

# Quantitative ultrasound and DXA measurements in aromatase inhibitor-treated breast cancer women receiving denosumab

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## Abstract

**Purpose** Denosumab has been proven to reduce fracture risk in breast cancer (BC) women under aromatase inhibitors (AIs). Quantitative ultrasound (QUS) provides information on the structure and elastic properties of bone. Our aim was to assess bone health by phalangeal QUS and by dual-energy X-ray absorptiometry (DXA), and to evaluate bone turnover in AIs-treated BC women receiving denosumab.

**Methods** 35 Postmenopausal BC women on AIs were recruited (mean age  $61.2 \pm 4.5$  years) and treated with denosumab 60 mg administered subcutaneously every 6 months. Phalangeal QUS parameters [Amplitude Dependent Speed of Sound (AD-SoS), Ultrasound Bone Profile Index (UBPI), Bone Transmission Time (BTT)] and DXA at lumbar spine and femoral neck were performed. Serum C-telopeptide of type 1 collagen (CTX) and bone-specific alkaline phosphatase (BSAP) were also measured. The main outcomes were compared with a control group not receiving denosumab ( $n = 39$ ).

**Results** In patients treated with denosumab, differently from controls, QUS and DXA measurements improved

after 24 months, and a reduction of CTX and BSAP was detected at 12 and 24 months in comparison to baseline ( $P < 0.05$ ). The percent changes ( $\Delta$ ) of QUS measurements were significantly associated with  $\Delta$ BMD at femoral neck, and  $\Delta$ CTX and  $\Delta$ BSAP were associated with  $\Delta$ BMD at lumbar spine ( $r = -0.39$ ,  $P = 0.02$ ;  $r = -0.49$ ,  $P = 0.01$ , respectively).

**Conclusions** Denosumab preserves bone health as assessed by phalangeal QUS and DXA. Since inexpensive and radiation-free, phalangeal QUS may be considered in the follow-up of AIs-treated BC women receiving denosumab.

**Keywords** Aromatase inhibitors · Denosumab · Quantitative ultrasound · Breast cancer · Bone mineral density · Bone turnover

## Introduction

Aromatase inhibitors (AIs) represent the gold-standard adjuvant treatment for postmenopausal women with hormone receptor-positive breast cancer (BC) and for the management of metastatic disease. Previous trials involving tamoxifen and AIs have shown the latter being associated to better disease-free survival, time to recurrence and time to metastasis [1–3]. Because of their use, accelerated bone loss and increased risk of fractures due to near-complete ablation of estrogen production have been reported, and increased awareness about AIs-induced osteoporosis has led to several recommendations for patient management during treatment with these drugs [4–8].

Denosumab is a fully human IgG2 monoclonal antibody that binds with high affinity and specificity to the tumor necrosis factor (TNF)-related cytokine receptor activator

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of nuclear factor kappa B ligand (RANKL), a key cytokine inducing differentiation of hematopoietic precursors common to the monocyte/macrophage and osteoclast lineages into multinucleated, active bone-resorbing cells [9, 10]. By preventing the interaction of RANKL with its receptor RANK, on osteoclasts and osteoclast precursors, denosumab reversibly inhibits osteoclast-mediated bone resorption [9].

Denosumab has been previously reported to reduce the risk of vertebral, non-vertebral, and hip fractures in postmenopausal women and to improve bone mineral density (BMD) in men with low bone mineral density [10, 11]. Also, denosumab has been recently proven to preserve bone mass and reduce the risk of vertebral fractures in men receiving androgen depletion therapy for prostate cancer and in BC women treated with AIs [12–14].

As known, a decreased BMD is the major risk factor for fractures, and measuring BMD by dual-energy X-ray absorptiometry (DXA) is considered the gold-standard tool in assessing osteoporosis [15].

Several studies have generally found significant correlations between quantitative ultrasound (QUS), DXA and prevalent vertebral fractures; the ten-year probability of clinical vertebral fractures was also associated to QUS [16–22]. QUS at phalangeal site was proven to be a reliable tool to screen for osteoporosis and to predict incident fractures independently from DXA; indeed, ultrasound transmission on bone tissue depends on physical properties of bone not measured by DXA but able to modulate bone strength and, consequently, the risk of fractures [18, 20]. However, a limited use in clinical practice of phalangeal QUS is due to questions related to the reproducibility of the measurements performed by different operators using the same device, or the performance of measurements using different devices applied to the same anatomical site, and the lacking of standardizing procedure for commercially available QUS devices.

