Antiresorptive Therapy and the Bone Turnover Markers—Assessed Fracture Risk in Postmenopausal Women

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Abstract

Purpose/Introduction: The aim of this study was to determine relationship of the bone markers levels with the fracture risk and treatment monitoring in patients with osteoporosis. Bone markers may point out to on specific aspects of bone quality, detecting changes of bone mineral density, thus providing prognostic perspective and accounting for a substantial proportion of fracture risk reduction.

Methods: The case-control study comprised data from 55 patients undergoing evaluation for osteoporosis at Medicus Universalis Polyclinic in Krusevac. Densitometric findings, P1NP, CTX and osteocalcin levels were determined in all patients twice – at the first assessment and 6 months after. While 30 patients took no medical therapy, 25 of them were treated with ibandronate.

Results: No convincing difference in densitometric measurements between patients with and without prevalent fractures were noted, while mean osteocalcin and P1NP levels were significantly lower ($p<0.05$) in osteoporotic patients who suffered fractures. A significant correlation between those bone turnover markers and T-score was established, especially in the second measurement and in patients treated with ibandronate.

Conclusion: In postmenopausal women and individuals with low BMD, the presence of increased bone turnover markers suggests an increased risk of fractures. Furthermore, these metabolic markers are useful in the monitoring of patients receiving antiresorptive therapy, wherein fast decline of their levels indicate favorable course. Their determination after 6 months offers the remarkable advantage in assessing the effectiveness of medical treatment comparing to 12–24 months required to document changes by BMD.

Introduction

With extended life expectancy, aging population in different regions of the world is facing almost epidemic incidence of osteoporosis. Causes are numerous; along with increased longevity, certain medications such as corticosteroids may have direct impact on bone mass; the same can be said about frequent routine obstetric interventions aiming to induce iatrogenic (surgical) menopause and changes in the way of life.

Estimated probability for future fractures in individuals over 50 is about 40% for female and 13% in male population. The biggest socio-epidemiological problem is hip fracture, taking in consideration high mortality rate, frequent sequels, and impaired quality of life; in addition, this type of fracture burdens society with high expenses of medical treatment (20-23). Osteoporosis, clinically well defined by low bone density (low BMD) and disrupted bone microarchitecture, (established by DXA measurements, with T- score below -2,5), leads to enhanced bone fragility, and consequent suspectibility for fractures (28). Fractures most often arise in locations characterized by previous quantitative and qualitative deterioration of trabecular bone.

Technological developments in bone mineral density (BMD) measurements have led to diagnostic criteria that are widely applied. The World Health Organization diagnostic criteria for osteoporosis includes a BMD that lies 2.5 standard deviations or more below the average value for young healthy women (a T-score of <-2.5 SD) [4, 5, 15, 16].

Markers of bone turnover are biochemical products released during bone formation or reabsorption. Commonly used bone resorption markers are degradation products of type I collagen 1 (5,6), but non-collagenous proteins such as osteoclast-derived enzyme tartrate-resistant acid phosphatase 5b (TRACP) have also been investigated as resorption markers.

These compounds may be easily determined (usually in blood or urine). They reflect bone metabolic activity although they themselves have no function whatsoever in regulation of skeletal metabolism. Markers of bone formation are direct or indirect products of the osteoblasts' activity expressed during various phases of their maturation and function. Type I collagen is an important component of bone matrix, and osteoblasts secrete its precursor - procollagen during the bone formation. The terminal peptides at each end of the procollagen molecule, procollagen type 1 pro-peptide (P1NP) and procollagen type I C pro-peptide (PICP), are cleaved by enzymes during bone matrix formation and released into the circulation (9-11).

Osteocalcin is another very sensitive and specific bone formation marker, also produced by osteoblasts. It is main non-collagen product of bone matrix. Osteocalcin demonstrates high calcium binding properties and serve as a messenger for calcitriol and leukocyte esterase inhibitor. It is excreted by the kidneys and its fragments may also be measured in urine. Newly formed osteoid undergoes maturation followed by mineralization. During this phase osteoblasts secrete alkaline phosphatase (ALP) into the extracellular fluid that may be detected in serum. In healthy adults, however, about half of the measured ALP is of hepatic origin, while the other half derived from the bone. Bone-derived isoform (BALP) is now widely available. Bone-derived alkaline phosphatase is very specific and sensitive marker of enhanced bone metabolism and reliable index of effectiveness of the antiresorptive therapy (34).

