



PRIMARY HYPERPARATHYROIDISM AND SERUM CALCIUM IN BREAST CANCER PATIENTS EVALUATED FOR LOW BONE MASS – A SINGLE CENTER EXPERIENCE

Marija Punda^{1,2}, Petra Petranović Ovčariček¹, Anita Tabain³, Klaas Pieter Koopmans⁴,
Gabriela Alfier⁵, Tomislav Jukić¹ and Ana Fröbe^{1,6}

¹Department of Oncology and Nuclear Medicine, Sestre milosrdnice University Hospital Center, Zagreb, Croatia;

²University of Applied Health Sciences, Zagreb, Croatia;

³Department of Radiology and Nuclear Medicine, PET/CT Center, Medikol Polyclinic, Zagreb, Croatia;

⁴Department of Nuclear Medicine and Radiology, Martini Hospital, Groningen, The Netherlands;

⁵Department of Surgery, University Hospital for Tumors, Sestre milosrdnice University Hospital Center, Zagreb, Croatia;

⁶School of Dental Medicine, University of Zagreb, Zagreb, Croatia

SUMMARY – The bone health guidelines for breast cancer (BC) patients recommend bone mineral density (BMD) testing. Patients with low BMD and elevated serum calcium levels (SCLs) are further evaluated for primary hyperparathyroidism (PHPT). We aimed to determine the prevalence of PHPT in treated BC patients with low BMD and analyze the association of SCLs with histopathologic tumor features and cancer treatment. This retrospective study included postmenopausal BC patients examined at Osteoporosis Clinic between 2013 and 2020. Clinical and BMD data were collected from patient medical records. Patients with biochemical suspicion of PHPT underwent standard parathyroid imaging procedures. Nine out of 137 (6.6%) patients were diagnosed with PHPT; 8/9 patients underwent parathyroidectomy and one patient was advised to follow-up. Among the rest of 128 non-PHPT patients, higher SCLs showed a trend of positive association with higher tumor grade and axillary lymph node involvement, and received immunotherapy, although without statistical significance. We found a higher prevalence of PHPT in treated BC patients compared to the general population. Higher SCLs show a trend of positive correlation with some more aggressive histopathologic tumor features and with immunotherapy. The results of this study suggest that assessment of SCLs should be routinely performed to rule out PHPT in treated BC patients with low BMD.

Key words: *Primary hyperparathyroidism; Serum calcium; Breast cancer; Bone mineral density; Cancer treatment*

Introduction

Breast cancer (BC) patients receiving advanced specific antineoplastic treatments are at an increased risk of accelerated bone loss, particularly with the use

of aromatase inhibitors (AI)¹. The bone health guidelines for women with BC recommend bone mineral density (BMD) measurement by dual-energy x-ray absorptiometry (DXA) and exclusion of secondary causes of low BMD such as primary hyperparathyroidism (PHPT)^{1,2}. Hypercalcemia is a common complication in BC patients, affecting up to 40% of them³. Increased serum calcium levels (SCLs) in BC patients are most commonly caused by skeletal metastasis, followed by PHPT, and in a certain proportion of

Correspondence to: *Marija Punda, MD*, Department of Oncology and Nuclear Medicine, Sestre milosrdnice University Hospital Center, Vinogradska c. 29, HR-10000 Zagreb, Croatia
E-mail: marija.punda@kbcs.m.hr

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cases by the production of humoral factors of malignancy such as parathyroid hormone-related protein (PTHrP)³⁻⁷. A few case reports describe that hypercalcemia mimicking PHPT is associated with the use of AI in hormone receptor-positive BC patients, but there is a lack of larger studies to confirm such relation^{8,9}. Studies that describe PHPT in BC patients are scarce and show conflicting results. Several reports documented an increased prevalence of PHPT in treated^{3-5,10} and untreated patients with BC¹¹, while others did not find such association in untreated patients^{12,13}.

