IQR= -3.15 to -2.70) and 12 months after the start of therapy it was -2.70 (IQR= -2.90 to -2.60).

### Table 2. Serum values of $\beta$-crosslaps (µg/L)

<table>
<thead>
<tr>
<th>$\beta$-crosslaps</th>
<th>N</th>
<th>M</th>
<th>SD</th>
<th>Min.</th>
<th>Max.</th>
<th>Percentiles</th>
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<td>25th</td>
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<tr>
<td>Start of therapy</td>
<td>73</td>
<td>0.65</td>
<td>0.57</td>
<td>0.1</td>
<td>4.25</td>
<td>0.38</td>
</tr>
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<td>6 months after therapy</td>
<td>73</td>
<td>0.48</td>
<td>0.40</td>
<td>0.11</td>
<td>2.77</td>
<td>0.28</td>
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<tr>
<td>12 months after therapy</td>
<td>73</td>
<td>0.36</td>
<td>0.32</td>
<td>0.04</td>
<td>2.11</td>
<td>0.16</td>
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### Table 3. Serum values of P1NP (mcg/L)

<table>
<thead>
<tr>
<th>P1NP</th>
<th>N</th>
<th>M</th>
<th>SD</th>
<th>Min.</th>
<th>Max.</th>
<th>Percentiles</th>
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<td>25th</td>
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<tr>
<td>Start of therapy</td>
<td>73</td>
<td>70.4</td>
<td>70.0</td>
<td>19.5</td>
<td>449.0</td>
<td>39.5</td>
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<tr>
<td>6 months after therapy</td>
<td>73</td>
<td>52.7</td>
<td>51.4</td>
<td>16.0</td>
<td>362.0</td>
<td>30.1</td>
</tr>
<tr>
<td>12 months after therapy</td>
<td>73</td>
<td>40.1</td>
<td>47.3</td>
<td>10.1</td>
<td>300.0</td>
<td>21.3</td>
</tr>
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</table>
Out of 73 patients included in this research, 58 of them (79.5%) have had improved vertebral T-score compared to the T-score at the start of the research, before inducing anti-resorptive therapy. (Table 4).

Wilcoxon signed-rank test was done and it showed significant increase in vertebral T-score values (0.20 SD) after 12 months of research compared to the T-score at the start of the research, \( z = 6.684, p < 0.001 \).

Mean value of femoral T-score (SD) at the start of the research was -2.57 ± 0.42 and 12 months after the start of therapy it was -2.45 ± 0.42.

Paired-samples T-test was done and it showed significant increase in femoral T-score values (-0.12, 95% CI, -1.72 to -0.694) after 12 months of research compared to the T-score at the start of the research (-2.57 ± 0.42); \( t(72) = -4.699, p < 0.001 \).

Out of 73 patients included in this research, 48 of them (65.8%) have had improved femoral T-score compared to the T-score at the start of the research, before inducing anti-resorptive therapy. (Table 5).

**Discussion**

Information on metabolic changes in bones can show us some early pathologic changes in bones and reveal potential risk of developing bone diseases. Also, by measuring bone markers concentration, it is possible to get information on response to therapy faster, considering that significant changes in bone markers can be detected about three months after inducing therapy for osteoporosis as supposed to changes in BMD (bone mass density) that can be detected after one or two years (with densitometry). That is why bone markers besides having a large role in early diagnostics of osteoporosis have an even bigger role in monitoring therapy response in osteoporosis. Response to therapy can indirectly be estimated by measuring values of bone formation and bone resorption markers. Large number of studies have shown significant changes in values of these markers in response to therapy with bisphosphonates.

In our study mean values of osteocalcin in serum have decreased from 33.7 ng/ml before the start of therapy to 18.5 ng/ml 12 months after inducing therapy. Values of osteocalcin have shown statistically significant difference in different intervals of these research.

In his study on bisphosphonates in prevention of postmenopausal osteoporosis published in 2002, Ravn P has proven significant changes in bone markers after the use of bisphosphonates. The study has shown decrease in osteocalcin and P1NP values after only six months of using Ibandronate 2.5 mg a day in prevention of postmenopausal osteoporosis. [15]

Significant reductions in serum values of P1NP were shown in this study. Mean values of serum P1NP at the start of the research were 56.6 ng/mL, and 12 months after the start of therapy there were 30.5 ng/mL, thus showing statistically significant difference in different stages of research (\( \chi^2 = 98.192, p < 0.001 \)).

