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Bone turnover markers in postmenopausal osteoporosis and their correlation with bone mineral density and menopause duration

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Abstract

Background: Biochemical bone turnover markers (BTMs) estimates the bone remodeling process, being valuable in the personalized approach of osteoporotic patients. *Aim*: The aim of the study was to evaluate the correlation between biochemical BTMs and bone mineral density (BMD), depending on menopause period, in postmenopausal osteoporotic women, compared to postmenopausal women without osteoporosis. *Patients, Materials and Methods*: The study included 149 untreated postmenopausal women, divided into three groups: group 1 (65 osteoporotic women with less than 10 years of menopause), group 2 (44 osteoporotic patients, with over 10 years of menopause), and the control group with 40 postmenopausal women without osteoporosis. *Results*: All BTMs levels were higher in the groups with osteoporosis, than in the control group. Lumbar BMD values correlated positively with deoxypyridinoline (DPD) and negatively with bone-specific isoform of alkaline phosphatase (BAP), tartrate-resistant acid phosphatase band 5b (TRAP 5b), osteocalcin (OC) and cross-linked *N*-telopeptides of type I collagen (NTX). Serum estradiol levels correlated positively with spine BMD in the whole study group (r=0.508, p=0.001). BTMs correlated positively with each other. Osteoporotic women with longer period of menopause presented significantly higher values of resorption markers (NTX and TRAP 5b), compared to the group with menopause duration less than 10 years. At a cutoff value of 12 µg/L, BAP presented 82.4% sensitivity and 62.5% specificity. *Conclusions*: Our study showed that BTMs correlated negatively with lumbar BMD and positively with each other. Resorption markers levels increase with duration of estradiol deprivation period.

Keywords: postmenopausal osteoporosis, bone turnover markers, bone mineral density, alkaline phosphatase.

Introduction

Osteoporosis is characterized by an altered architecture and decreased bone mineral density (BMD), harboring a high risk for fractures. There are many risk factors for osteoporosis, among them being the menopause and age. After menopause, bone remodeling increases, due to estrogen deficiency. This process represents an independent factor for fracture risk. The alteration of bone architecture contributes independently of decreased BMD, to the increased bone fragility and risk for fractures. During the first assessment of osteoporosis, the determination of BMD represents a static parameter, providing information about a particular site at some point in time. Bone turnover is a dynamic process, which can be evaluated by several biochemical markers or by invasive methods (histomorphometry) [1].

Bone turnover markers (BTMs) are products released during bone remodeling process. They can reflect osteoclasts activity (named markers of bone resorption) or osteoblasts activity (markers of bone formation). Although the determination of collagen metabolism parameters and BTMs are useful in the diagnosis and monitoring of several bone disorders (*e.g.*, metastases, bone Paget's disease), they cannot be used to diagnose osteoporosis. High levels of BTMs do not necessarily mean bone loss. This will be evident only if resorption exceeds bone formation [2]. The levels of BTMs reflect the bone remodeling process within several days up to months, before decreasing BMD can be observed. Nonetheless, BTMs could predict to some extent the rate of future bone loss, evaluate the risk of future fractures, and contribute to the monitoring the response to antiosteoporotic therapy [3–5].

Some authors recommend BTMs as useful parameters in deciding the treatment of osteoporosis. Patients with high turnover could benefit from antiresorptive therapy, while those with low turnover rate should be treated with an anabolic agent [6, 7].

BTMs present some advantages, being non-invasive, affordable, and repeatable determinations.

Over the last decades, many serum and urine BTMs have been studied. Osteoblastic activity (and thus, bone formation) can be evaluated by the serum level of bone-specific isoform of alkaline phosphatase (BAP). The role of this enzyme in the process of bone mineralization is still not fully understood, but has a major role in the degradation of the mineralization inhibitor pyrophosphate, at alkaline pH [8].

Osteocalcin (OC) is a non-collagenous hydroxyapatite-binding protein, secreted by the osteoblasts. However, OC is released also into the circulation from the bone matrix, during bone resorption, thus reflecting the bone turnover process *per se*, rather than bone formation [7]. Bone formation markers levels vary with age. After a long stable period in young decades, the serum levels of OC begin to increase in women after menopause. By an unexplained mechanism, in most of the women, serum levels of OC return to premenopausal amounts 15–20 years after the menopause onset.

The best bone resorption markers are considered collagen degradation products, which are released into the circulation and eliminated by urine. One of the most abundant amino acid of type I collagen is hydroxyproline, which can be measured in the urine. However, as hydroxyproline is not specific to bone collagen, the urinary levels are influenced by dietary intake. In addition, the accurate determination requires expensive methods (high-pressure liquid chromatography). Other resorption markers that are easier to determine are cross-linked *N*-telopeptides of type I collagen (NTX) and *C*-terminal telopeptides of type I collagen (CTX) [8, 9].