There are a lot of discussions about whether both PHPT and hypercalcemia are the cause or consequence of BC. Few studies describe positive association of pre-diagnostic SCLs with tumor volume and BC aggressiveness^{14,15}. On the other hand, Martin *et al.*¹² did not find a relation of SCLs with tumor size. However, both cancer treated³ and untreated BC patients¹⁶ have been shown to have higher SCLs compared to non-BC controls. The first aim of this study was to determine the prevalence of PHPT in treated BC patients evaluated for low BMD. The second aim was to determine the association of SCLs with cancer treatment and histopathologic tumor features.

Patients and Methods

Patient selection

Retrospectively, we initially examined 170 postmenopausal BC patients evaluated for low bone mass at our Osteoporosis Clinic. Patients were recruited from two tertiary oncologic institutions in Zagreb, Croatia, in the period between January 2013 and October 2020. After exclusion of 33 patients based on exclusion criteria, 137 patients were examined. Patients with biochemical suspicion of PHPT underwent standard parathyroid imaging procedures (n=9) and they were analyzed separately. The association of SCLs with cancer treatment and histopathologic tumor features was analyzed in 128 non-PHPT patients.

Inclusion/exclusion criteria

The inclusion criteria were as follows: patients with BC stages I-III, at least 2 months after BC diagnosis and under cancer therapy, postmenopausal ≥ 12 months, and with at least two examinations at Osteo-

porosis Clinic. The exclusion criteria were as follows: single visit at Osteoporosis Clinic (n=12), history of previous malignancy (n=12), bone metastasis involvement (n=6), and use of glucocorticoids (n=2) and anti-epileptics (n=1), which can affect BMD.

BMD assessment

Spine (L1-L4) and hip (total hip and femoral neck) BMD was assessed by DXA Hologic Delphi C (Bedford, USA) and values were expressed as T-scores according to the World Health Organization classification system¹⁷. For the purpose of this study, a cut-off T-score < -2.0 was used as suggested in the current bone health guidelines for BC patients¹.

Data collection

Clinical data were collected from patient medical records and using a questionnaire drawn up before DXA scanning. The following data were included: time from BC surgery, type of BC, cancer treatment (neoadjuvant chemotherapy, postoperative chemotherapy, radiotherapy, immunotherapy, LHRH therapy), adnexectomy, use of aromatase inhibitors and bisphosphonates, vitamin D supplementation, history of adult non-traumatic fracture, current and past cigarette smoking.

Histology type of breast cancer, tumor size, tumor grade, and lymph node status were obtained from the histopathology report. Tumor stage was classified according to the American Joint Committee on Cancer TNM staging system¹⁸. Grades of tumor were expressed as grade 1 (well-differentiated), 2 (moderately differentiated), and 3 (poorly differentiated). Hormone receptor status was abstracted from histopathology report as positive or negative for estrogen receptor (ER), progesterone receptor (PR), or HER-2 receptor.

Laboratory testing

Data on blood levels of SCL and serum 25(OH) vitamin D, alkaline phosphatase (ALP), serum phosphate, creatinine, and estimated glomerular filtration rate (eGFR) were obtained from medical records. The date of laboratory testing was within a year of BMD measurement. In patients with increased SCLs (≥ 2.54 mmol/L), ionized serum calcium and intact parathyroid hormone (PTH) were measured in order to exclude or confirm PHPT.

Laboratory analyses

Serum calcium levels (normal range 2.14-2.53 mmol/L) were measured with atomic absorption spectrophotometry (AAS) on a Microlab 530B (Hamilton®, USA), 25-hydroxyvitamin D by the chemiluminescent microparticle immunoassay (CMIA) method on Architect i2000SR (Abbott, Abbott Park, IL, USA) using the original manufacturer's reagents (insufficiency <25, suggested level ≥75 nmol/L), ALP (normal range 54-119 U/L) with colorimetric IFCC method.

Ionized serum calcium was measured with a potentiometric method using ion-selective electrode (ISE) technology on a Rapidpoint 400 (Siemens AG, Munich, Germany) (normal range 1.18-1.32 mmol/L), and intact parathyroid hormone (PTH) levels on a Cobas e 411 automatic analyzer (Roche Diagnostics GmbH, Mannheim, Germany) by ECLIA method with commercial PTH STAT reagent (normal range 15-65 pg/mL).