Recently, we demonstrated that phalangeal QUS measurements were able to detect the AIs-induced changes of bone, and were associated to DXA [23]. The possibility to evaluate the effects of bone-active drugs by QUS was also proven in postmenopausal women [24, 25]. There are no data on whether phalangeal QUS may be affected under denosumab in AIs users.

Aim of this research was to evaluate bone status by QUS and DXA in BC women receiving denosumab to counteract the expected accelerated bone loss induced by AIs.

## Materials and methods

Our prospective study included a group of Caucasian women ( $n=38$ ), with early BC (carcinoma in situ and

stage I and stage II breast cancers) who started (within the last 12 months previous enrollment) adjuvant treatment with AIs (i.e. anastrozole, letrozole, exemestane). Recruited subjects were all postmenopausal women referring to the outpatients clinics for the prevention and treatment of osteoporosis at the Department of Clinical and Experimental Medicine of Messina University (Messina, Italy). Low bone mass (osteopenia or osteoporosis in accordance with the WHO criteria) with at least one prevalent morphometric vertebral fracture were required to participate in this study, in accordance with the Italian drug reimbursement issues at the time of recruitment. Exclusion criteria were: other malignancies, known or suspected bone metastases, liver or renal failure, hyperthyroidism, hyperparathyroidism, hypercalcemia or hypocalcemia, use of medications affecting bone and mineral metabolism within the last 6 months (including corticosteroids, heparin, and anticonvulsants), ongoing or previous use of active bone agents (e.g. bisphosphonates, selective estrogen receptor modulators, strontium ranelate, teriparatide or PTH, calcitonin).

Recruited women received subcutaneous denosumab 60 mg every 6 months (Dmab group), and were compared, for ethical reasons, with a historical control group ( $n=39$ ) of AIs-treated BC postmenopausal women attending the same center and matched for age and anthropometric features, but without vertebral fractures at baseline evaluation.

All the participants received an oral supplementation of cholecalciferol (25,000 IU bimonthly); moreover, women with an estimated poor dietary calcium intake were accordingly supplied with calcium carbonate (500–1000 mg, daily) to reach the recommended daily allowance of calcium.

The study was conducted in accordance with the ethical standards of our institutional research committee and with the 1964 Declaration of Helsinki and its later amendments, and written informed consent was obtained from all the participants.

QUS measurements were obtained at the proximal phalangeal metaphysis of the last four fingers of the non-dominant hand using a DBM Sonic Bone Profiler (Igea, Carpi, Italy) as previously described [26]. The following parameters were measured: amplitude-dependent speed of sound (AD-SoS), bone transmission time (BTT), fast wave amplitude (FWA), signal dynamic (SDy), and ultrasound bone profile index (UBPI) automatically calculated ( $UBPI = -(0.0018 \times SDy - 0.0560 \times FWA) + 0.0560 - 1.1467 \times BTT + 3.0300$ ). The coefficient of variation (CV) for AD-SoS was of 0.9%.

BMD was assessed by a dual-energy X-ray absorptiometry (DXA) scan device (Hologic Discovery) at the lumbar spine (L1-L4) in AP projection and at the femoral neck. Calibration of DXA densitometer was carried out on a daily

basis in accordance with the manufacturer’s indications, and the CV was 0.5% with the standard phantom.

Fracture assessment was based on the analysis of lateral radiographs of the dorsal and lumbar spine according to the Genant’s semiquantitative visual score: vertebral fractures were defined as height reductions in the anterior, middle or posterior height compared with the same or adjacent vertebra of 20–25% or more on radiographs. QUS, DXA and vertebral morphometry by X-ray were performed at baseline and after 24 months.