An osteoclast-specific product is tartrate-resistant acid phosphatase band 5b (TRAP 5b), which correlates with the number and the activity of osteoclasts. Bone resorption starts after the osteoclasts attach to the bone surface and enzymes (including TRAP 5b), respectively acids are released into the space between the ruffled border of the osteoclasts and the bone. Increased levels of TRAP 5b were associated with high bone turnover states and bone metastases [10, 11].

During bone collagen catabolization, cross-links between collagen molecules are released: pyridinoline (less specific for the bone) and deoxypyridinoline (DPD) (with higher specificity, as it can be found in bone and dentin) [8, 9].

Vitamin D plays an important role in calcium homeostasis and bone remodeling, although the exact mechanisms are not known [12]. Low 25-hydroxy vitamin D [25(OH)D] levels have a deleterious effect on bone. It is largely accepted that levels of 20 ng/mL are sufficient for the general population. This threshold was associated with decreased fracture risk [13]. Other studies suggested higher values (30 ng/mL) to maintain bone health. Vitamin D deficiency causes hypocalcemia and secondary hyperparathyroidism, leading to decreased BMD [14].

Measuring BTMs and BMD early in the postmenopausal period could add valuable information about the bone-remodeling rate, in order to decide the optimum moment to introduce the treatment.

Aim

The aim of the study was to evaluate the correlation between biochemical BTMs and BMD, in relationship with estrogens deprivation period, in postmenopausal osteoporotic women, compared to postmenopausal women without osteoporosis. In addition, we try to determine the significance of BTMs, as well as the best BTM in osteoporosis assessment.

Patients, Materials and Methods

The study included 149 untreated postmenopausal women, divided into three groups, based on the *T*-score determined by dual-energy X-ray absorptiometry (DXA) osteodensitometry and duration of estrogen deficiency: group 1 included 65 osteoporotic women with less than 10 years of estrogen deficiency; group 2 was composed of 44 osteoporotic patients, with over 10 years of menopausal period; the control group (group 3) included 40 post-menopausal women without osteoporosis. The patients evaluated in the Outpatient Department of Endocrinology of the Emergency County Hospital, Timişoara, Romania, from September 2016 to October 2018. Menopause was defined as cessation of menses for at least one year.

The exclusion criteria were as follows: patients with secondary causes of osteoporosis (primary hyperparathyroidism, Cushing's syndrome, rheumatoid arthritis, malabsorption, hyperprolactinemia, etc.), bone diseases (Paget's disease, multiple myeloma, metastatic breast cancer), fractures within the last 12 months, chronic kidney disease, collagen diseases, severely deficient patients [25(OH)D less than 10 ng/mL], immobilized patients. Women under treatment with bone affecting medication were also excluded: current/previous antiosteoporotic agents, calcium supplements, estrogens, corticosteroids, thyroxine, heparin, and anticonvulsants.

Osteodensitometry was performed using a DXA bone densitometer (Hologic, QDR Inc., Bedford, MA, USA). BMD (g/cm^2) was measured at the lumbar spine (L1–L4 lumbar vertebra), respectively at the hip (total and neck) levels. The coefficient of variation (% CV) of the BMD for the lumbar spine, respectively for the hip, was 1%.

The *T*-score was calculated by comparing the BMD measured in the analyzed patients with the BMD average of the healthy, same-gender young adult. For women, the reference database was represented by white women aged between 20 and 29 years.

The *Z*-score was calculated by comparing the BMD measured in the analyzed patients with the BMD average in the reference population, of the same gender, age and ethnic category.

Body mass index (BMI, kg/m^2) was calculated as the ratio of body mass (kg) to height (m). Normal weight were considered when BMI was 18.5–24.9 kg/m^2, overweight when BMI was 25–29.9 kg/m^2, and obesity at BMI over 30 kg/m^2 [*World Health Organization* (WHO), 2004].

Osteoporosis was defined as a BMD (expressed as *T*-score) below 2.5 standard deviation (SD) the average value for young women, measured at lumbar spine or at hip [15].

Serum estradiol was measured by enzyme-linked immunosorbent assay (ELISA), with intra- and inter-assay CV of 7.25% and 6.8%, respectively (kit from HUMAN Gesellschaft für Biochemica und Diagnostica, GmbH, Wiesbaden, Germany).

Serum intact parathyroid hormone (PTH) (1–84) was determined by chemiluminescent immunoassay (DiaSorin Inc., Stillwater, MN, USA), with intra- and inter-assay CV of <8% and <14%, respectively. Normal values were considered less than 65 pg/mL.

Serum 25(OH)D was determined by ELISA, using commercial kit (DiaSorin Inc.). Sufficient level was considered over 20 ng/mL, insufficiency was defined as values between 10 and 20 ng/mL, while deficiency was diagnosed at less than 10 ng/mL levels.