Additionally, the levels of serum phosphate (normal range 0.79-1.42 mmol/L), creatinine (49-90 μmol/L) and eGFR (normal ≥90, mild loss 60-89, mild to moderate 45-59, moderate to severe 30-44, severe loss of kidney function 15-29 mL/min/1.73 m²) were recorded.

Parathyroid imaging methods

Patients with biochemical suspicion of PHPT underwent dual-phase [^{99m}Tc]Tc-MIBI SPECT/CT parathyroid scintigraphy, high-resolution cervical ultrasonography (cUS), and ultrasound-guided fine-needle aspiration cytology with measurement of PTH levels in the needle washout (FNA-PTH).

Dual-phase [^{99m}Tc]Tc-MIBI SPECT/CT parathyroid scintigraphy was performed on a Symbia SPECT/CT Gamma Camera, with injected activity between 600-900 MBq (depending on body mass) with early (10-15 min post-injection) and delayed (1.5-2.5 h) acquisition. In one case of negative [^{99m}Tc]Tc-MIBI SPECT/CT, the patient underwent [¹⁸F] fluorocholine PET/CT scan in another institution. High-resolution cUS was performed on a Xario Toshiba ultrasound machine using a linear 7-12 MHz probe and in certain cases ultrasound-guided (using small convex 7 MHz probe) fine-needle aspiration cytology along with measurement of PTH levels in the needle washout (FNA-PTH).

Ethical issues

The study was conducted in accordance with ethical standards set by the institutional Ethics Committee and the Helsinki Declaration from 1975, as revised in 1983. Informed consent was not needed since this was a retrospective study, and all procedures were done according to the institutional standard diagnostic protocol.

Statistical analysis

Data were assessed with Kolmogorov-Smirnov test and according to the findings, appropriate non-parametric tests according to the findings were used in the analysis. Differences in continuous data were analyzed with Mann-Whitney U test, while Fisher-Freeman-Halton test was used to assess difference in categorical clinical and socio-demographic values. Spearman's correlation coefficients Rho were used to analyze the correlation between clinical variables and SCLs. Patients were divided into two groups according to the distribution of SCLs, as follows: patients with SCLs below and equal to 2.49 (1st and 2nd tertile) and patients with SCLs higher than 2.49 (3rd tertile). All p values below 0.05 were considered significant. IBM SPSS Statistics version 25.0 was used in all statistical procedures.

Results

Results are presented as those for PHPT patients and non-PHPT patients.

PHPT patients

Out of 137 patients examined, nine (6.6%) were diagnosed with PHPT. Clinical data on PHPT patients are shown in Table 1. The patients (mean age 62.78, range 55-72 years) were diagnosed with PHPT after a median follow-up of 12 (range 2-40) months following BC surgery. The mean breast tumor size was 1.89 (range 0.9-2.8) cm; one patient had multifocal ductal carcinoma *in situ* and one had bilateral BC. Four out of nine patients had axillary lymph node involvement. All patients had both ER and PR positive tumors, and 4 of them had associated HER-2 positive receptors. The mean serum calcium, ALP and PTH levels were 2.77 (range 2.39-2.97) mmol/L, 78.78 (range 59-105) U/L, and 146 (range 81-294) pg/mL,

Table 1. Clinical profile and parathyroid status in nine breast cancer patients with primary hyperparathyroidism

BC patient	Age (yrs)	BC stage	BC to PHPT diagnosis (months)	Serum PTH 15-65 (pg/mL)	Serum calcium 2.14-2.53 (mmol/L)	DXA-BMD T-score LS/neck	US diameter of the parathyroid (mm)	Cancer treatment	RT	Treatment for PHPT
1	71	II	35	119.4	2.85	-3.0/-2.5	7	AI	+**	PTX
2	64	I	26	81.0	2.78	-2.4/-1.6	8	AI	+*	PTX
3	59	I	2	182.3	2.77	-2.1/-2.6	8	AI	+	PTX
4	55	II	12	82.0	2.39	-2.4/-1.9	4	TF, AI, CT	-	Follow-up
5	56	III	26	99.1	2.69	-0.4/0.7	11	NAC, AI, IM	+	PTX
6	67	II	40	102.0	2.60	-2.0/-1.6	8	CT, AI, IM	+*	PTX
7	63	II	11	294.0	2.93	-2.9/-2.9	17	AI, CT	+*	PTX
8	58	I	10	112.8	2.97	-2.8/-1.7	32	NAC, AI, IM	-	PTX
9	72	I	8	249.0	2.94	-1.6/-1.7	15	CT, IM	+*	PTX

