Osteoporosis and Bone Metabolism in Treatment-naive Primary Prostate Adenocarcinoma Patients

VIOLETA BOJINCA[1,2], CLAUDIU POPESCU[2]*, ANDRA RODICA BALANESCU[1,2], SERBAN MIHAI BALANESCU[2,4], MIHAI BOJINCA[2,5]

[1]Sfanta Maria Clinical Hospital, Internal Medicine and Rheumatology Department, 37-39 Ion Mihalache Blvd., 011172, Bucharest, Romania

[2]Carol Davila University of Medicine and Pharmacy, 8 Eroii Sanitari Blvd., 050474, Bucharest, Romania

[3]Ion Stoia Clinical Centre for Rheumatic Diseases, 5 Thomas Masaryk Str., 030167, Bucharest, Romania

[4]Elias Hospital, Department of Cardiology, 17 Marasti Blvd., 011461, Bucharest, Romania

[5]Dr Ion Cantacuzino Hospital, Department of Internal Medicine and Rheumatology, 5-7 Ion Movila Str.,030167,Bucharest, Romania

The objectives of the study were to evaluate bone metabolism in primary prostate cancer (PCa) patients prior to any treatment and to compare estrogens and anti-androgens in terms of bone metabolism. The study prospectively included consecutive patients with primary PCa who were proposed for radical prostatectomy and androgen deprivation therapy (ADT; either estrogens-group E, or anti-androgens -group A) and age-matched controls. Bone markers (osteoprotegerin -OPG; osteocalcin; deoxypyridinoline) were measured before treatment and after 6 months. Bone mineral density (BMD) was measured by dual X-ray absorptiometry before treatment and after 12 months (osteoporosis was defined as a spine or hip T score ≤ -2.5). Continuous variables are reported as mean ± standard deviation. The study included 30 controls (aged 70 ± 6 years), 15 patients treated with estrogens (aged 71 ± 6 years) and 15 patients with anti-androgens (aged 72 ± 5 years). At baseline, 0% of controls, 33.3% of group E (p = 0.002 versus controls) and 53.3% of group A (p = 0.0001 versus controls) had osteoporosis. In group E, compared to baseline, OPG (4.67 ± 1.38 versus 5.27 ± 1.89; p = 0.043) and DPD (6.85 ± 3.24 versus 8.63 ± 2.42; p = 0.008) increased, while spine (0.99 ± 0.32 versus 0.94 ± 0.31; p = 0.019) BMD decreased. In group A, compared to baseline, OPG (6.37 ± 3.04 versus 5.02 ± 1.12; p = 0.041), spine (1.03 ± 0.15 versus 0.89 ± 0.15; p = 0.0003) and hip (0.82 ± 0.18 versus 0.75 ± 0.17; p = 0.003) BMD decreased. Osteoporosis is prevalent among hormone-naïve PCa patients. Estrogens are associated with an increase of serum OPG, while anti-androgens with a decrease of serum OPG. Irrespective of ADT type, BMD still decreases in primary PCa patients.