For the entire study group, we measured OC and BAP as formation markers and TRAP 5b, NTX and serum DPD as resorption markers, respectively.

For the determination of BTMs, blood samples were collected in the morning (at 8–9 a.m.), in fasting conditions. Immediately after centrifugation at 2000×g, the serum was frozen and stored at -80°C.

Serum BAP was determined by the MicroVue BAP human ELISA kit, with intra- and inter-assay CV of 5.0–5.8% and 4.8–5.2%, respectively (MicroVue™ BAP, MDSS GmbH, Hannover, Germany).

Serum DPD was determined by ELISA (MyBioSource Inc., San Diego, CA, USA), with intra- and inter-assay CV of ≤8% and ≤12%, respectively.

TRAP 5b was measured using a two-step MicroVue TRAP 5b assay (MicroVue™ TRAP 5b, MDSS GmbH, Hannover, Germany), with intra- and inter-assay CV of 2.2–3.6% and 3–4.6%%, respectively.

OC was measured by ELISA (Nordic Bioscience Diagnostics A/S, Herlev, Denmark), with intra- and inter-assay CV of 2–3.4% and 3.6–6.4%, respectively.

Serum NTX was determined by ELISA technique, using Osteomark kit (Osteomark, Ostex International Inc., Seattle, WA, USA), with intra- and inter-assay CV of of 4.6% and 6.9%, respectively.

Each patient signed an informed consent. The study was performed in conformity with the ethical recommendations of the *Helsinki Declaration*, being approved by the local Ethics Committee.

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) *ver.* 20.0 for Windows (IBM, Armonk, NY, USA).

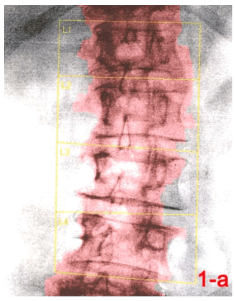
Continuous variables with normal distribution were expressed as mean±SD (min.–max.). Group means were compared using Student's *t*-test for normally distributed variables, respectively Mann–Whitney *U*-test for non-normally distributed variables. Analysis of variables among groups was performed using one-way analysis of variance (ANOVA). To compare categorical variables, we applied λ^2 test. The correlations between BTMs, respectively BTMs and BMD were evaluated using Pearson's coefficient or nonparametric Spearman's coefficient, as appropriate. The receiver operating characteristic (ROC) curve analysis was used to evaluate the discriminative power of each studied BTM for osteoporosis. Significance was established at $p<0.05$.

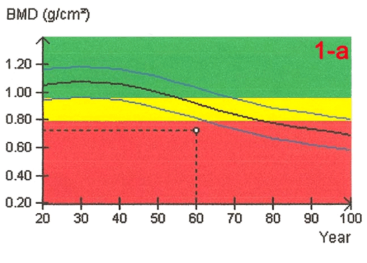
Results

The mean age of the osteoporosis group was 61.5± 6.8 years (range 54–79 years, median 60 years). The mean age of the control group was 60±7.1 years, range 50–77 years, median 58 years), not statistically different from the osteoporotic group ($p>0.05$).

The mean age was 57.8±2.29 years for group 1 and 65.5±4.7 years for group 2, respectively ($p<0.0001$). Serum calcium and creatinine levels did not differ among the groups. PTH levels did not differ among the groups (group 1: 42.7±20.2 pg/mL, group 2: 48.0±16.6 pg/mL, and group 3: 49.2±19.3 pg/mL, respectively, $p=0.82$).

BMI was significantly lower in the osteoporotic group (25.1±4.5 kg/m^2) than in the controls (29.2±5.1 kg/m^2) ($p<0.0001$). No significant difference was noted between groups 1 and 2. Obese patients with BMI over 30 kg/m^2 showed higher BMD values (0.841±162 g/cm^2) than normal weight patients (0.770±0.142 g/cm^2) ($p=0.07$). BTMs values did not significantly differ in overweight and normal weight patients (Figures 1 and 2).