BC = breast cancer; PHPT = primary hyperparathyroidism; DXA = dual energy x-ray absorptiometry; BMD = bone mineral density; LS = lumbar spine; neck = femoral neck; US = ultrasound; RT = radiotherapy; AI = aromatase inhibitors; CT = chemotherapy; TF = tamoxifen; NAC = neoadjuvant chemotherapy; IM = immunotherapy; PTX = parathyroid adenectomy; *ipsilateral parathyroid adenoma; **bilateral PTX

respectively. The mean level of 25-hydroxyvitamin D was 54.5 (range 24-93) nmol/L. All but one patient had low DXA T-scores at lumbar spine (mean, SD -2.18±0.81) and at femoral neck (-1.76±1.04). At the time of PHPT evaluation, 8/9 patients were taking AIs for a median of 11 (range 1-38) months, and one patient was evaluated before starting AI therapy.

Patients had previously undergone neoadjuvant chemotherapy (2/9 patients), chemotherapy (4/9 patients), and 4 of them received immunotherapy. Post-operative radiotherapy was performed in 7 out of 9 patients. In 4/7 patients having undergone radiotherapy, the parathyroid lesion was ipsilateral to the treated BC side. In one patient, two parathyroid adenomas were found, i.e. on the irradiated ipsilateral and on the contralateral side.

Primary hyperparathyroidism was confirmed in 8/9 patients based on concordant [^{99m}Tc]Tc-MIBI SPECT/CT, cUS, and FNA-PTH findings, and they underwent surgery for parathyroid adenoma. One patient (no. 4) with recurrent nephrolithiasis had negative [^{99m}Tc] Tc-MIBI SPECT/CT and PHPT was diagnosed based on [¹⁸F]fluorocholine PET/CT scan at another institution. In that patient, a lesion of 4 mm in diameter was also detected by cUS (not suitable for FNA), and follow-up was recommended.

Non-PHPT patients

The 128 non-PHPT patients (median age 63.0 years; IQR 54.8-70.0) were evaluated after a median of 2.2 years (range 2-84 months) following BC diagnosis. The preoperative BC staging was as follows: stage I: 52 (40.6%), stage II: 43 (33.6), and stage III: 33 (25.8%) patients. Histopathology revealed axillary lymph node involvement in 59 (46.1%) patients. Recurrent and bilateral BC was found in 8 and 5 patients, respectively. The majority of patients had hormone-positive BC (96.9% ER positive and 83.6% PR positive), while HER-2 receptors were positive in 34 (26.6%) cases.

Non-PHPT breast cancer patients: higher vs. lower calcium level group

Serum calcium of patients with values in the top tertile (>2.49, n=39) were compared to those in the 2 lower tertiles (≤2.49, n=89) (Table 2). On univariate analysis, higher SCLs showed trends of positive asso-

Table 2. Comparison of two groups of patients according to serum calcium values regarding tumor characteristics and cancer treatment

