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Denosumab and bone and muscle health in Older Women with Breast Cancer: A Geriatric Perspective

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Abstract

Background: In recent years, a steady increase has been observed in the prevalence of elderly women affected by hormone-sensitive breast cancer. The standard therapy for this condition—aromatase inhibitors—is well known for its adverse effects on bone metabolism, which are particularly pronounced in postmenopausal women who are already characterized by skeletal fragility. Concurrently, growing attention has been devoted to to muscle health and to the concomitant presence of alterations in both bone and muscle metabolism, which in certain conditions lead to a state known as osteosarcopenia—a syndrome associated with adverse clinical outcomes such as increased risk of falls, fractures, and disability. Among the therapeutic strategies recommended by current guidelines to prevent or counteract Cancer Treatment–Induced Bone Loss (CTIBL), denosumab, a potent antiresorptive agent, plays a pivotal role. However, its potential effects on muscle tissue have been investigated only recently, with heterogeneous and sometimes conflicting results. Moreover, studies conducted in elderly patients with breast cancer treated with denosumab often lack data on geriatric phenotypes and comprehensive geriatric assessment (CGA)—both essential for appropriate clinical characterization of this population.

Objectives: To evaluate the impact of denosumab on bone and muscle health in elderly women with breast cancer receiving aromatase inhibitors, to assess its potential effects on other geriatric domains, including frailty, to analyse treatment adherence rates and the main causes of non-compliance.

Methods: We conducted a retrospective observational study involving patients attending the Oncogeriatrics Outpatient Clinic at IRCCS San Martino Hospital (Genoa) within the “Breast and Bone” project between April 2021 and September 2024. Inclusion criteria: women aged ≥65 years, with non-metastatic breast cancer under aromatase inhibitor therapy, followed for at least one year by our clinic, and who provided informed consent. Participants were divided into two subgroups: “Denosumab” group- patients who had initiated and continued denosumab treatment for at least one year, and “No Denosumab” group- patients who had never initiated denosumab or had received only one injection. For both groups, we analysed bone mineral density (BMD) in patients with two Dual Energy X-ray Absorptiometry (DXA) scans—one prior to therapy initiation and another after at least one year of treatment with denosumab. Collected data included medical history, osteoporosis risk factors, DXA T-scores for lumbar spine, total hip and femoral neck, occurrence of new fractures, biochemical markers of bone turnover when available,

therapy-related adverse events, and multidimensional assessment with calculation of the Frailty Index (FI) score. Muscle function was evaluated using handgrip strength (HG) and Timed Up and Go (TUG) tests. Additionally, adherence data and reasons for non-adherence were recorded.

Results: Between April 2021 and September 2025, 220 patients were evaluated within the “Breast and Bone” project. Of these, 120 met inclusion criteria: 80 in the “Denosumab” group and 40 in the “No Denosumab” group. The mean age was 76.9 years in both groups. In the “Denosumab” group (n=56 with paired DXA), a significant increase in BMD T-score was observed between baseline and follow-up: lumbar spine: mean +0.38, 95% CI 0.21–0.55 (normalized +0.19, CI 0.11–0.28), total hip: mean +0.33, 95% CI 0.18–0.49 (normalized +0.19, CI 0.11–0.28). In the “No Denosumab” group, a non-significant trend toward BMD decline was detected. The between-group differences in densitometric changes were statistically significant across all DXA sites. No significant variations were observed in muscle strength or gait speed between the two groups. Similarly, no statistically significant differences emerged in frailty trajectories or other CGA domains at 1- and 2-year follow-up. Adherence analysis: among the 40 patients in the “No Denosumab” group, 27 (22.5% of total) never initiated treatment due to: ongoing or planned dental procedures / dentist’s recommendation (7), fear or refusal of new therapies (19), hospitalization or worsening general condition (1). Of the remaining 13 who received only one injection, delays between indication and initiation were attributed to dental issues (3) or patient choice (8); 2 discontinued after the first dose upon dentist’s advice. No major adverse events or fractures were reported. In conclusion, denosumab confirmed its efficacy in improving BMD in elderly women with breast cancer, while its effects on sarcopenia appeared inconclusive, likely reflecting heterogeneity in patient phenotypes.

Introduction

1. CTIBL in hormone-receptor-positive breast cancer

Overall, it is estimated that approximately 80% of breast cancers in older women are hormone receptor–positive (Estrogen receptor-positive/Progesterone receptor-positive: ER+/PR+), with a higher prevalence compared with younger age groups. [1] The incidence of ER-positive breast cancer has been increasing over several decades, particularly for low-grade and early-stage tumors. [2] In Italy, breast cancer incidence continues to rise, whereas mortality rates are declining.