At baseline, 12 and 24 months, C-telopeptide of type 1 collagen (CTX), as a marker of bone resorption, bone-specific alkaline phosphatase (BSAP), as a marker of bone formation, 25-hydroxyvitamin D (25(OH)D), albumin-corrected calcium, phosphorus, and creatinine, were measured. CTX (Roche, Basel, Switzerland) and BSAP (Beckman Coulter, Fullerton, California) were measured by enzyme-linked immunosorbent assay, with intra-assay coefficient of variation CV of 1.6–3% and inter-assay CV of 1.3–4.3% for CTX, and intra-assay CV of 2.3–3.7% and inter-assay CV of 4.9–9.8% for BSAP. 25(OH)D were detected by high-performance liquid chromatography; calcium, phosphorus and creatinine were measured using standard laboratory techniques.

Statistical analyses were performed using MedCalc software (version 10.2.0.0; Mariakerke, 173 Belgium). The normal distribution of values was verified with the Kolmogorov–Smirnov test. Comparisons between the groups were performed using Student’s *t* test or Mann–Whitney test as appropriate. A repeated-measures ANOVA model was used to assess the changes of parameters within each group over time. Spearman’s coefficient was used to measure the degree of association between two variables. Values of *P* < 0.05 were considered to indicate statistical significance. All reported *P* values were two-sided.

**Results**

A total of thirty-five BC postmenopausal women, in the Dmab group, completed the study; three patients were

excluded from the final analysis: two women because of introducing drugs potentially involving bone metabolism (heparin, corticosteroid), the other one because of having moved to other city and, thus, missed to follow-up. The main clinical characteristics of participants are shown in Table 1. At baseline, no significant differences were detected between Dmab and control groups, except for the above-mentioned absence of vertebral fractures in controls.

DXA measurements are presented in Table 2. At the end of the study, BMD values at lumbar spine and femoral neck were significantly improved (*P* < 0.05) in the Dmab group (+3.4 and +1.5%, respectively), but resulted significantly worsened in controls (–2.7 and –3.0%, respectively). The same trend was observed in QUS measurements (Table 2), with a significant increase (*P* < 0.05) in AS-SoS, UBPI and BTT values (+3.8, +6.4 and +8.4%, respectively) in the Dmab group, and a significant reduction (*P* < 0.05) of these values (–3, –6.4 and –7%) in controls. A significant reduction of bone turnover markers was also observed at 12 and 24 months (Table 3). 25(OH)D levels rose over the observation period in both groups, but no significant changes of calcium, phosphorus and creatinine were detected. In the Dmab group, the percent changes ( $\Delta$ ) of QUS measurements (AD-SoS, UBPI and BTT) were significantly associated with  $\Delta$  BMD at femoral neck, and the highest degree of association was observed for AD-SoS (*r* = 0.5, *P* = 0.003), as shown in Fig. 1.

$\Delta$ CTX and  $\Delta$  BSAP were associated with  $\Delta$  BMD at lumbar spine (*r* = –0.39, *P* = 0.02; *r* = –0.49, *P* = 0.01, respectively).

During the study period, none of the patients reported any new vertebral fractures.

**Discussion**

In postmenopausal patients with hormone receptor-positive early-stage BC who receive therapy with AIs, the subcutaneous administration of denosumab every 6 months significantly improved bone health as suggested both by DXA and QUS evaluations. Accordingly to other authors,

**Table 1** Clinical characteristics of recruited BC women on aromatase inhibitors

	Dmab ( <i>n</i> = 35)	Controls ( <i>n</i> = 39)	<i>P</i> values
Age (years)	61.2 ± 4.5	62.3 ± 5.6	0.36
Time since menopause (years)	11.6 ± 4.9	12.13 ± 6.8	0.70
BMI (kg/m <sup>2</sup> )	26.1 ± 3.2	26.45 ± 4.96	0.72
Smoking [ <i>n</i> (%)]	5 (14.8)	7 (16.6)	0.97
Patients with 1 prevalent Vfx [ <i>n</i> (%)]	24 (68.5)	0	
Patients with more than 1 prevalent Vfx [ <i>n</i> (%)]	11 (31.5)	0	

Data are expressed as mean ± SD or *n* (%)  
 Dmab denosumab, Vfx vertebral fracture

**Table 2** Bone status evaluated by quantitative ultrasound at phalangeal site and by DXA at lumbar spine and femoral neck in AIs-treated BC women receiving denosumab and in controls