Variable	Serum calcium ≤2.49 (mmol/L) n=89	Serum calcium >2.49 (mmol/L) n=39	p value*
Breast cancer histology^b:			
Ductal invasive	71 (79.8)	33 (84.6)	0.791
Lobular invasive	7 (7.9)	2 (5.1)	
Other	11 (12.4)	4 (10.3)	
Axillary involvement ^b :	38 (42.7)	21 (53.8)	0.244
Tumor stage^b:			
I	39 (43.8)	13 (33.3)	0.415
II	29 (32.6)	14 (35.9)	
IIIA	9 (10.1)	8 (20.5)	
IIIB	2 (2.2)	0 (0.0)	
IIIC	10 (11.2)	4 (10.3)	
Tumor size^b:			
≤2 cm	58 (65.9)	23 (59.0)	0.582
>2 cm	30 (34.1)	16 (41.0)	
Tumor grade^b:			
1	14 (17.5)	2 (5.7)	0.182
2	41 (51.3)	23 (65.7)	
3	25 (31.3)	10 (28.6)	
Ki-67 (%) ^a	30.06±19.93	27.76±12.40	0.794
ER positive ^b	86 (96.6)	38 (97.4)	0.809
PR positive ^b	72(84.7)	35(92.1)	0.260
HER-2 positive ^b	21(23.6)	13(33.3)	0.251
Operation^b:			
Unilateral	79 (88.8)	36 (92.3)	0.815
Recurrent	6 (6.7)	2 (5.1)	
Bilateral	4 (4.5)	1 (2.6)	
AI therapy ^b	86 (96.6)	39 (100)	0.245
Neoadjuvant chemotherapy ^b	13 (14.6)	7 (17.9)	0.632
Chemotherapy ^b	54 (60.7)	18 (46.2)	0.127
Immunotherapy ^b	20 (22.5)	14 (35.9)	0.113
LHRH therapy ^b	4 (4.5)	3 (7.7)	0.464
Adjuvant radiotherapy ^b	67(75.3)	31(79.5)	0.605
Adnexectomy ^b	7 (7.9)	3 (7.7)	0.973
AI therapy duration (months) ^a	20.93±17.64	20.44±18.52	0.814

^aMann-Whitney U test, values are expressed as mean ± SD; ^bFisher-Freeman-Halton test, values are expressed as n (%); *p<0.05 was considered statistically significant; Ki-67 = cell proliferation marker; AI = aromatase inhibitors; ER = estrogen receptor; PR = progesterone receptor; HER-2 = herceptin-2 receptor; LHRH = luteinizing hormone-releasing hormone

ciation towards higher tumor grade, axillary lymph node involvement, received immunotherapy, HER-2 and PR receptor positivity, however, the results did not reach statistical significance. Although patients with

Table 3. Comparison of two groups of patients according to serum calcium values regarding clinical characteristics

Variable	Serum calcium ≤2.49 (mmol/L) n=89	Serum calcium >2.49 (mmol/L) n=39	p value*
Age (years) ^a	64.0 (55.0 to 71.0)	62.0 (54.5 to 69.0)	0.604
BMI (kg/m ²) ^a	26.3 (23.6 to 28.8)	25.4 (22.6 to 28.1)	0.455
Time from surgery (months) ^a	22.0 (12.0 to 34.0)	20.0 (13.0 to 30.5)	0.913
Ionized calcium (mmol/L) ^a	1.2 (1.2 to 1.3)	1.3 (1.2 to 1.3)	0.076
Phosphate (mmol/L) ^a	1.2 (1.0 to 1.3)	1.2 (1.0 to 1.3)	0.213
ALP level (U/L) ^a	71.0 (61.0 to 86.0)	83.5 (71.8 to 99.0)	0.009
Creatinine (μmol/L) ^a	65.0 (58.0 to 71.0)	70.0 (66.0 to 76.0)	0.002
eGFR (mL/min/1.73m ²) ^a	87.0 (78.0 to 97.5)	79.5 (71.0 to 89.0)	0.010
25(OH) vitamin D (nmol/L) ^a	72.2 (53.0 to 90.5)	70.5 (57.0 to 82.8)	0.566
PTH (pg/mL) ^a	44.4 (34.9 to 58.9)	49.0 (38.4 to 64.2)	0.109
Femoral neck T-score ^a	-1.8 (-2.2 to -1.0)	-1.9 (-2.3 to -1.3)	0.406
Total hip T-score ^a	-1.0 (-1.5 to -0.4)	-0.9 (-1.6 to -0.4)	0.901
L1-L4 T-score ^a	-2.2 (-2.9 to -1.5)	-2.2 (-2.8 to -1.4)	0.986
T-score <-2.0 at any site ^b	66 (74.2)	29 (74.4)	0.981
Smoker ^b	21 (23.6)	10 (25.6)	0.735
Previous adult fracture ^b	18 (20.2)	5 (12.8)	0.315
Vitamin D supplementation ^b	81 (91.0)	37 (94.9)	0.454
Bisphosphonate treatment ^b	32 (36.0)	6 (15.4)	0.019