Keywords: androgen deprivation therapy, prostate cancer, osteoporosis

During the past half century, the prevalence of prostate cancer (PCa) has been steadily increasing, following the distribution of socio-economic status. In 2012, western Europe had an incidence of PCa of above 67.2/100000 inhabitants, while the reported incidence of PCa in Romania was below 32.4/100000 inhabitants [1]. Known risk factors include age, family history of PCa and smoking [2]. It is also known that patients with acromegaly are at high risk of developing prostate disorders compared with healthy subjects [3,4]. There are some cases reported about prostate cancer with metastases to the kidney and so renal impairment [5], rising the prevalence of bone demineralization. Depending on patient profile and tumor characteristics, routine treatment options include surgery (*e.g.* prostatectomy, orchiectomy) [6], radiotherapy, chemotherapy and pharmacologic androgen deprivation therapy (ADT). There are several options for systemic ADT: estrogens (which the current recommendations do not recommend as first line treatment because of their thromboembolic risk [7,8]); luteinizing-hormone-releasing hormone (LHRH) agonists (synthetic LHRH analogues); LHRH antagonists (*e.g.* degarelix); anti-androgen drugs (*e.g.* medroxyprogesterone, cyproterone, megestrol, abiraterone, nilutamide, flutamide, bicalutamide, enzalutamide). Cohort studies have shown that the use of ADT is associated with a higher risk of several long-term adverse events, such as cardiovascular disease (*e.g.* heart failure [9], arrhythmia, conduction disorder etc. [10]), dementia [11], osteoporosis (with a prevalence of 9-53% depending on disease characteristics [12]) with a high risk of pathological fractures [13] and interestingly even rheumatoid arthritis [14], endocrine dysfunction [15,16] with increased risk of developing metabolic syndrome [17]. Recent studies suggest that the use of ADT increase the risk of kidney dysfunction appearance [18]. In all of these conditions, the duration of ADT is a key element of risk generation, suggesting a strong causal relationship in which the outcome changes proportionally with the exposure: on one hand, it was recently reported that ADT duration is a significant predictor of osteoporosis prevalence and low bone mineral density (BMD) in the usual regions of interest (ROI; lumbar spine and hip) [19]; on the other hand, compared to continuous ADT, intermittent ADT seems to generate a lower risk of cardiovascular disease and fragility fracture [20]; lastly, in terms of BMD loss, acute and chronic ADT produce similar effects, suggesting that osteoporosis develops rapidly after treatment initiation, but this bone loss shows a degree of reversibility upon ADT discontinuation [21]. The rate of bone loss in PCa patients on ADT seems to be comparable to the overall rate of bone loss in post-menopausal women [22]. The association of ADT and osteoporosis is clinically relevant for fragility fracture risk, a fact that has been proven by observational studies that report an increased risk for any fracture [23] with the necessity for different osteosynthesis methods [24,25], due to subjacent affected bone mineralization, for all types of ADT [26]. Since patients with PCa have a higher mortality risk caused by PCa-related and non-related events [27], adding the risk of morbidity and mortality associated

*email: claudiu.popescu@reumatologiedrstoia.ro

All authors had equal contribution

with osteoporotic fractures [28] will significantly reduce the survival of PCa patients. Interestingly, there are reports that osteoporosis is more prevalent among non-metastatic PCa patients even before ADT [29,30], which suggests an imbalance between the coupling between bone formation and resorption as a possible secondary trait of PCa in the absence of external hormonal interventions. Therefore, the first objective of this study was to evaluate bone metabolism in primary PCa patients prior to any treatment intervention (surgical or pharmacological). Since selective estrogen receptor modulator (SERM) treatment is proven to be effective in postmenopausal osteoporosis [31] but also in men with PCa [32] and since ADT should increase loss of bone independently of age and PCa by the lack of protective signalling through androgen receptors in trabecular bone [33], we would expect different bone metabolism outcomes in PCa patients treated with these principles. Therefore, the second objective of this study was to compare estrogen and anti-androgen therapy in primary PCa in terms of bone metabolism.