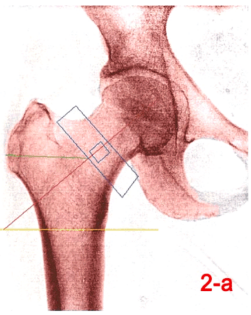


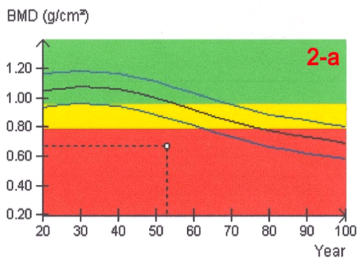


1-b

R.O.I.	BMD (g/cm²)	BMC (g)	Area(cm²)	ZScore	TScore
L2	0.721	14.106	19.57	-1.80 (78 %)	-3.21 (66 %)
L3	0.687	11.077	16.13	-2.11 (74 %)	-3.57 (63 %)
L4	0.764	13.397	17.55	-1.42 (82 %)	-2.75 (71 %)
Total	0.725	38.580	53.25	-1.77 (78 %)	-3.17 (67 %)
L1	0.673	10.611	15.78	-2.24 (73 %)	-3.67 (62 %)

Figure 1 – ***Patient, L.M., with postmenopausal osteoporosis, 60.5-year-old, menopause installed at 46.5 years, estrogen deprivation interval of 14 years: (a) Image of bone mineral density – total BMD = 0.725 g/cm^2 at lumbar level; (b) Assessment of lumbar T-score.***





2-b

R.O.I.	BMD (g/cm²)	BMC (g)	Area(cm²)	ZScore	TScore
Neck	0.554	3.030	5.47	0.15 (103 %)	-2.58 (65 %)
G.T	0.515	4.817	9.36	-0.74 (87 %)	-2.98 (63 %)
InterTro	0.738	17.287	23.42	-0.43 (92 %)	-3.29 (62 %)
Total Hip	0.657	25.134	38.25	-0.41 (92 %)	-3.13 (62 %)

Figure 2 – ***Patient, S.C., with postmenopausal osteoporosis, 53.5-year-old, menopause installed at 42.5 years, estrogen deprivation interval of 11 years: (a) Image of bone mineral density – total BMD = 0.657 g/cm^2 at femoral level; (b) Assessment of lumbar T-score.***

In our study group, BMI values did not correlate significantly with BMD or BTMs values. BMD values (in both sites: lumbar spine and femoral neck, respectively) were significantly lower in osteoporotic women as compared to postmenopausal women without osteoporosis. Moreover, a significant difference was noted between groups 1 and 2, BMD decreasing with the increased period of estrogens deprivation (Table 1).

Table 1 – *BMD and BTM values for patients with osteoporosis and for control subjects, respectively*

Parameter (mean±SD)	Osteoporosis group (*n*=109)	Control group (*n*=40)	*p*-value
Lumbar BMD [g/cm^2]	***0.691±0.087***	0.897±0.124	<0.0001
	Group 1: 0.652±0.051		
	Group 2: 0.581±0.060		
	p<0.0001		
Neck BMD [g/cm^2]	***0.623±0.041***	0.789±0.111	<0.0001
	Group 1: 0.631±0.102		
	Group 2: 0.594±0.062		
	p<0.0001		
OC [ng/mL]	***24.33±12.10***	20.01±8.99	0.028
	Group 1: 24.4±10.2		
	Group 2: 22.6±6.7		
	p=NS		
DPD [nmol/L]	***5.54±2.05***	4.94±1.26	0.036
	Group 1: 5.1±2.2		
	Group 2: 5.8±1.6		
	p=NS		
BAP [µg/L]	***15.04±4.06***	13.57±2.36	0.006
	Group 1: 14.92±5.17		
	Group 2: 15.13±3.03		
	p=NS		
NTX [nmol/L]	***17.44±4.9***	15.8±4.17	0.062
	Group 1: 15.9±4.6		
	Group 2: 18.3±4.4		
	p=0.007		
TRAP 5b [U/L]	***4.72±1.94***	3.92±0.94	0.006
	Group 1: 6.25±1.13		
	Group 2: 7.23±1.39		
	p=0.001		

BTM: Bone turnover marker; BMD: Bone mineral density; SD: Standard deviation; OC: Osteocalcin; DPD: Deoxypyridinoline; BAP: Bone-specific isoform of alkaline phosphatase; NTX: Cross-linked *N*-telopeptides of type I collagen; TRAP 5b: Tartrate-resistant acid phosphatase band 5b; NS: Not significant; *p*-values between osteoporosis groups 1 and 2; *p*-values between osteoporosis groups and control group; *p*-values <0.05: significant difference; *p*-values >0.05: NS difference from control group; *p*-values were determined using the Student's *t*-test.

Estradiol levels were significantly decreased for group 2 (20.3±3.2 pg/mL) than for group 1 (26.6±3.03 pg/mL) (p<0.0001).

Based on 25(OH)D levels, we found that the majority of the patients (in both groups, osteoporotic and control) were insufficient or deficient. Ninety (82.5%) osteoporotic patients and 32 (72.7%) patients from the control group, respectively, presented 25(OH)D less than 20 ng/mL (p=0.18, Fisher's exact test). The osteoporotic group had lower values of vitamin D (20.6±6.35 ng/mL) than the control group (22.36±6.61 ng/mL) (p=0.1). Vitamin D did not correlate significantly with BMD or BTMs values.