^aMann-Whitney U test, values are expressed as median (interquartile range, IQR); ^bFisher-Freeman-Halton test, values are expressed as n (%); *statistically significant p values are shown in bold (p<0.05); BMI = body mass index; ALP = alkaline phosphatase; eGFR = estimated glomerular filtration rate; PTH = parathyroid hormone

higher SCLs had a somewhat higher proportion of stage III BC (30.8% *vs.* 23.5%) we did not find significant positive association. There was no apparent correlation between SCLs and duration of AI treatment (p=0.814). In Table 3, two groups of patients are compared according to SCLs with respect to other clinical characteristics. The group with higher SCLs compared to the group with lower SCLs had significantly higher ALP levels (83.5 (IQR 71.8-99.0) *vs.* 71.0 (IQR 61.0-86.0); p=0.009) and creatinine levels (70.0 (66.0-76.0) *vs.* 65.0 (58.0-71.0); p=0.002), while eGFR was lower (79.5 (71.0-89.0) *vs.* 87.0 (78.0-97.5); p=0.010). On the other hand, the group with lower SCLs had higher bisphosphonate use compared to the other group (32 (36.0%) *vs.* 6 (15.4%); p=0.019). Low bone mass with a T-score <-2.0 at any of the measured sites was found in 95 (74.2%) patients. The mean densitometry T-scores at all measured regions did not differ significantly between the two serum calcium groups.

Discussion

Primary hyperparathyroidism is the most common cause of benign hypercalcemia and affects about 1% of adults, mostly postmenopausal women¹⁹. We found PHPT in 6.6% of our BC patients, which is consistent with several reports on treated BC. Fierrabraci *et al.*³ report a prevalence of 7% among 100 cancer treated BC patients, and no case among 102 age-matched healthy subjects or 60 thyroid cancer patient controls. These patients had previously undergone surgery, radiotherapy, chemotherapy, or hormonal therapy with tamoxifen. In another study that examined 200 patients within 5 years of BC diagnosis, PHPT was observed with an overall prevalence of 4%⁴. Camacho *et al.*¹⁰ found PHPT in 4.7% out of 64 patients compared to 1.7% out of 174 non-BC controls. However, 97% of these patients were observed before starting adjuvant treatment with AIs. In the study by Tanaka⁵, PHPT was diagnosed in 7 (2.88%) out of 243 BC patients.

Based on their findings, the onset of PHPT was not related to previous cancer treatments.

The occurrence of PHPT was also observed among untreated BC patients, raising the question of whether PHPT is the cause or consequence of BC. In the study by Martin *et al.*¹², PHPT was diagnosed in 3/190 patients with untreated BC and none among 172 non-BC controls (median age 71, range 65-93 years). Berardi *et al.*¹⁶ found no case of PHPT either among 186 patients with untreated BC or among controls including 122 women before surgery for thyroid cancer and 111 patients with benign thyroid diseases. During a retrospective review of BC patients admitted to the Memorial Sloan-Kettering Cancer Center during the 1959-1986 period, PHPT was found in 35 (0.15%) BC patients, which was similar to the incidence in the total population (0.14%)¹³. The patients with PHPT had an earlier stage of the disease (0-II), however, no information on previous cancer treatment was discussed.

Although the majority of our PHPT patients had an earlier stage of the disease (4 patients in stage I and 4 patients in stage II), 6/9 patients had undergone two or more forms of systemic treatment. Compared to previously reported studies, our patients had received some new-advanced systemic treatments such as neoadjuvant chemotherapy, immunotherapy, and use of AIs.

Besides systemic treatments, prior neck and breast irradiation has been reported as a possible factor responsible for the occurrence of PHPT. In our study, in 4 out of 7 patients having undergone neck/breast irradiation, the parathyroid lesion was ipsilateral to the treated BC side. In the study that evaluated patients having undergone parathyroidectomy after previous surgery and neck/breast irradiation, the side of radiation therapy was strongly associated with the side of the subsequent parathyroid adenoma²⁰. Latency of about 10 years from neck irradiation and about 8 years between breast irradiation and diagnosis of PHPT has been reported^{20,21}. However, in our group of PHPT patients, the diagnosis was proven much earlier, after a median of 12 months after radiation therapy.