Experimental part

Materials and methods

The study prospectively observed and screened consecutive male patients diagnosed with primary PCa who were proposed in a real-life setting for radical prostatectomy and ADT by their attending urologists/ oncologists. The following patients were excluded at screening or during follow-up: patients with secondary bone lesions on whole body scintigraphy at the time of screening; patients who underwent chemotherapy before screening or who were proposed for chemotherapy; patients without prostate adenocarcinoma, as confirmed by histopathological examination of prostatectomy specimens in the 4 Bucharest university urology departments from which the patients originated. After the clinical diagnosis of PCa, within one week prior to prostatectomy and prior to pharmacological treatment (either estrogens or anti-androgens, according to real-life indications of attending urologists/oncologists), the patients were referred for laboratory determinations (serum and urine samples, which were taken in the same day for each patient) and imaging (bone densitometry, which was carried out no later than 1 day from the laboratory samples). Depending on the recommendation of each attending physician, the prostate cancer group was split in two: group A included patients who were assigned to receive treatment with non-steroidal anti-androgens (flutamide); group E included patients who were assigned to receive treatment with synthetic estrogens (chlorotrianisene or polyestradiol phosphate). For reference, the study also prospectively screened age-matched controls randomly evaluated in the rheumatology department for osteoarthritis symptoms, without history or treatment for osteoporosis and without history or present prostate disease (group C) and with normal screening prostate-specific antigen levels for age. The following additional exclusion criteria were applied to both categories of patients (current/history of): auto-immune inflammatory joint or bowel disease, kidney disease, liver disease, type 2 diabetes mellitus, hyperparathyroidism, hyperthyroidism, hypogonadism, malabsorption, malnutrition, treatment with glucocorticoids, bisphosphonates, anticonvulsants, and methotrexate. Each patient gave written informed consent and the study was approved by the local ethics committee.

Bone markers

Bone markers were measured in a single laboratory at baseline (before pharmacological treatment) for patients in all the subgroups and 6 months later for patients in groups E and A. Serum osteoprotegerin (OPG) and serum osteocalcin (OC) were determined using commercially available enzyme linked immunosorbent assays (respectively produced by Biomedica Medizinprodukte Wien, Austria and Osteometer BioTech A/S Herlev, Denmark). Urine deoxypyridinoline (DPD) was determined using a chemiluminescence assay (produced by Chiron Diagnostics Corporation East Walpole, USA). The tests were carried out in accordance to the manufacturer's instructions. Each biologic sample (blood, urine) was tested in the laboratory in the same day it was obtained.

Dual X-ray absorptiometry

Each subject underwent two dual X-ray absorptiometry (DXA) scans: upon study inclusion (baseline, pre-treatment) and after 12 months (end of study, post-treatment). All the scans were performed by a single certified clinical densitometrist using a single General Electric Lunar Prodigy machine. Daily calibration and quality control tests were performed according to the manufacturer's recommendations. The regions of interest included the spine (lumbar vertebra 1 to 4) and the non-dominant hip neck, in which BMD (expressed in g/cm^2) and T scores (expressed in standard deviations - SD) were measured. The patients were required to wear light clothing, without metal or plastic, and were scanned in the morning, after nocturnal fast, micturition and 5 min of supine rest on the examination table, in the absence of radioactive or radiocontrast investigations in the prior week. Osteoporosis was defined according to the World Health Organization statement as a spine or hip T score equal to or less than -2.5 SD [34].

Statistics

Distribution normality was assessed using descriptive statistics, normality plots and Lillefors corrected Kolmogorov-Smirnov tests. Continuous variables were normalized by eliminating outliers and they were consequently reported as *mean (SD).* Qualitative variables were expressed as *absolute frequency (fraction of subgroup).* The difference of bone markers and DXA measurements between subgroups (for example the difference between controls and patients treated with estrogens) was assessed by independent samples t tests, while their difference within the same subgroup at different time points (for example before and after androgen depleting pharmacological treatment in the estrogen group) was assessed by paired-samples t tests. Fischer's exact tests were used to assess the difference of osteoporosis prevalence between subgroups (for example the difference between controls and patients treated with anti-androgens), while the difference of osteoporosis prevalence within the same subgroup at different time points were assessed by McNemar tests. All tests were considered significant if $p < 0.05$ and were done using IBM SPSS v.20 (IBM Inc., Armonk, New York, 2010) for Windows.