All BTMs levels were higher in the groups with osteoporosis than in the control group.

Osteoporotic women with longer period of menopause presented significantly higher values of resorption markers. Mean NTX levels for group 1 were 15.9±4.6 nmol/L and for group 2, 18.3±4.4 nmol/L (p=0.007). Mean values of TRAP 5b for group 1 were 6.25±1.13 U/L and for group 2, 7.23±1.39 U/L (p=0.001). DPD levels did not differ between groups 1 and 2. Regarding osteoformation markers (OC, BAP), the values did not differ significantly for group 1 compared to group 2 (Table 1).

In the osteoporotic group, 10 (10.09%) patients presented in the past atraumatic fractures of the limbs, and one presented hip fracture. In the control group, only one patient presented an upper arm fracture. BTM and BMD values did not differ significantly between the patients with fractures, compared to those without fractures.

Age correlated positively, but non-significantly with BAP and NTX levels.

The duration of estradiol deprivation correlated negatively (but not significantly) with BMD and BTMs levels.

Serum estradiol levels correlated positively with spine BMD (but not with femoral neck BMD) in the whole study group [r=0.508, 95% confidence interval (CI) 0.211–0.719, p=0.001].

Serum OC correlated with BAP only in osteoporotic group, regardless of age and the duration of menopause (r=0.570, 95% CI 0.361–0.7, p<0.0001). BMD values (lumbar, and to a lesser extent hip) correlated positively with DPD and negatively with BAP, TRAP 5b, OC and NTX levels. BTM correlated positively with each other. Serum TRAP 5b correlated the best with BAP values (r=0.814, 95% CI 0.745–0.866, p<0.0001) (Table 2).

Table 2 – *The correlations between BMD and BTM values for osteoporotic patients*

Parameter	Lumbar BMD	Femoral neck BMD	BAP	TRAP 5b	OC	DPD	NTX
Femoral neck BMD	0.540	–					
BAP	-0.137	NS	–				
TRAP 5b	-0.619	-0.389	0.814	–			
OC	-0.421	-0.230	0.570	0.581	–		
DPD	0.322	0.105	0.660	0.711	0.491	–	
NTX	-0.191	NS	0.702	0.750	0.512	0.702	–

BMD: Bone mineral density; BTM: Bone turnover marker; BAP: Bone-specific isoform of alkaline phosphatase; TRAP 5b: Tartrate-resistant acid phosphatase band 5b; OC: Osteocalcin; DPD: Deoxypyridinoline; NTX: Cross-linked *N*-telopeptides of type I collagen; NS: Not significant; Relationships between the rates of bone loss and the serum levels of BTMs in postmenopausal women with osteoporosis; *r*: The Pearson's correlation coefficient was tested by one-way analysis of variance (ANOVA).

ROC curve analysis was used to determine the discriminative power of each evaluated parameter for osteoporosis.

The accuracy of BTM for identifying patients with osteoporosis in our study showed that at a cutoff value of 4.25 U/L, TRAP 5b presented a sensitivity of 73.6% and a specificity of 60% [area under ROC curve (AUC) 0.725, 95% CI 0.638–0.809, p=0.0001]. For DPD, the accuracy was low, at a cutoff value of 4.83 nmol/L, the sensitivity was 60% and the specificity 47.4% (AUC 0.581, p=0.115). A better specificity showed OC (80%), but with

low sensitivity (40%), for a cutoff value of 27 ng/mL (AUC 0.603, 95% CI 0.503–0.703, p=0.046). Decreasing the cutoff level to 19.7 ng/mL, sensitivity increases slightly (62.3%), but the specificity drops to 50%. For NTX, at a value of 17.45 nmol/L, the specificity was 73.5%, but with low sensitivity (50%) (AUC 0.603, p=0.111). At 14.75 nmol/L, the sensitivity increases to 70%, but the specificity decreases to 46.9%. At a cutoff value of 12 μg/L, BAP presented 82.4% sensitivity, with 62.5% specificity (AUC 0.778, 95% CI 0.697–0.859, p<0.0001) (Figure 3).

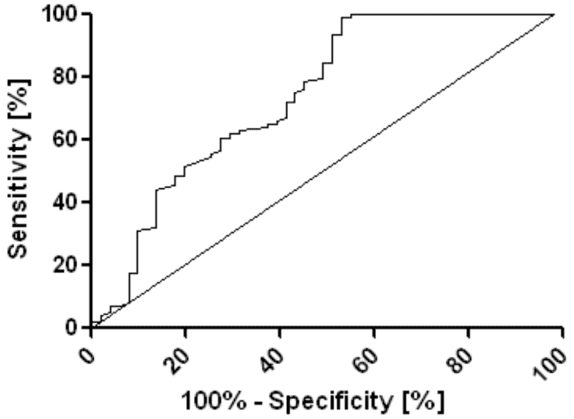


Figure 3 – ***ROC curve analysis for BAP levels. ROC: Receiver operating characteristic; BAP: Bone-specific isoform of alkaline phosphatase.***