![Figure 3. Dot plot of serum values of P1NP](image-url)

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Out of 73 patients included in this research, 48 of them (65.8%) have had improved femoral T-score compared to the T-score at the start of the research, before inducing anti-resorptive therapy. (Table 5).

**Table 4. Values of vertebral T-score**

<table>
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<tr>
<th>Vertebral T-score</th>
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<td></td>
<td>50th (Median)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>75th</td>
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<tr>
<td>Start of therapy</td>
<td>73</td>
<td>-2.98</td>
<td>0.50</td>
<td>-4.60</td>
<td>-2.20</td>
<td>-3.15</td>
</tr>
<tr>
<td>12 months after therapy</td>
<td>73</td>
<td>-2.76</td>
<td>0.47</td>
<td>-4.20</td>
<td>-1.40</td>
<td>-2.90</td>
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</table>

**Table 5. Values of T-score of the femur**

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<thead>
<tr>
<th>Femoral T-score</th>
<th>N</th>
<th>M</th>
<th>SD</th>
<th>Min.</th>
<th>Max.</th>
<th>Percentiles</th>
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<td>50th (Median)</td>
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<td>75th</td>
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<tr>
<td>Start of therapy</td>
<td>73</td>
<td>-2.57</td>
<td>0.42</td>
<td>-3.50</td>
<td>-1.20</td>
<td>-2.80</td>
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<tr>
<td>12 months after therapy</td>
<td>73</td>
<td>-2.45</td>
<td>0.42</td>
<td>-3.20</td>
<td>-1.00</td>
<td>-2.70</td>
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Henry G. Bone and associates in their study in 2004. about 10 year experience with alendronate in therapy of postmenopausal osteoporosis have shown significant decreases in bone markers values, specifically P1NP values, after use of oral alendronate (5,10,20 mg daily). Study has also shown stabilisation of values in the period of 10 years of therapy with alendronate. [16]

In their study on effects of alendronate and hormone replacement therapy, alone and in combination on bone mass and markers of bone turnover in elderly women with osteoporosis published in 2003. Sirpa Evio and ass. have shown significant reductions in values of bone markers after 12 months of therapy. Reduction of values specifically of P1NP values were more significant in a group of patients that were on therapy with alendronate compared to those on the combination of alendronate and hormone replacement therapy. [17]

Mean value of serum β-crosslaps in this study was 0.55 ng/mL at the start of the research, and 12 months after the start of therapy it was 0.28 ng/mL and it showed statistically significant difference in different periods of research (χ²=81.986, p < 0.001).

E. Kučukalić-Selimović et al. have shown significant decreases (49%) in serum values of β-crosslaps in their study published in 2011., as well as reduction in values of serum osteocalcin in patients with osteoporosis on therapy with alendronate. [18]

In this study improvement in vertebral bone mass was detected in 79.5% (58 out of 73 patients) of patients 12 months after inducing therapy with alendronate. Improvement in vertebral bone mass was also detected in 65.8% (48 out of 73 patients) of patients.

There were statistically significant changes in vertebral ( z=6.684, p < 0.001.) as well as in femoral T-score (t(72)= -4.699, p < 0.001).

Results of the study that Pyon Ey has published 2006. have shown significant improvement in bone mass after use of ibandronate in therapy for osteoporosis in the period of 12 months. [19]

P.Ravn et al. in their research from 1996. have shown increase in vertebral bone mass as well as femoral bone mass in woman suffering from osteoporosis that were treated with ibandronate in period of 12 months. [20]

**Conclusions**

Results in this study have showed that dynamics of bone markers significantly reflects therapeutic effect of bisphosphonate therapy in postmenopausal osteoporosis. Considering all results, it is necessary to induce bone markers in the management of osteoporosis, as it is a good diagnostic tool that gives us insight in bone formation process and it enables us to early detect effects of therapy for osteoporosis.

**Declaration of interest**

Authors declare no conflict of interest.

**References:**


[17] Sirpa Eviö, Aila Tiitinen, Kalevi Laitinen, Olavi Ylikorkala, and Matti J. Välimäki Effects of Alendronate and Hormone Replacement Therapy, Alone and in Combination, on Bone Mass and Markers of Bone Turnover in Elderly Women with Osteoporosis; The Journal of Clinical Endocrinology & Metabolism, Volume 89, Issue 2 pp. 626–631