	Groups	Baseline	24 months
Phalangeal QUS measurements			
AD-SoS (m/s)	Dmab	1851 (1773 to 1929)	1915 (1806 to 2030) <sup>**</sup>
	Controls	1863 (1791 to 1941)	1812 (1744 to 1900) <sup>#</sup>
UBPI (U)	Dmab	0.31 (0.20 to 0.50)	0.35 (0.22 to 0.53) <sup>**</sup>
	Controls	0.28 (0.19 to 0.45)	0.22 (0.17 to 0.41) <sup>#</sup>
BTT (μs)	Dmab	1.17 (1.05 to 1.31)	1.37 (1.16 to 1.50) <sup>**</sup>
	Controls	1.20 (1.05 to 1.50)	1.07 (0.98 to 1.20) <sup>#</sup>
T-score (SD)	Dmab	-3.47 (-4.99 to -1.75)	-3.11 (-4.72 to -1.47) <sup>**</sup>
	Controls	-4.09 (-5.09 to -2.06)	-4.11 (-5.21 to -3.06) <sup>#</sup>
DXA measurements			
L1–L4 BMD (g/cm <sup>2</sup> )	Dmab	0.83 (0.79 to 0.90)	0.88 (0.85 to 0.94) <sup>**</sup>
	Controls	0.87 (0.80 to 0.90)	0.83 (0.79 to 0.89) <sup>#</sup>
L1–L4 T-score (SD)	Dmab	-2.0 (-2.37 to -1.35)	-1.5 (-1.87 to -1.10) <sup>**</sup>
	Controls	-1.7 (-2.3 to -1.3)	-2.1 (-2.4 to -1.40) <sup>#</sup>
Femoral neck BMD (g/cm <sup>2</sup> )	Dmab	0.64 (0.60 to 0.68)	0.67 (0.62 to 0.70) <sup>#</sup>
	Controls	0.67 (0.61 to 0.71)	0.63 (0.59 to 0.69) <sup>#</sup>
Femoral neck T-score (SD)	Dmab	-2.0 (-2.21 to -1.50)	-1.6 (-2.10 to -1.32) <sup>#</sup>
	Controls	-1.6 (-2.10 to -1.31)	-2.0 (-2.22 to -1.37) <sup>#</sup>

Data are expressed as median (IQR)

QUS quantitative ultrasound, Dmab denosumab

\**P* < 0.05 vs. controls, #*P* < 0.05 vs. baseline

**Table 3** Laboratory data of study population

	Groups	Baseline	12 months	24 months
Corrected serum calcium (mg/dl)	Dmab	9.18 ± 0.38	9.11 ± 0.44	9.19 ± 0.47
	Controls	9.22 ± 0.43	9.19 ± 0.34	9.08 ± 0.48
Phosphorus (mg/dl)	Dmab	3.56 ± 0.47	3.59 ± 0.42	3.61 ± 0.51
	Controls	3.61 ± 0.49	3.56 ± 0.41	3.62 ± 0.42
Creatinine (mg/dl)	Dmab	0.82 ± 0.21	0.84 ± 0.19	0.83 ± 0.18
	Controls	0.78 ± 0.19	0.83 ± 0.20	0.81 ± 0.19
25(OH)D (ng/ml)	Dmab	25.43 ± 6.12	29.32 ± 5.52*	34.51 ± 6.41 <sup>*#</sup>
	Controls	26.09 ± 7.57	32.98 ± 7.54	37.43 ± 6.12
CTX (ng/ml)	Dmab	0.68 (0.47–0.83)	0.61 (0.39–0.78) <sup>*†</sup>	0.44 (0.35–0.60) <sup>*#†</sup>
	Controls	0.63 (0.37–0.79)	0.71 (0.44–0.85) <sup>*</sup>	0.73 (0.42–0.86) <sup>*</sup>
BSAP (ng/ml)	Dmab	15.3 (13.7–17.3)	14 (13.1–15.1) <sup>*</sup>	13.9 (12.9–17.2) <sup>*†</sup>
	Controls	15.16 (13.1–17.5)	15.89 (13.9–18.4)	16.04 (14.2–18.9) <sup>*</sup>