The pyridinium cross-links, pyridinoline (PYD) and deoxypyridinoline (DPD), formed during the maturation of bone collagen, are present in significant amounts in bone and dentine. These compounds are released during bone resorption and excreted in urine in the free and peptide-bound forms without being metabolized. (36) The peptide-bound forms of PYD and DPD include the C-terminal and N-terminal cross-linking telopeptides (CTX, NTX) of the type I collagen. These molecules are also released into the circulation and subsequently excreted in urine (9). Estrogen deficiency, associated with menopause, results in age related increase in bone remodeling and imbalance between bone formation and resorption.

Aim

The aim of this study was to determine relationship of the bone markers levels with the fracture risk and treatment monitoring in patients with osteoporosis and to present our experience in overall research

agenda.

Methods

The case-control study comprised analytical data gathered from 55 patients undergoing evaluation and treatment protocols for osteoporosis at Medicus Universalis Polyclinic in Krusevac. All patients were previously diagnosed and staged with secondary osteoporosis by the experienced rheumatologist. Study was approved by Ethical Committee at Medical Faculty, University of Pristina – K.Mitrovica.

A diagnostic, therapeutic and follow-up protocol was formed for each patient. They were divided in 2 groups determined by presence of the osteoporotic fractures. Densitometric findings, P1NP, CTX and osteocalcin levels were determined in all patients twice – at the first assessment and 6 months after. While 30 patients took no medical therapy, 25 of them were treated with ibandronate. Within this subgroup the densitometry and blood samples were obtained at the first assessment and 6 months after the initiation of therapy.

For the verification of secondary osteoporosis, we used KSA GE – LUNOR densitometer; the densitometry was performed at spinal and hip osteoporosis predilection sites.

Bio-humoral indicators were obtained by chemiluminescence, using Roche ELECSYS Immunoassay Analyzer.

Statistical analysis: Descriptive statistical parameters - arithmetic mean and standard deviation (SD) as well as determination of frequencies by observation characteristics were used to summarize the obtained information.

Testing of statistical significance of differences between mean values for data with normal distribution was performed by Student's t-test, while Kruskal-Wallis and Man-Whitney's U test were used for data that did not fit normal distribution. The Wilcoxon equivalent pair test was used to test for statistical significance of differences between dependent samples. For non-ordinal and non-parametric data, differences between the frequencies were evaluated by the Hi square test.

Pearson's linear correlation coefficient was used to calculate correlations.

The software programs SPSS and Instat were used for statistical processing.

Results

T-score, CTX, osteocalcin and P1NP levels were obtained from the group of 54 female and one male patient (Table 1), age 57.85 ± 0.9. Mean BMI was slightly above the normal (26.04 ± 4.18 kg/m^2). About 9% of female patients were in pre-menopause, 15% had menopause with premature onset, while the rest had normal menopause. Densitometry revealed normal bone mass in 2 patients, osteopenia in 35 of

them, while 18 patients had osteoporosis manifested on densitometry. About one – tenth of patients had prevalent (pre-existing) fractures.

Power analysis for the given data sample (55 patients) showed the following results: >0.85 for osteocalcin mean values, >0.95 for P1NP mean values and >0.80 for osteodensitometric measurements distribution (maximal power of the analysis is 1). These data confirmed the adequate sample size for the specified analyses.

There was no significant difference in densitometric measurements in patients with and without prevalent fractures; mean osteocalcin level ($p<0.05$) and P1NP level was significantly higher in patients suffering pre-existing fractures. ($t=2.530$, $DF=53$, $p< 0.05$), while beta-CTX levels showed no significant differences (Table 2).

Table 3 delineates correlation coefficients between T-score and biochemical markers (CTX, osteocalcin and P1NP) in all patients with osteoporosis, regardless of ibandronate application. In the first assessment the correlation between T-score and biochemical markers of osteoporosis was weak and insignificant (r 0.17-0.20, $p>0.05$); in the second measurement performed six months after the first one, T score values were significantly related to CTX, osteocalcin and P1NP levels (r between 0.31 and 0.39, $p<0.01$ and 0.05 respectively).

In patients not treated with ibandronate, the correlation between T-score and osteocalcin, P1NP and CTX in the first measurement was insignificant, whereas a significant (low-to-middle level) correlation between T-score and those BTMs was estimated in the second measurement (Table 4). Also, middle-to-high correlation between bone turnover markers was found in first as well as in second assessment.