Patients with hormone receptor–positive (HR+) breast cancer are particularly susceptible to treatment-related bone loss as a consequence of commonly used adjuvant therapies, including endocrine agents, chemotherapy, and targeted treatments (the so called “cancer treatment-induced bone loss", CTIBL), thereby contributing to an increased prevalence of risk of fragility fractures in this population. Among women treated with aromatase inhibitors, bone loss occurs at a rate approximately two- to four-fold higher than that observed with normal age-related skeletal decline. Furthermore, in patients at elevated risk who continue aromatase inhibitor treatment for extended durations, up to ten years, fracture risk is estimated to rise by approximately 2–4% annually.[3] This bone loss is superimposed on the physiological postmenopausal decline, which is estimated at approximately 1–2% per year, rendering this population particularly vulnerable.[4] The accelerated reduction in bone mineral density results in a significantly increased risk of fragility fractures during and after the period of intensive treatment.[5] In a cohort of postmenopausal women receiving aromatase inhibitor therapy, more than 60% exhibited reduced bone mineral density prior to treatment initiation (osteopenia or osteoporosis), and approximately 7–8% already had radiographically evident vertebral fractures.[6]

Bone loss associated with aromatase inhibitor therapy is predictable, clinically relevant, and preventable; therefore, current international guidelines recommend early assessment of bone health and timely initiation of therapy when indicated, particularly in older patients. [5, 7, 8]

2. Muscle health and sarcopenia in older women with breast cancer

Accumulating evidence indicates that sarcopenia is a common and clinically relevant condition in patients with breast cancer, occurring both in early and advanced disease. In the overall breast cancer setting, several systematic reviews and meta-analyses have been conducted. With regard to prevalence, the study by Min Kyeong Jang published in 2024 reported a mean prevalence of sarcopenia of 32.5% among patients with stage I–III breast cancer. [9] More recently, a systematic review by Roberto M. and colleagues analysed 16 studies including 6,130 patients, the majority of whom (5,284) had non-metastatic breast cancer. [10] Notably, a similar prevalence of sarcopenia was observed (33%), with 95% of sarcopenic patients presenting with non-metastatic disease, contrary to what might be expected. Across studies, sarcopenia was associated with a higher risk of mortality and disease progression or relapse, and sarcopenic patients were more likely to develop grade 3–4 treatment-related toxicities.

Low skeletal muscle mass and impaired muscle quality have been consistently associated with reduced treatment tolerance, functional decline, and poorer survival outcomes. [11] In early-stage breast cancer, sarcopenia and unfavourable body composition have been linked to worse prognosis even after adjustment for body mass index, highlighting the limitations of BMI as a surrogate of nutritional and functional status. In hormone receptor–positive disease, endocrine therapies—particularly aromatase inhibitors—may further contribute to loss of lean mass and deterioration of muscle function, potentially exacerbating frailty in older patients (table 1).

Author (Year)	**Study design**	**Population (n); HR+ cancer/ Endocrine therapy (ET) (%)**	**Assessment of sarcopenia / muscle**	**Key outcomes**	**Main findings**
Pedersini et al. (2024)[12]	Observational, single-centre prospective cohort study	Early breast cancer survivors, postmenopausal (n=194); All HR+ aromatase inhibitors (AI) users (100%)	DXA: total fat mass and total lean mass in the whole body and in the body districts: right and left arms, right and left legs, trunk, and head; trunk to appendicular fat; total fat mass to square of height (fat mass index, FMI); total lean mass	Body composition changes, increased prevalence of sarcopenia, obesity and sarcopenic obesity	AI therapy was associated with reduced lean mass and unfavourable body composition, suggesting increased risk of sarcopenia