Data are expressed as mean ± SD or median (IQR) as appropriate

Dmab denosumab, CTX C-telopeptide of type 1 collagen, BSAP bone-specific alkaline phosphatase

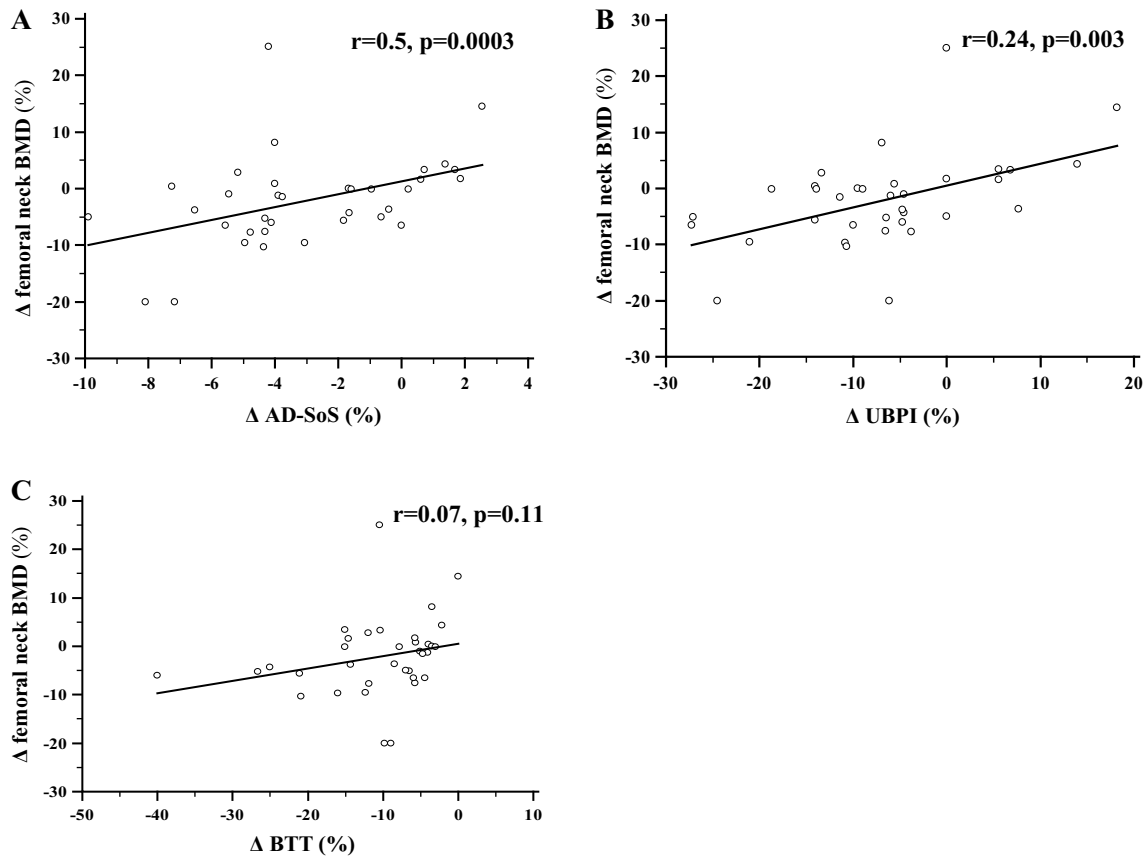
\**P* < 0.05 vs. baseline; #*P* < 0.05 vs. 12 months; †*P* < 0.05 vs. controls

denosumab has been confirmed to be effective in improving BMD, by reducing bone turnover [13, 14]; moreover, for the first time, we have shown the potential ability of phalangeal QUS to detect bone changes due to denosumab, over a period of accelerated bone loss in postmenopausal BC survivors on AIs.

As known, in addition to DXA, QUS at the phalanges provides additional data useful to the study of bone fragility, possibly by capturing more and different information

on the physical properties of bone tissue (e.g. structure and elasticity) that contribute to bone strength and are not recognized by DXA.