Results and discussions

General characteristics

The study sample included 30 controls (with a mean age of 70 years), 15 patients in group E (with a mean age of 71 years) and 15 patients in group A (with a mean age of 72 years, table 1). All the patients survived the 12-month

	control (**C**) n = 30	estrogen (**E**) n = 15	anti-androgen (**A**) n = 15	$P_{E\ versus\ C}$	$P_{A\ versus\ C}$	$P_{A\ versus\ E}$
age	70 (6)	71 (6)	72 (5)	0.439	0.212	0.668
OPG1	5.10 (2.17)	4.67 (1.38)	6.37 (3.04)	0.512	0.158	0.059
OPG2	-	5.27 (1.89)	5.02 (1.12)	-	-	0.425
OC1	11.77 (2.70)	12.48 (5.62)	11.65 (2.44)	0.623	0.898	0.606
OC2	-	12.01 (2.39)	12.02 (1.65)	-	-	0.989
DPD1	6.83 (2.70)	6.85 (3.24)	7.35 (2.74)	0.984	0.575	0.648
DPD2	-	8.63 (2.42)	8.77 (2.24)	-	-	0.872
sBMD1	1.09 (0.16)	0.99 (0.32)	1.04 (0.16)	0.201	0.302	0.698
sBMD2	1.01 (0.15)	0.94 (0.31)	0.89 (0.15)	0.126	0.136	0.704
sT1	-0.10 (1.31)	-1.06 (1.29)	-1.30 (1.71)	0.029	0.016	0.685
sT2	-0.14 (1.25)	-1.03 (1.47)	-1.62 (1.51)	0.035	0.009	0.312
hBMD1	0.69 (0.07)	0.65 (0.08)	0.81 (0.18)	0.107	0.002	0.004
hBMD2	0.66 (0.10)	0.63 (0.14)	0.75 (0.17)	0.138	0.059	0.049
hT1	-0.03 (1.32)	-1.68 (1.28)	-1.84 (1.52)	0.0003	0.0003	0.771
hT2	-0.09 (1.15)	-1.63 (1.44)	-1.81 (1.54)	0.001	0.001	0.757
OP1	0 (0%)	5 (33.3%)	7 (46.7%)	0.002	0.0001	0.449
OP2	2 (6.7%)	8 (53.3%)	5 (33.3%)	0.001	0.019	0.332

Table 1
GENERAL CHARACTERISTICS AND SUBGROUP COMPARISONS

*Notes: age is expressed in years, OPG in nmol/L, OC in ng/mL, DPD in nmol/mmol, BMD in g/cm^2, T scores in SD; P values represent the significance of either independent samples t tests (for comparing means of continuous variables) or Fischer's exact tests (for comparing OP prevalence among subgroups); prior to statistical testing, the variables were normalized by extracting square roots (age) or by eliminating outliers (all the rest) and consequently they have been reported as "mean (SD)"; OP prevalence is reported as absolute frequency (fraction of subgroup); abbreviations: **1**- baseline; **2** - after 6 months for bone markers and after 12 months for BMD measurements; **A** - anti-androgen group; BMD – bone mineral density; **C** - control group; **E** – estrogen group; DPD - deoxypyridinoline; h - hip; OC - osteocalcin; OP- osteoporosis; OPG - osteoprotegerin; s – spine; SD- standard deviation; versus- versus.*

observation period and there were no fragility fractures recorded during this time.

Inter-group comparison

There were insignificant age differences between either two groups (table 1). Compared with controls at baseline, there were no significant differences of bone markers (DPD, OC, OPG) in both study groups (E and A, table 1). Compared with the control group, both at baseline and after 12 months (table 1), patients from the E and A groups had lower spine and hip T scores and higher prevalence of osteoporosis. Unexpectedly, patients from group A had significantly higher mean hip BMD than controls at baseline, a difference which was maintained after 12 months but which became statistically insignificant.

When comparing group E with group A (table 1), there were only two noticeable differences: group A patients had significantly higher hip BMD at baseline and after 12 months and tended to have higher baseline OPG levels (barely missing significance level), a difference which disappeared after 6 months.

Intra-group comparison

At baseline, there were no osteoporosis cases in the control group, while after 12 months 2 subjects (6,7%) developed osteoporosis (p = 0.202). Similarly, osteoporosis prevalence increased insignificantly in both the E group (5 patients - 33.3% at baseline and 8 patients - 53.3% after 12 months) and the A group (7 patients - 46.7% at baseline and 9 patients - 60.0% after 12 months; Figure 1).