In patients treated by ibandronate, the correlation between T-score and osteocalcin, P1NP and CTX in the first measurement (Table 5) was insignificant, similarly to those not receiving therapy. Also, CTX, osteocalcin and P1NP values were positively associated among themselves in this group. In the second assessment in patients previously treated with ibandronate T-score values were still not significantly related to BTM levels, while the biochemical markers themselves correlated significantly (except for lack of correlation between CTX and P1NP).

Discussion

Although BMD is considered golden standard in osteoporosis monitoring, it may reveal only 66-74 % osteoporotic changes. (9); moreover, normal BMD does not exclude individual fracture probability. Bone markers level reflect total bone turnover, rather than resorption/formation balance in individual bone remodeling unit (35). Bone turnover influences microarchitecture of matrix, mineral content and mineralization process. There are some misconceptions in discerning bone strength from bone density: strong bone doesn't always mean optimal density. (12) Bone quality is not precisely defined – it is described as the combination of all those properties that make bone resistant to fractures, independently of BMD. Bone strength is determined *in vivo* by bone markers turnover assessment, while bone geometry

and microarchitecture may be estimated by evaluation of biochemical markers levels or using (more invasive) histomorphometric analysis of materials from bone biopsies.

Biochemical markers may predict bone fractures in older women, including clinical vertebral fractures (38) That is evident mainly in individuals with low BMD with high resorptive markers levels. The wide variety of interpretations and overlapping of fracture risks makes reliable prediction of fractures difficult, especially when one relies on just one assessment of bone markers/resorptive markers. Biochemical markers are valuable parameters in prediction fracture risk, and they could provide good insight only if they are considered concurrently with other risk indicators (such as low BMD, personal and family history for maternal bone fractures, and low body mass)(38.40). There are still some uncertainties related to routine clinical utility of biochemical markers of bone turnover. These are related to their biological variability and multiple methodologies used for their analysis (for example, in the case of osteocalcin determination) (14).

High levels of bone turnover markers (BTMs) may predict fracture risk independently from bone mineral density in postmenopausal women. (11,13). Although they are not new in clinical practice, there is ambiquity concerning the specific information each of the individual markers provide. BTMs may provide pharmacodynamic information on short – term response to osteoporosis treatment after only few months; in comparison, similar information provided by BMD requires a year at least. BMTs are especially helpful in making the decision on starting antiresorptive treatment. Also, BTMs are widely used for treatment monitoring, especially in post-menopausal women. However, there may be some limitations to their clinical utilization (variable standardization of analytic procedures, limited data of comparison of treatments using the same BTM and inadequate quality control) (14) These limitations may be, in part, overcome by unifying international standards. IOF/IFCC recommend one bone formation marker (s-P1NP) and one bone resorption marker (s-CTX) to be used as reference; they should be measured by standardized assays in future studies in order to improve precision and systematization of data, enabling routine clinical application of and comparability of outcomes. (9)

Biochemical markers are not meant to replace well established and broadly used imaging procedures (DEXA and CT) in determining bone mineral density (BMD). They are rather a new catalysts in already existing broad diagnostic repertoire.

Risk of clinical fractures enhances with age - in elderly women, (34). It is equal in women with normal and in those with moderately increased values of markers. The risk doubles in women with high values of resorptive markers. The role of resorptive bone markers in fracture risk development is still not quite clear. Excessive research has been carried out on topic of osteoporosis: data suggests that efficiency of antiresorptive therapy depends on the ability to maintain or increase BMD and suppress osteoclastic bone turnover (33.36). Occurrence of vertebral and non-vertebral fractures in women on antiresorptive treatment could be only partly explained with increased BMD (41) so bone markers have been introduced in order to obtain better insight in the relationship between changes in bone turnover and vertebral fractural risk. Data from the literature (37) point to serum osteocalcin as the most reliable marker. In

contrast to these results, BMD changes in the neck of the femur after 12 and 24 months did not correlate well with fracture risk (41)

Decrease in CTX (mean values 60%) and NTX (51%) for 3-6 months of ibandronate treatment correlate significantly ($p<0.05$) with decrease of the vertebral fracture risk (75% for one year and 50% for three years) (42).

BTM might be a useful tool in predicting fracture risk and for monitoring antiresorptive treatment. Increased bone turnover leads to demineralization and bone mass reduction and deteriorates bone microarchitecture and strength, thus enhancing fracture susceptibility.