			to square of height (lean mass index, LMI); the sum of arms and legs lean mass to square of height (appendicular lean mass index, ALMI).		
Saraf et al. (2024)[13]	Multicenter retrospective cohort study	Early-stage breast cancer (n=305); HR+ on adjuvant endocrine therapy (aET) (100%)	Skeletal muscle index (SMI) derived by cross-sectional radiation simulation imaging	Toxicity related aET discontinuation	Low muscle mass associated with toxicity-related early discontinuation of aET and shorter time to aET toxicity
Caan et al. (2018) [11]	Retrospective cohort study	Non-metastatic breast cancer (stage II-III) (n=3241); HR+ subgroup included (73%)	CT-based muscle mass and radiodensity (cross-sectional area of muscle and adipose tissue in centimeters squared at the third lumbar vertebra (L3)), SMI was defined as muscle area at L3 in cm2 divided by height in meters squared. Skeletal muscle radiodensity (SMD) represented muscle quality and was measured using the average radiation attenuation of tissue in Hounsfield Units. Visceral (intra-abdominal) adipose tissue (VAT); subcutaneous adipose tissue (SAT); and	Overall survival and all-cause mortality	Low muscle mass and low muscle quality associated with poorer survival even in early disease. Sarcopenia was associated with a 41% (HR, 1.41; 95% CI, 1.18-1.69) greater risk of dying compared with those without sarcopenia. Similarly, an increase in 1 standard deviation of SMI (7.2) or absolute

			intramuscular adipose tissue (IMAT) were all quantified separately. Total adiposity (TAT) was the sum of VAT, SAT, and IMAT		muscle area (19.9 cm^2) was associated with a 13% and 10% decreased risk of death.
Aleixo et al. (2022)[14]	Observational study	Early breast cancer (stage I-III) (n=482); HR+, AI and tamoxifene (100%)	SMI by bioelectrical impedance spectrometry (SMM kg)/ (patient height, m^2). Sarcopenia: SMI ≤ 6.75 kg/m^2	Toxicity related aET discontinuation	Sarcopenic patients showed higher incidence of grade 3–4 endocrine therapy–related toxicities and more frequent treatment discontinuations or switches due to adverse effects
Du L. (Li P.) et al. (2024)[15]	Retrospective study	Early breast cancer (n=285); all types, HR+ included (not known proportion)	SMI by CT, defined as skeletal muscle area (cm^2) at the L3 vertebral level normalized to height (m^2). The cutoff values were 40.8 cm^2/m^2 for males and 34.9 cm^2/m^2 for females, following the EWGSOP2 guidelines.	Overall survival (OS); impact of multimodal intervention on outcomes	Sarcopenia significantly reduced OS rates at 1 year, 3 years, and 5 years compared to non-sarcopenia patients (P<0.001). Patients in the multimodal intervention group showed better outcomes, including

					improved ALB levels, reduced complication rates, and fewer chemotherapy side effects
Adams S.C. et al (2016)[16]	Multicenter randomized controlled trial	Early-stage breast cancer (stage I-IIIa) (n=200); all types, HR+ included (72%)	SMI by DXA was defined as the ratio of lean body mass (kg)/height (m)2. Upper extremities and lower extremities muscle disfunction (indicators of dynapenia status) were defined according to the ratio of weight lifted (kg)/body mass (kg). Sarcopenia was defined according to being either >1 SD (Class I) or >2 SD (Class II) below age- and sex-based normative values. Dynapenia was defined as being >1 SD below age- and sex-based normative values	Effects of resistance exercise program or aerobic exercise program on sarcopenia and effect of changes in muscle mass on quality of life (QoL)	Resistance exercise training consistently demonstrated superior effects to usual care for improving sarcopenia-related outcomes; the greatest QoL improvements were associated with the reversal of sarcopenia status

Table 1. Most relevant studies on sarcopenia in breast cancer patients treated with aromatase inhibitors.

3. Osteosarcopenia

Osteosarcopenia is defined as a geriatric syndrome characterized by the concurrent presence of osteoporosis (or low bone mass/strength) and sarcopenia (loss of skeletal muscle mass, strength, and/or physical performance) in the same individual. This combined phenotype is associated with a higher risk of falls, fragility fractures, functional decline, disability, and mortality than either condition alone.[17, 18]

Figure 1. Osteosarcopenia and its consequences.

Although most studies have evaluated sarcopenia and osteoporosis as separate entities, emerging data support the concept of an integrated bone–muscle unit, suggesting that the coexistence of muscle and bone loss may confer additive negative effects on clinical outcomes in breast cancer patients.

4. Role of Denosumab in CTIBL

Antiresorptive therapy plays a critical role in mitigating the accelerated bone loss induced by aromatase inhibitors in breast cancer patients. [19] [20] Among the available agents, denosumab has emerged as one of the leading options due to its efficacy in increasing bone mineral density and reducing fracture risk, with a favourable safety profile when administered at osteoporosis doses. Its biannual subcutaneous administration also enhances patient adherence, making it a valuable component of comprehensive bone health management in HR+ breast cancer. The main studies illustrating the effects of denosumab on bone in early breast cancer patients receiving aromatase inhibitors are summarized in Table 2.