Compared to baseline (table 2), in the E group at 6 months OPG and DPD levels increased significantly (fig. 2), while OC had an insignificant decrease. Regarding bone measurements at 12 months, spine BMD decreased significantly (fig. 2), hip BMD decreased insignificantly, spine and hip T scores remained virtually the same.

Compared to baseline (table 2), in the A group at 6 months OPG decreased significantly (fig. 3), while OC and DPD increased insignificantly. Regarding bone measurements at 12 months, spine and hip BMD decreased

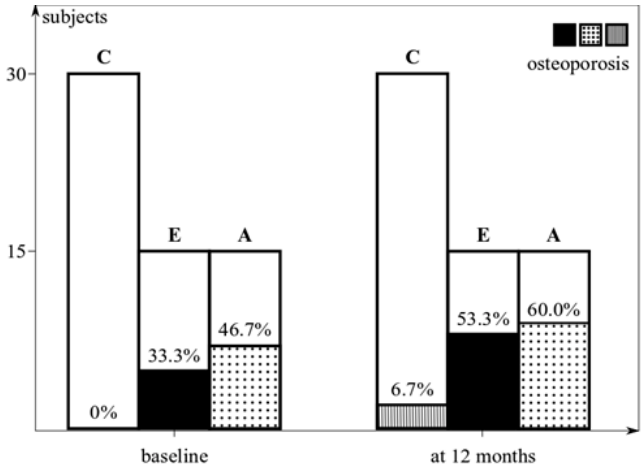

Fig. 1. The prevalence of osteoporosis in the 3 studied groups (controls- **C** = 30 subjects; patients treated with estrogens - **E** = 15 patients; patients treated with anti-androgens - **A** = 15 patients), at baseline and after 12 months (from radical prostatectomy and respective hormonal treatment)

significantly (fig. 3), spine and hip T scores had an insignificant variation.

The first objective of this study was to evaluate bone metabolism in primary PCa patients prior to any treatment intervention (surgical or pharmacological). Compared to controls, we found no significant differences of bone markers (OPG, OC, DPD) at baseline in primary PCa patients, instead we observed a higher prevalence of osteoporosis (33.3-53.3%) and lower spine and hip T scores. Recent studies have observed a comparable osteoporosis prevalence of up to 33-38% in hormone-naive PCa patients [29,30], suggesting further awareness of poor bone health in PCa patients and screening DXA scans prior to ADT, a recommendation which we find suitable for modern preventive medicine standards.

The lack of significant differences of bone markers (*e.g.* OPG, OC, DPD) between controls and PCa patients before treatment which we observed and which confirms other literature reports [35], suggests other biological

	treatment		
1. *estrogen group*	*before*	*after*	*p*
OPG	4.67 (1.38)	5.27 (1.89)	0.043
OC	12.48 (5.62)	12.01 (2.39)	0.725
DPD	6.85 (3.24)	8.63 (2.42)	0.008
sBMD	0.996 (0.319)	0.935 (0.311)	0.019
sT score	-1.06 (1.29)	-1.03 (1.47)	0.649
hBMD	0.647 (0.075)	0.626 (0.136)	0.632
hT score	-1.68 (1.28)	-1.63 (1.44)	0.924
OP	5 (33.3%)	8 (53.3%)	0.256
2. *anti-androgen group*			
OPG	6.37 (3.04)	5.02 (1.12)	0.041
OC	11.65 (2.44)	12.02 (1.65)	0.587
DPD	7.35 (2.74)	8.77 (2.24)	0.108
sBMD	1.034 (0.154)	0.899 (0.153)	0.0003
sT score	-1.30 (1.71)	-1.62 (1.51)	0.071
hBMD	0.814 (0.180)	0.747 (0.166)	0.003
hT score	-1.84 (1.52)	-1.81 (1.54)	0.961
OP	7 (46.7%)	9 (60.0%)	0.431