Discussions

Osteoporosis and its complication, the fracture, present a significant health and economic impact. Postmenopausal osteoporosis develops mainly due to estrogen deficiency, associated with increased bone remodeling, with enhanced bone resorption and bone loss. In early menopause, despite bone resorption, bone formation is preserved. In elderly women, the two processes are uncoupled, reduced bone formation being overcome by increased bone resorption [16]. The bone loss is rapid in the first years after menopause occurs (reaching its maximum at 3–4 years), then progressively decreases for some years, later remaining quite stable around 1–1.5% per year [17].

BMD measurement using DXA is currently the "gold standard" for positive diagnosis of osteoporosis. However, more than half of atraumatic fractures do not meet the DXA criteria for osteoporosis, based on BMD values. Therefore, other markers evaluating osteoporosis raised great interest in the past decades [18].

DXA osteodensitometry cannot identify all the patients with risk of fracture. In patients with fragility fractures, but without osteoporosis on DXA, high-resolution peripheral quantitative computed tomography (CT) showed irregular endosteal margins, with semilunar defects. The enhanced endosteal remodeling involves further trabecular thinning, loss of trabecular connections, cortical thinning, and increased intracortical porosity. This increased bone cells activity can be assessed by BTMs [19].

In the last years, personalized treatment in osteoporosis has become a reasonable approach. This implies a correct assessment of risk for future fractures, because this could help to select the most suitable treatment for a specific patient. Low BMD is a modest predictor of fractures, so other tools developed in time, such as Fracture Risk Assessment (FRAX®) tool [20].

FRAX® tool calculates the fracture risk, based on several parameters, in addition to BMD: gender, age, family history, secondary causes of osteoporosis, medication, smoking, past fracture, and BMI [21]. However, it does not include BTMs, calcium intake, or vitamin D insufficiency. Due to the paucity of data, the determination of BTMs is not included in the FRAX® [1, 2]. Not all the properties of the bone can be assessed in clinical practice. While the bone strength could be appreciated indirectly by BMD, the BTMs reflect the bone metabolism. The diagnostic and prognostic value of BTMs is still disputed. There are significant analytical and biological variability dynamics. Epidemiological studies have confirmed that BTMs can represent an important tool in assessing the fracture risk, independently of BMD [22]. The prediction of fracture risk in elderly women could be improved by using several BTMs, rather than only one. In early postmenopausal period, risk of osteoporosis should be evaluated using BMD combined with BTM in order to start the prevention therapy [23, 24].

Some authors classified the osteoporosis, based on the rate of turnover, as normal, increased or decreased turnover, although after menopause bone loss prevails over bone formation [25–27].

Fisher *et al.* classified the bone turnover status into six subtypes, based on procollagen type 1 *N*-terminal propeptide (P1NP) and *β*-CTX values, associating these types with the risk of different types of fractures and other comorbidities. For example, type 4B, defined as high bone turnover (and P1NP/*β*-CTX <100), was associated with increased risk of fractures [overall risk (OR) for hip fractures 2.5] [27].

One of the main issues regarding BTMs are represented by the reference values for a specific population, as they are influenced by numerous factors. The levels can be altered in specific circumstances, such as: immobilization, bone fractures (in the first six months), bone disorders (Paget's disease, multiple myeloma, bone metastases), endocrine disorders (like hyperparathyroidism), chronic kidney disease, etc. Moreover, other conditions can influence BTMs levels: smoking (increases the level), fasting state, the diurnal rhythm (*e.g.*, CTX presents a peak in the morning, the level decreasing in the afternoon) [28, 29].

BTMs have some limitations: reanalytical and analytical variability, lack of standardized assays, insufficient data of the BTMs response to the various treatments. This leads to heterogeneous results for the same sample [30]. Although BTMs were associated with increased risk of fracture in several studies, there are still not enough data to support the inclusion of BTMs in fracture prediction models [31]. There is still a debate regarding the best BTM, which reflects bone remodeling with good sensitivity and specificity. Therefore, the "reference BTM", which can assess the fracture risk, has not been identified yet [32].

International Osteoporosis Foundation (IOF) and *International Federation of Clinical Chemistry and Laboratory Medicine* proposed serum CTX and P1NP as reference markers of bone resorption and formation

[33, 34]. However, despite this recommendation to use serum CTX and P1NP as reference markers in observational and interventional studies, they are not widely used. Furthermore, Crandall *et al.* found that these BTMs did not correlate with hip fracture risk in postmenopausal women [35].