Both cortical and trabecular bone determine bone strength; 80% of bone is cortical, and up to 70% of all appendicular bone loss is cortical and occurs mainly by intracortical remodeling; the increase in intracortical porosity reduces bone strength exponentially [27]. The phalanx of adult subjects can be expected to mostly



**Fig. 1** Correlations between percent changes ( $\Delta$ ) of femoral neck BMD detected by DXA and the phalangeal QUS parameters AD-SoS (*panel a*), UBPI (*panel b*) and BTT (*panel c*) in AIs-treated BC women receiving denosumab

consist of cortical bone; thus, phalangeal QUS may be affected when cortical bone is impaired [28]. We previously observed a positive association between the percent changes of AD-SoS and femoral neck BMD in AIs users, consistent with the wide representation of cortical bone tissue in the femur [23]. Here, we observed an improvement of QUS parameters over a 24-month course of denosumab in BC women on AIs. In comparison with alendronate, denosumab has been proven to reduce remodeling more rapidly, more completely and to decrease intracortical porosity to a greater extent [29]; in fact, in comparison with trabecular bone, less bisphosphonate may be incorporated into the large mineralized cortical bone matrix because of less surface area per unit volume of mineralized bone matrix upon which bisphosphonates can be adsorbed. A more pronounced effect on cortical bone is, thus, expected for denosumab, and reversing cortical porosity may contribute to further reduction of fracture risk. Since AD-SoS is mainly affected by cortical area, cortical bone density, and cortical porosity, it may be a potentially useful parameter to explore the skeletal effects over time of denosumab when

administered to prevent bone loss in AIs-treated BC women.

Previously, it was observed by other authors using high-resolution quantitative computed tomography (QCT), that both volumetric BMD and cortical thickness were deteriorated more dramatically than detected by DXA, in BC women treated with exemestane [30]. Similarly, the trabecular bone score (TBS), a recently developed analytical tool that performs novel grey-level texture measurements on DXA images, capturing information related to trabecular microarchitecture, has been proven to be significantly decreased at the lumbar spine and hip, irrespective of DXA-measured BMD [31]. Considered altogether, these data suggest that the predictive performance of BMD measured by DXA may be affected in this cohort of women. Furthermore, it could be speculated that the denosumab-mediated fracture risk reduction that was observed by Gnani A et al. in BC postmenopausal women with baseline apparently normal BMD at the same extent of women with low BMD could be ascribed to favorable changes of bone properties



beyond DXA-derived BMD, but further bone evaluations with QUS, QCT or TBS were not performed in their study [14].

At baseline, we observed suboptimal 25(OH)D serum levels in BC women involved in this research; however, 25(OH)D levels rose over time due to cholecalciferol supplementation, and improving vitamin D status could have contributed in boosting the effects of denosumab; it was also reported that vitamin D increases aromatase expression in bone, leading to a reduction of the deleterious effects of estrogen deprivation in bone caused by the AIs, and may play a role in musculoskeletal pain management [32].

We acknowledge that our research has some limitations: the small sample size, the absence of a matched control group with regard to prevalent vertebral fractures, the observation period not long enough to account for fractures and the lack of DXA data on distal forearm, which is rich in cortical bone and would have allowed additional correlations with our QUS measurements. On the other hand, phalangeal QUS, a radiation-free and inexpensive tool, for the first time, has been highlighted to be helpful in the follow-up of bone health evaluation in BC postmenopausal women on AIs and starting treatment with denosumab. Data from phalangeal QUS may be useful in studying the bone quality and, therefore, in the fracture risk assessment in this particular set of patients.

In conclusion, over a period of 2 years, denosumab at a dose of 60 mg twice a year has been confirmed to be able to improve bone health as recognized by phalangeal QUS and DXA measurements. Further researches with larger numbers of patients treated for longer periods are needed to determine if the changes over time in phalangeal QUS measurements are associated with fracture risk reduction in this cohort of women.

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#### Compliance with ethical standards

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments.

**Informed consent** Informed consent was obtained from all participants included in the study.

**Conflict of interest** Catalano Antonino, Gaudio Agostino, Morabito Nunziata, Basile Giorgio, Agostino Rita Maria, Xourafa Anastasia, Atteritano Marco, Morini Elisabetta, Natale Giuseppe and Lasco Antonino declare that they have no conflicts of interest.

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