Conclusion

In postmenopausal women and individuals with low BMD, the presence of increased bone turnover markers suggests an increased risk of fractures. Furthermore, these metabolic markers are useful in the monitoring of patients receiving antiresorptive therapy, wherein fast decline of their levels indicate favorable course. Their determination after 6 months offers the remarkable advantage in assessing the effectiveness of medical treatment comparing to 12–24 months required to document changes by BMD.

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Declarations

Conflict of interests disclosure: Prof. Ljiljana Smilic, Dr Tanja Smilic, Prof. Aleksandar Jovanovic, Prof. Snezana Markovic – Jovanovic, Professor Mirkovic Zlatica, Dr Mirkovic Jana, Dr Jaksic Bojan, Dr Boban Bisevac and Dr Jelena Filimonovic declare that they have no conflict of interests to disclose regarding publishing / printing this manuscript.

Declarations: The authors hereby confirm that the manuscript is not being submitted to any other medical journal and that submission to other journals will not be made until a decision is reached by BMC Women's Health..

This manuscript is original and does not duplicate similar manuscripts published or being considered for publication by other scientific journal(s).

All authors listed in the manuscript take part in research, drafting, statistical analysis, translations and design of the manuscript. They all agree and accept responsibility for the contents of the manuscript submitted to BMC.

This case-control study was conducted after permission of the Ethics Committee of the Medical Faculty in Pristina, Kosovska Mitrovica, and with a written consent of the subjects. The survey was conducted respecting ethical principles for human clinical trials by the Declaration of Helsinki of 1983 [14].

The authors hereby declare that they do not have any financial interest in publishing the results of this study and that they have had no specific source of funding for the research related to this study.

Data availability: https://1drv.ms/w/s!AnToNOskCFMggf0o7Tqikd6XUWgZ-Q?e=R88W9I

Tables

Table 1: Basic clinical characteristics of the patients

Gender	*Female*	54
	Male	1
Age		57.85 ± 0.9 years
Menopause	*Pre-menopause*	9,3%
	Early	14.8%
	Normal	75.9%
BMI		26.04 ± 4.18
Densitometry	*Normal bone mass*	3.6%
	Osteopenia	63.6%
	Osteoporosis	32.7%
Prevalent fractures	*No*	89.1%
	Yes	10.9%

Table 2: Mean osteocalcin, P1NP and beta-CTX levels in patients with and without prevalent fractures

	Osteocalcin *(Mean ± SD)*
No fractures	27.18 ± 10.67
Prevalent fractures	17.42 ± 10.78
t = 2.113, p< 0.05	

	P1NP
No fractures	45.5 ± 23.67
Prevalent fractures	20.79 ± 4.96
t=2.530, p< 0.05	

	Beta-CTX
No fractures	0.43 ± 0.4
Prevalent fractures	0.61 ± 0.15
t=1.47, p = 0.08	

Table 3: Correlation of T- score and biochemical markers in all patients with osteoporosis (both treated with ibandronate and those without therapy) in the first assessment and second asserssment (six months later)

a. first assessment

		CTX	Osteocalcin	P1NP
T- score	r	-0.195	-0.207	-0.177
	p	0.154	0.130	0.197

b. second assessment

		CTX	Osteocalcin	P1NP
T score	r	0.348	0.391	0.316
	p	0.01*	0.003*	0.019*

Table 4: Correlation of T- score and biochemical markers in osteoporotic patients not treated with ibandronate

a. the first assessment

		CTX	Osteocalcin	P1NP
T- score	r	0.083	0.061	-0.030
	p	0.662	0.747	0.876
CTX	r		0.680	0.512
	p		0.000*	0.004*
Osteocalcin	r			0.891
	p			0.000*

b) second assessment (six months after the first one)

		CTX	Osteocalcin	P1NP
T score	r	0.139	0.159	0.182
	p	0.464	0.402	0.337
CTX	r		0.468	0.184
	p		0.009*	0.332
Osteocalcin	r			0.357
	p			0.053

Table 5: Correlation of T- score and bone turnover markers in osteoporotic patients treated with ibandronate

a. before the initiation of ibandronate

		CTX	Osteocalcin	P1NP
T- score	r	-0.444	-0.368	-0.427
	p	0.026*	0.070	0.033*
CTX	r		0.776	0.667
	p		0.000*	0.000*
Osteocalcin	r			0.843
	p			0.000*

b. six months after the initiation of ibandronate

		CTX	Osteocalcin	P1NP
T score	r	0.283	0.122	-0.006
	p	0.171	0.560	0.977
CTX	r		0.558	0.286
	p		0.004*	0.165
Osteocalcin	r			0.651
	p			0.000*