Table 2
COMPARISON OF BONE MARKERS AND DENSITY BEFORE AND AFTER SPECIFIC TREATMENT FOR EACH STUDY GROUP

Notes: OPG is expressed in nmol/L, OC in ng/mL, DPD in nmol/mmol, BMD in g/cm² and T scores in SDs; P values represent the significance of either independent samples t tests (for comparing means of continuous variables) or McNemar's tests (for comparing OP prevalence before and after treatment); prior to statistical testing, continuous variables were normalized by eliminating outliers and consequently they have been reported as mean (SD); abbreviations: BMD - bone mineral density; DPD - deoxypyridinoline; h - hip; OC - osteocalcin; OP osteoporosis; OPG - osteoprotegerin; s - spine; SD - standard deviation.

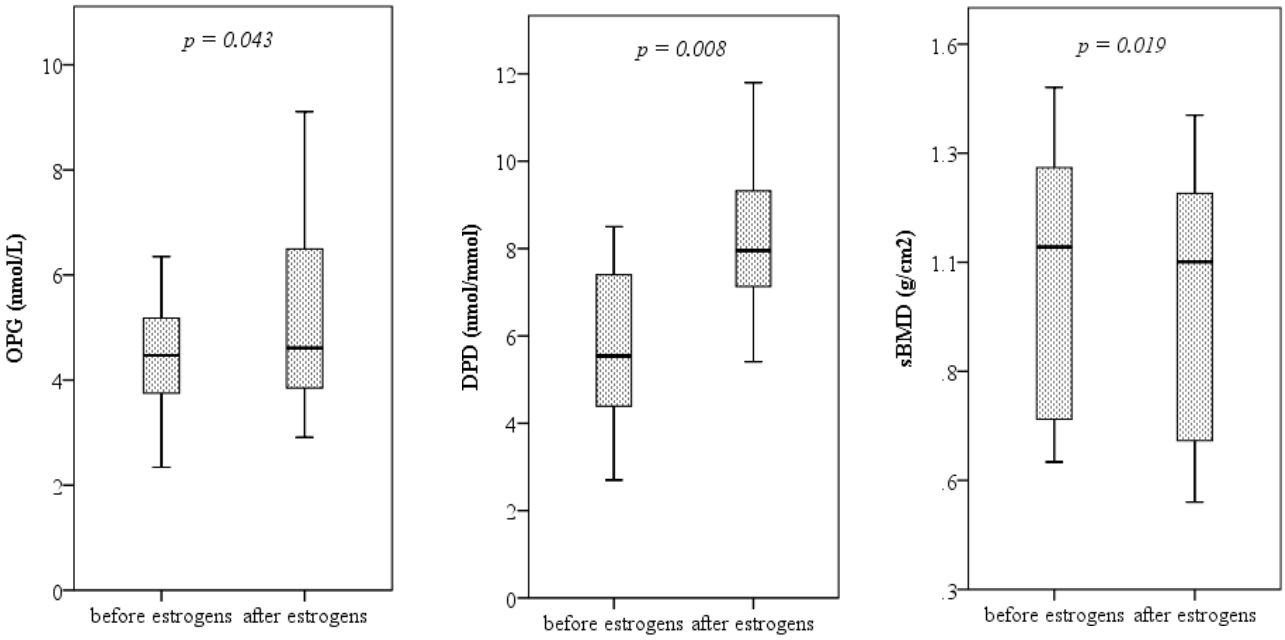

Fig. 2. Estrogen group (E) - significant differences between bone markers (OGP, DPD) and spine BMD before and after 6 months of treatment. P values represent the significance of paired-samples t tests. *Abbreviations*: BMD - bone mineral density; DPD-deoxypyridinoline; OPG-osteoprotegerin