Antineoplastic treatment with the use of AIs in hormone-receptor positive BC is known to be associated with accelerated bone loss and an increased risk of osteoporosis and subsequent fractures²². We found a high prevalence of low bone mass (74.2%) expressed as

a T-score of <-2.0 at any of the measured sites among our non-PHPT BC patients. The joint guidelines underline the importance of bone health evaluation in BC patients and recommend antiresorptive therapy in those having a baseline T-score of <-2.0 or independent of T-score in patients with two or more clinical risk factors for fracture¹. The current guidelines for BC patients published by the Croatian Oncology Society recommend osteoporosis treatment in women receiving AIs with a T-score in osteoporosis range (<-2.5). In patients with normal BMD (T-score >-1.0) and osteopenia (T-score >-2.4), densitometry after 2 years is recommended, without suggesting referral to endocrinologist²³. Therefore, in a certain number of BC patients, bone health evaluation including biochemical screening for SCLs and PHPT would be missed before advanced bone loss occurs.

Hypercalcemia of malignancy (HCM) is most often associated with malignancies of the lung, breast, head and neck, cervix, prostate, urinary tract cancers, and multiple myeloma. Common causes of HCM are humoral hypercalcemia of malignancy (HHM) and local bone osteolysis. HHM refers to systemic secretion of PTHrP by malignant tumors and is responsible for 80% of HCM cases. PTHrP increases calcium resorption in the kidney and stimulates osteoblasts to secrete receptor activators of nuclear factor- κ B ligand (RANKL). RANKL binds to the RANK receptor on osteoclasts, thus stimulating bone resorption. Osteolysis, which accounts for 20% of HCM cases, is mediated by local tumor cell secretion of osteoclast-activating cytokines. Tumor-mediated cytokines cause increased secretion of RANKL by osteoblasts, which stimulates osteoclast differentiation and increased bone resorption²⁴.

Several studies indicated a higher prevalence of increased SCLs in BC patients, but there are a lot of controversies on whether those SCLs are caused by cancer treatment, as well as regarding its relation to histopathologic tumor features^{3,13}. Although expected, we did not confirm a relation between increased SCLs and duration of AI treatment. There are several case reports on hypercalcemia that appeared after treatment with AIs in patients with BC^{8,9}. In these reports, after exclusion of other possible causes, AI-related hypercalcemia was confirmed by 'dechallenge and rechallenge' AI-testing, i.e. a fall of SCLs after withdrawal and an increase after re-intake of anastrozole⁸ or letro-

zole⁹. The possible mechanisms by which AIs can cause hypercalcemia are unknown. One likely hypothesis is that AIs interfere with the calcium-sensing receptors on renal tubular cells thus decreasing renal excretion of calcium²⁵. To the best of our knowledge, the studies that include a larger group of AI-treated BC patients to confirm such a relation are lacking. Since the majority (91.2%) of our patients were receiving AIs, we were unable to make comparison with untreated patients and therefore to rule out whether higher SCLs were AI treatment-induced.

Although within an age-reference range, the values of creatinine and ALP were higher and eGFR was lower in the BC group with higher SCLs. The given results might be the consequence of increased bone resorption and altered renal metabolism of calcium, probably through the action of the systemic treatments including AIs received. In addition, we observed a significant positive correlation between lower SCLs with the use of bisphosphonate treatment. This might be explained by the inhibitory effect of bisphosphonates on bone resorption by osteoclasts and the subsequent decrease of SCLs²⁶.

In our study sample of BC patients, higher SCLs showed trends of association with some indicators of tumor aggressiveness such as tumor grade and axillary lymph node involvement, however, we did not confirm a significantly positive correlation. Our results are in concordance with those obtained in several studies performed in treated and untreated BC patients^{3,12}. Few other studies report on the positive association between SCLs and histopathologic features of the tumor. In the prospective study performed by Almquist *et al.*¹⁵, prediagnostic SCLs were positively associated with increased tumor aggressiveness in premenopausal and/or overweight women. A positive correlation between hypercalcemia and tumor stage was also observed by Hassan *et al.*²⁷, who involved two-thirds of patients in the advanced stage of BC. A similar finding has been reported in the cross-sectional study of 555 postmenopausal women whose baseline SCLs positively correlated with tumor volume¹⁴.