The determination of BTMs in individual patients may be useful in selected cases, as in women with BMD values considered not a risk factor for fractures. In this situation, biochemical BTMs could help in the assessment of fracture risk and treatment decision. The serum level of BTMs increase gradually with age, especially those that reflect the bone resorption [36].

When we divided the osteoporotic group into two subgroups, according to their period of menopause (years of estrogen deprivation), the lumbar spine and neck BMD values decreased significantly in the group with over than 10 years of menopause. NTX and TRAP 5b showed also significant increase in women with more than 10 years of menopause, compared to those with shorter period of menopause. This is in accordance with other studies that showed similar results [36]. BAP, OC and DPD did not show significant differences between subgroups. Kumar *et al.* confirmed progressive increasing BTMs levels with longer menopause duration [36].

In the present study, osteoporotic women presented significantly lower BMI values (25.1±4.5 kg/m^2) compared with non-osteoporotic women (29.2±5.1 kg/m^2) (p<0.0001). On the other hand, BMD values were higher in obese women, compared to normal weight subjects.

Traditionally, obesity was considered to be a protective factor against osteoporosis, delaying its onset [37, 38]. Nonetheless, due to the complex relationship between the adipose tissue and bone, the published data reporting the correlation between BMI and the incidence of fractures are conflicting. A large study, including data from 25 prospective cohorts (mainly population-based), concluded that, in general, increased BMI has a protective role for most fragility fractures. Low BMI was associated with increased risk for hip fractures, high BMI being a risk factor for upper arm fractures [39].

We did not observe any correlation between BTMs values and BMI in the osteoporotic, respectively control group. In contrast, Guañabens *et al.* found that low BMI was associated with higher BTMs [40]. Kumar *et al.* found a negative correlation between BMI and NTX levels [36]. Papakitsou *et al.* reported a negative correlation between BMI and carboxy-terminal propeptide of type 1 procollagen (P1CP) [41]. Eastell *et al.* showed that postmenopausal women with a high BMI have lower levels of OC (probably related to increased hormone secretion from adipocytes that influence osteoblast/osteoclast activity) [24].

In the present study, the highest values of BAP were registered in the group with over 10 years of menopause (15.13±3.03 μg/L), although there was no statistically significant difference between groups 1 and 2. Many studies confirmed that in osteoporotic patients, BAP increases with age. The increase of BAP in postmenopausal women can be explained by the disappearance of the inhibitory effect of estrogen on bone turnover process [17].

Mean OC levels in our osteoporotic groups were significantly higher (24.33±12.1 ng/mL) than in control group (20.01±8.99 ng/mL) (p=0.028). Higher values of OC in osteoporotic patients were confirmed by numerous studies [42].

However, the values did not differ between groups 1 and 2. In contrast, Park *et al.* found that OC levels in women with over 10 years of menopause were significantly lower than OC levels in the group of 6–10 years of menopause. They concluded that the highest values of BTMs were seen after 8–9 years of menopause (after 10 years), bone turnover rate started to decline [42].

We did not find any statistical differences regarding BTMs or BMD in patients with fractures *versus* those who did not present fractures. This could be explained by the small number of patients with fractures in the studied groups (only 11 in osteoporotic group).

As there is a significant overlap in BMD values between patients with fractures and subjects without fractures, other risk factors for fractures must be taken into consideration [7]. Several studies, like *Epidémiologie de l'Ostéoporose* (EPIDOS) and *Os des Femmes de Lyon* (OFELY), showed a significant correlation between resorption BTMs and the risk of fragility fractures. Women with femoral BMD less than 2.5 SD and high serum CTX or high urinary DPD levels, presented an increased risk for hip fracture, as compared to patients with only low BMD or high BTMs levels [43–45].

In the present study, we did not find a significant correlation between age and BTMs values. Previous studies demonstrated that bone formation markers (OC and P1NP) decreased with age, until 44 years; after this age, they increase significantly, especially in the fifth decade. The same tendency was observed also for CTX levels [46]. Lumachi *et al.* found that OC and BAP correlated positively with age, only in postmenopausal women aged over 59 years, concluding that increased formation markers later in postmenopausal period, mirrors the increased bone turnover [47]. Atalay *et al.* showed that the highest OC and BAP levels were measured in women within 1–5 years after menopause occurrence, reflecting the increased bone turnover [48, 49]. We found a positive correlation (although non-significant) between age and BAP, respectively NTX values. This is in contrast with data published by Kumar *et al.*, who found a negative correlation between NTX levels and age, in postmenopausal women [36].

In our study, BTMs correlated better with lumbar spine BMD than with femoral neck BMD (Table 2). This is in accordance with other studies that demonstrated a stronger correlation of BTMs values with lumbar spine BMD than with other bone sites [50]. In a meta-analysis published in 2012, Biver *et al.* found that in postmenopausal osteoporosis, the strongest negative correlation was between BMD and BAP, OC, CTX and NTX, respectively [51]. Other authors did not find significant correlation between BTMs and BMD values [47, 52].