Author, Year	**Study Type**	**Cohort: N, Characteristics**	**Key Findings (Denosumab vs Comparator)**

Ellis GK et al., 2008 [21]	RCT, double-blind	252 postmenopausal women with HR+ early breast cancer on AI, with osteopenia	Denosumab 60 mg every 6 months vs placebo: lumbar spine BMD increased by +5.5% (12 m) and +7.6% (24 m) (p<0.0001). Significant gains at total hip (4.7% difference at 24 m), femoral neck (3.6% difference at 24 m), and total body (4.2% difference at 24 m). Bone turnover markers decreased. No vertebral fractures were reported over 24 months. Nonvertebral fractures were reported and confirmed in eight patients (6%) in each treatment group at 24 months. Major nonvertebral fractures, defined as those occurring at the pelvis, distal femur, proximal tibia, ribs, proximal humerus, forearm, and hip, were reported and confirmed in three patients (2%) in the denosumab group and in five patients (4%) in the placebo group. No increase in adverse events or osteonecrosis of the jaw (ONJ).
Gnant M. et al., 2015 (ABCSG-18) [22]	Multicenter RCT, double-blind	3425 postmenopausal women with HR+ early BC on AI therapy	Denosumab 60 mg every 6 months vs placebo: at 36 m, patients in the denosumab group had a relative increase in BMD of 10.02% (95% CI 9.04–11.01) at the lumbar spine, 7.92% (6.87–8.97) at the total hip, and 6.51% (5.62–7.39) at the femoral neck, as compared with those in the placebo group (all adjusted p values <0·0001). Reduced risk of first clinical fracture by ~50% (*HR* =0.50, 92 vs 176 fractures; p<0.0001). Overall lower number of fractures in the denosumab group (92) than in the placebo group (176) was similar in all patient subgroups, including in patients with a BMD T-score of -1 or higher at baseline (n=1872, HR 0.44 [95% CI 0.31-0.64], p<0·0001) and in those with a BMD T-score of less than -1 already at baseline (n=1548, HR 0·57 [95% CI 0.40-0.82], p=0.002). Denosumab treatment also significantly reduced the incidence of new vertebral fractures in the vertebral fracture analysis set at 36 months

			(n=1644), with 27 fractures in 835 patients in the denosumab group compared with 49 in 809 patients in the placebo group (odds ratio 0.53 [95% CI 0.33–0.85], p=0.009) No significant safety issues reported.
De Sire A. et al., 2022 [23]	Systematic review of RCTs (15 studies)	Postmenopausal women with early BC on AI (various populations)	Review found both bisphosphonates (BPs) and denosumab increased BMD; as for denosumab: Ellis et al. [21] reported significant differences between groups after 2 years of treatment (12 months: 5.5%; p<0.0001; 24 months: 7.6%; p<0.0001). On the other hand, hip BMD increased accordingly in both total hip site (12 months: p<0.0001; 24 months: 4.7%; p<0.0001) and femoral neck site (12 months: p<0.0001; 24 month: 3.6%; p<0.0001). Similarly, Gnant et al.[22] underlined a significant difference between groups at 36 months (12 months: -1.81% vs +3.94%; p<0.0001; 24 months: -2.44% vs +5.85%; p<0.0001; 36 months: -2.75% vs +7.27%; p<0.0001). Hip BMD results were in line with the previous results with a significant increase in the denosumab group (12 months: -1.20% vs +2.67%; p<0.0001; 24 months: -2.5% vs +3.70%; p<0.0001; 36 months: -3.32% vs +4.60%; p<0.0001)).; only denosumab significantly reduced fracture risk in trials.
Mastrantoni L. et al., 2023 [24]	Meta-analysis (ABCSG-18, D-CARE)	2 phase III RCTs (n ≈ 7929 women; HR+ and others)	Denosumab 60 mg/6m vs placebo: no Disease-Free Survival (DFS)/OS benefit. Denosumab addition to standard of care anticancer therapy significantly reduced fracture incidence in the overall population (RR: 0.787; 95% CI: 0.696–0.890) and in pre- (RR: 0.771; 95% CI: 0.589–1.009) and postmenopausal patients (RR: 0.794; 95% CI: 0.692–0.912). Denosumab treatment also delayed the time to first fracture (HR: 0.760; 95% CI: 0.666–0.869), with a consistent treatment effect among pre- (HR: 0.740; 95% CI: 0.557–0.984, $p < 0.01$) and