mechanisms of prevalent osteoporosis among treatment-naive primary PCa patients, namely an imbalance of bone formation – bone resorption by overactivity of latter. In support of this hypothesis, fundamental science has shown that OPG is directly expressed by prostate cancer cells at the primary tumor site [36], but OPG shows relatively increased levels, significant correlations and predictive power only in relation to advanced/metastatic PCa (*i.e.* secondary bone tumors) [35,37-40], for which there also seems to be a genetic predisposition [41]. Since cancer, irrespective of type and patient gender, is associated with a higher prevalence of osteoporosis compared to non-cancer subjects [42], we may assume that PCa is not different in this respect and that it generates this risk by overexpressing bone resorption signals. Alteration of calcium metabolism does not seem to be involved (PCa had the lowest prevalence of malignancy-associated hypercalcemia [43], even though osteolytic cytokines produced by PCa cells may be involved [44]). In these circumstances, we may hypothesize common environmental factors and chronic diseases for osteoporosis and PCa (*e.g.* smoking, diabetes mellitus [45], chronic haemodialysis [46-48] and/or specific PCa-produced osteoclast activators, one of which can be receptor activator of nuclear factor κ-B ligand (RANK-L) [49] which can exert its effects through the alternative MYC/ERRá pathway [50-51]. Future research should address the study of cytokine production profile of primary PCa cells in order to offer reasonable evidence for a specific PCa osteoporosis-inducing effect.

The second objective of this study was to compare estrogen and anti-androgen therapy in primary PCa in terms of bone metabolism. We observed that in the estrogen-treated group (E), OPG and DPD increased after 6 months and spine BMD decreased after 12 months, while in the anti-androgen-treated group (A), OPG decreased after 6 months and spine and hip BMD decreased after 12 months. There are several discussion points which arise from these observations.

Serum OC, a marker of metastatic PCa [52-53] and a predictor of hormone treatment response [54], does not seem to be influenced by ADT in primary PCa, maybe