In our study, BAP correlated negatively (r=-0.137, p=0.01) with lumbar BMD, but did not correlate with neck BMD. Other reports [48, 53] support our finding, but also conflicting results have been published. Ikeuchi & Umesaki demonstrated that BAP correlates positively with lumbar BMD in premenopausal women, but after

menopause, the correlation becomes negative [53]. Biver *et al.* found a moderate negative correlation between BMD and BAP, OC and serum CTX, and urinary NTX, respectively [51].

NTX levels correlated negatively with lumbar BMD values in our patients (r=-0.191, p=0.01). A Chinese community-based population study, including 1724 post-menopausal women, showed that serum β-CTX and P1NP correlated negatively with lumbar and spine BMD [54]. The negative correlation between BMD and NTX was confirmed by other authors [36].

In our study group, we found an inverse relationship between OC and BMD (both lumbar and neck BMD). This negative correlation was confirmed also in other studies [46, 49]. Other authors did not find any relationship between OC and BMD or age [50].

Moreover, serum OC correlated positively with BAP in our study (r=0.553, p<0.0001).

Serum TRAP 5b correlated significantly with other BTMs, the best correlation was with BAP levels (r=0.814, p<0.0001). Lumbar BMD correlated negatively with TRAP 5b levels (r=-0.619, p=0.001). These correlations were reported also by other authors.

Nenonen *et al.* showed that baseline lumbar BMD correlated significantly with TRAP 5b, serum CTX and OC values, and concluded that the best BTMs to be evaluated during alendronate treatment were TRAP 5b, serum CTX and P1NP [55].

Although parathormone plays an important role in bone remodeling process, in our patients, BTMs values did not correlate with PTH levels. Similar results were communicated by other studies [56]. In elderly women, the increase in bone turnover can be attributable partly to vitamin D, respectively calcium deficiency, associated with secondary hyperparathyroidism [52, 57]. Our high prevalence of vitamin D insufficiency in the whole study group is in accordance with many publications [52]. A retrospective study showed that, in Romania, the levels of vitamin D are suboptimal in postmenopausal women. Mean values for fifth decade were 26.5±10.9 ng/mL, remaining relatively stable in the subnormal range in the following decades [58].

In our study, the levels of 25(OH)D did not correlate significantly with BMD or PTH and BTMs, respectively. Some studies (including elderly osteoporotic women) found that patients with vitamin D insufficiency presented higher BTMs levels [57]. Zhao *et al.* found that vitamin D correlated negatively with β-CTX and P1NP levels [54]. Kharroubi *et al.* found a positive correlation between vitamin D levels and lumbar spine BMD (but not with hip BMD) in osteoporotic postmenopausal women. However, they did not find any correlation between vitamin D status and BTMs values [52].

Eastell *et al.* found that the levels of BTMs (CTX, NTX, P1NP, BAP) were significantly higher in subjects with vitamin D insufficiency, compared with those with normal levels, mainly as a result of higher PTH. All BTMs were higher in subjects with spine osteoporosis compared to those non-osteoporotic subjects [24]. Many authors did not confirm a correlation between BMD and serum 25(OH)D. The bone is also greatly influenced by the locally produced active metabolite, 1,25-dihydroxy vitamin D3 [1,25(OH)2D3], thus a true correlation could not be excluded [59].

ROC curve analysis showed that for identifying the osteoporotic patients in our study group, the best parameters were BAP (AUC 0.778, p<0.0001) and TRAP 5b (AUC 0.725, p=0.0001). Atalay *et al.* published similar results for BAP (for neck osteoporosis AUC was 0.882, p=0.002 and for lumbar spine osteoporosis AUC was 0.873, p=0.012, respectively) [48]. In contrast with this study, which showed that OC would be a good diagnostic tool to identify patients with osteoporosis (AUC 0.949, p=0.003), we did not find a very good discriminatory ability of OC (AUC 0.603, p=0.046).

Conclusions

The mean value of lumbar and femoral neck BMD decreased significantly as the duration of menopause increased. Persistent increasing values of resorption markers (NTX, TRAP 5b), in the late postmenopausal years, suggest that bone resorption continues to be high many years after menopause occurs. The inverse correlation between BMD and BTMs suggests that determining serum BTMs could define better the bone metabolism and future risk for fractures. We consider that currently applying the DXA technique as a screening method for the entire female population in the postmenopausal period is a viable para-clinic option, because DXA still represents the "gold standard" in diagnosing osteoporosis.

Conflict of interests

The authors have no conflict of interests to declare.

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Authors' contribution

Camelia Vidiţa Gurban and Melania Olga Balaş have equally contributed to this study.

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