Our study had some limitations. Since it was retrospective, we could not exclude the possible selection bias. We were unable to compare treated to untreated BC patients since all patients had received some anti-neoplastic treatment before referral for bone loss evaluation. Another limitation was the lack of specific

bone ALP and PTHrP data since these are not routinely performed in our hospital. Since SCLs were obtained during regular follow-up at different timelines from starting AI treatment, we could not determine whether there was any fluctuation of SCLs over time that was not captured.

Our study also had strengths. It underlined the value of biochemical screening for SCLs and PHPT among BC patients examined for low BMD. In addition, it produced data on received cancer treatment, including advanced therapy with AIs and immunotherapy, as well as on histopathologic features of BC in relation to SCLs.

Conclusions

We found a remarkable prevalence of primary PHPT of 6.6% of treated BC patients, which is much higher than the incidence in the general adult female population without BC (about 1%). Based on our findings, the possible trigger effect of the concomitant systemic treatment or BC irradiation for the development of PHPT cannot be excluded. Higher SCLs showed trends of association with the indicators of tumor aggressiveness such as tumor grade and axillary lymph node involvement, as well as with immunotherapy. Prospective studies should better clarify the causative relationship between AI treatment and SCLs. The given results emphasize the importance of biochemical screening for SCLs and PHPT in BC patients evaluated for low bone mass.

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Sažetak

PRIMARNI HIPERPARATIREOIDIZAM I SERUMSKI KALCIJ U BOLESNICA S RAKOM DOJKE TIJEKOM PROCJENE NISKE KOŠTANE MASE – ISKUSTVO JEDNOG CENTRA

M. Punda, P. Petranović Ovčariček, A. Tabain, K.P. Koopmans, G. Alfier, T. Jukić i A. Fröbe

Smjernice o koštanom zdravlju za bolesnice s rakom dojke (RD) preporučuju ispitivanje mineralne gustoće kostiju (*bone mineral density*, BMD). U bolesnica s niskom vrijednosti BMD-a i povišenom razinom kalcija u serumu (RKS) dodatno se procjenjuje primarni hiperparatireoidizam (PHPT). Cilj je bio utvrditi učestalost PHPT-a u liječenih bolesnica s RD i sniženom vrijednosti BMD-a te analizirati povezanost RKS s histopatološkim značajkama tumora i onkološkim liječenjem. Retrospektivna studija obuhvatila je postmenopausalne bolesnice s RD koje su pregledane u Ambulanti za osteoporozu između 2013. i 2020. godine. Klinički podaci i podaci o BMD prikupljeni su iz medicinske dokumentacije. Bolesnice s biokemijskom sumnjom na PHPT podvrgnute su standardnim postupcima snimanja paratireoidnih žlijezda. U devet od 137 (6,6%) bolesnica dijagnosticiran je PHPT; 8/9 bolesnica podvrgnuto je paratireoidektomiji, a jednoj je bolesnici savjetovano praćenje. U ostalih 128 bolesnica bez PHPT-a više RKS pokazale su trend pozitivne povezanosti s višim gradusom tumora, zahvaćenošću aksilarnih limfnih čvorova i primljenom imunoterapijom, iako nije postignuta statistička značajnost. Utvrdili smo veću učestalost PHPT-a u liječenih bolesnica s RD u odnosu na opću populaciju. Više RKS pokazuju trend pozitivne korelacije s nekim agresivnijim histopatološkim značajkama tumora i imunoterapijom. Rezultati ove studije upućuju na to da bi se kod liječenih bolesnica s RD i s niskom vrijednosti BMD-a trebala rutinski provoditi procjena RKS kako bi se isključio PHPT.

Ključne riječi: *Primarni hiperparatireoidizam; Serumski kalcij; Rak dojke; Mineralna gustoća kostiju; Liječenje karcinoma*