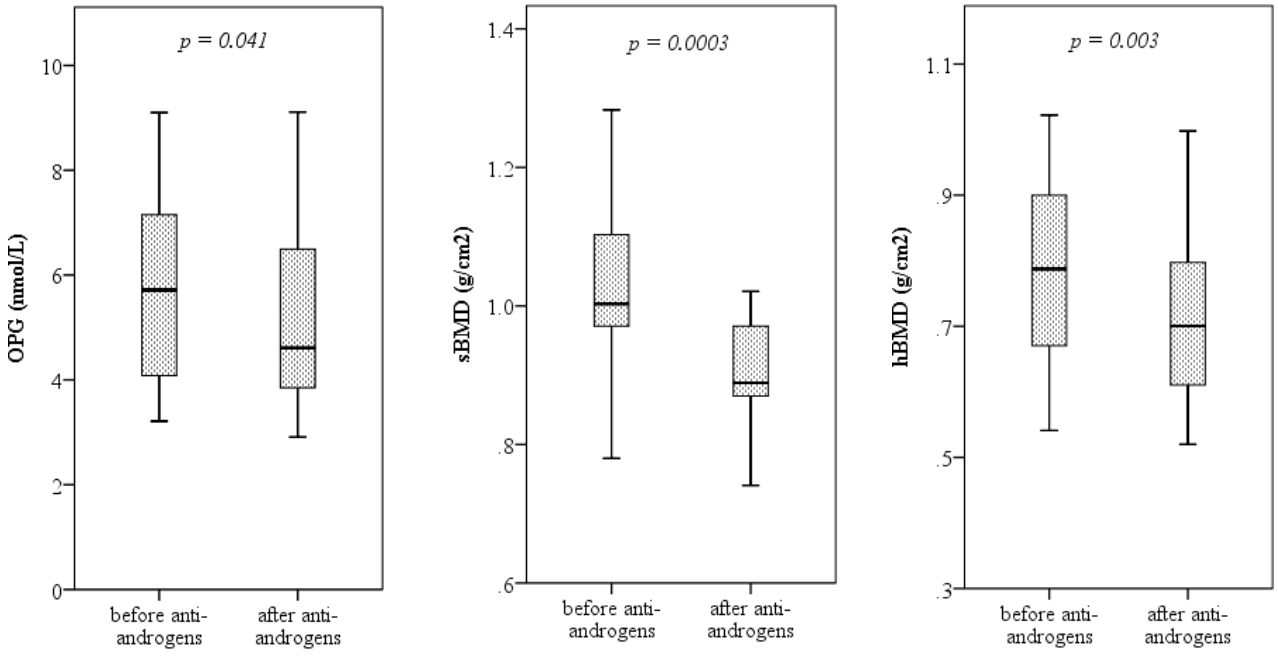

Fig. 3. Anti-androgen group (A) – significant differences between bone markers (OGP) and bone densitometry (spine and hip BMD) before and after 6 months of treatment. P values represent the significance of paired-samples t tests. *Abbreviations*: BMD - bone mineral density; OPG -osteoprotegerin

because, even though it is expressed by local PCa cells, it is incompletely spliced [55].

The rise of OPG after 6 months of estrogen is consistent with literature data which show that estrogen stimulates OPG production in vitro [56] and in vivo in male patients [57-58], including PCa patients on ADT [59]. Treatment-induced rise of serum OPG with estrogen in our primary PCa patients seems to have been insufficient to counteract the bone resorption process (maybe PCa-specific) evidenced by a parallel rise in urinary DPD, resulting overall in bone loss. Since a randomized controlled trial of raloxifene (SERM) in patients with primary PCa reported a decrease of urinary DPD [60], the rise of DPD we observed in the E group could be a biological sign of incident occult metastatic disease in the studied patients, since DPD is a marker and predictor of metastatic PCa [61-63].

Vandyke et al. [64] showed that androgen inhibits OPG production of in vitro PCa cells and Khosla et al. [58] reported that testosterone decreases OPG levels in vivo in men, while we observed that anti-androgens decrease serum OPG over 6 months in primary PCa patients – an observation which would be in accordance with the other findings of positive correlation of OPG-testosterone levels in non-PCa subjects [57] and in PCa patients [59]. This apparent contradiction with fundamental research, in ideal study conditions, suggests either a different effect of androgens and anti-androgens in vivo or in vitro (i.e. anti-androgens may increase OPG in vitro – a further research topic) or the lack of sizeable effect of anti-androgens on OPG levels in our patients, the decrease of OPG being PCa-related or generally cancer-related.

Observational studies have shown that BMD testing and osteoporosis treatment are sub-optimal in PCa patients on ADT [65]. Given the detrimental effects of fragility fractures and the evidence of a high proportion of inappropriate ADT in PCa [66], DXA scans of PCa patients should become routine medical practice, before, during and after ADT. There are two reasons for this suggestion: the underestimated recognition of osteoporosis among PCa patients is reversible with educational strategies [67]; anti-osteoporotic drugs (e.g. denosumab [68-70], alendronate [68], zoledronat, risedronate [71], pamidronate [72]) are effective in reducing BMD loss in PCa patients.

Therefore, primary prostate adenocarcinoma is an aggressive type of cancer, leading to multiple complications and disabling conditions, especially bone fragility, like others malignant aggressive disorders [73-76].

There are several study limitations which could have influenced the results and their relevance. First of all, the study sample was small (as a consequence of funding availability), a limitation which could influence statistics judgments of null hypotheses. Because of study design, there was no information regarding smoking status of subjects and patients and the testing of RANK-L was not available.

Conclusions

Osteoporosis is prevalent among hormone-naïve PCa patients through pro-resorptive signals. Appropriate DXA screening, prophylactic and curative treatment are warranted. Estrogen treatment of primary PCa seems to be associated with an increase of serum OPG, while anti-androgen treatment seems to decrease serum OPG. However, irrespective of ADT type, BMD still decreases in primary PCa patients, which suggests a PCa-specific mechanism of stimulating bone catabolism.

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