			postmenopausal patients (HR: 0.764; 95% CI: 0.657–0.887). At 72 months, cumulative incidence of fractures was 14.2% for denosumab and 10.7% for placebo, with a 3.5% absolute difference. No difference was observed between denosumab and placebo arms in terms of all adverse events (AEs) (RR: 1.003; 95% CI: 0.995–1.012) and serious adverse events (SAEs) (RR: 1.016; 95% CI: 0.951–1.086) and no differences were observed for ONJ and atypical femoral fracture (AFF) between the 60-mg every 6-month schedule and placebo.
Catalano A. et al, 2017 [25]	Prospective study	38 postmenopausal women with HR+ early BC on AI therapy vs 39 women of historical cohort	Denosumab 60 mg/6m vs no therapy (control historical group). BMD values at lumbar spine and femoral neck were significantly improved ($P < 0.05$) in the Denosumab group (+3.4 and +1.5%, respectively) but resulted significantly worsened in controls (−2.7 and −3.0%, respectively). A significant reduction of bone turnover markers was also observed at 12 and 24 months. None of the patients reported any new vertebral fractures.
Nakatsukasa K. et al., 2019 [26]	Non-randomized prospective study	93 postmenopausal women with early BC on AI	Denosumab 60 mg/6 m: the percentage change in BMD at the lumbar spine from baseline over 12 months before and after the initiation of denosumab was 7.0% (95% CI 5.8–8.3) and 6.0% (95% CI 4.6–7.5), respectively, with the difference being nonsignificant (1.0% mean difference between AI with denosumab and before denosumab; $P = 0.2994$). By the end of the study, increase of BMD of the right femoral neck and left femoral neck were 3.3% (95% CI 2.2–4.4) and 4.1% (95% CI 2.7–5.5), respectively, although the difference between the two sides was not significant. Levels of bone markers (TRAP5b and BAP) rapidly reduced by denosumab, with a mean percentage reduction of 59.5 and 49.8%,

			respectively after 12 months. At month 12, non-traumatic clinical fractures were not observed in patients receiving AI and denosumab. No CTC grade 3, 4, or 5 adverse events occurred, no ONJ was observed.
Ouchi Y. Et al., 2020 [27]	Prospective non-randomized clinical trial	74 postmenopausal women with early BC on AI	Denosumab 60 mg/6 m. At 36 months, BMD of the lumbar spine, right femoral neck, and left femoral neck increased by 8.8% (95% CI 7.6-10.1), 4.3% (95% CI 3.0-5.5), and 3.1% (95% CI 2.1-4.1), respectively. The use of denosumab was found to rapidly reduce the levels of the markers of bone turnover (TRAP5b and BAP) at 6 and 12 months and slightly increase them at 18, 24, and 36 months. AFF did not occur at 36 months in patients receiving AI and denosumab. No CTC grade 3, 4, or 5 adverse events occurred, no ONJ was observed.
Scaturro et al., 2022 [28]	Retrospective case-control study	59 BC post-menopausal women, treated with AIs therapy	Study group (starting Denosumab $\leqslant$ 12 months after AIs) and control group (> 12 months). Early Denosumab showed a significant positive effect on both LS (+0.169, p= 0.044) and FN (+0.234, p= 0.024) T-score variations. At 18 months Late Denosumab group showed 2 (6.5%) incident hip fractures and 4 (12.9%) vertebral fragility fractures.
Mazziotti G. et al., 2022 [29]	Prospective study	567 women with early BC on AI	Denosumab vs BPs (oral or iv) vs no therapy: during follow-up, treatment with denosumab or BPs induced a significant increase in LS, FN and TH BMD, whereas a significant decrease in BMD at all skeletal sites occurred in subjects who were not treated with bone-active drugs. The entity of increase in BMD at LS and TH was significantly greater in subjects treated with denosumab as compared to those treated with BPs. Higher risk of vertebral fractures in subjects who were untreated (32/184 vs. 22/383; P<0.001). Fracture risk was significantly decreased by denosumab [OR

			0.22; P< 0.001] and zoledronate (OR 0.27; P = 0.035), but not by oral BPs (P = 0.317).
Shibata M. et al., 2023 [30]	Single-center retrospective study	55 postmenopausal women with early BC on AI with low T score	Denosumab 60 mg/6m: BMD increased at 18 and 24 months; lumbar spine increased by 7.40% (95% CI 5.35–9.44), femoral neck increased by 5.24% (95% CI 1.72–8.76) after 24 months of denosumab. The u-NTX levels showed a significant decrease 1-month post-initiation of treatment and were lower than before the baseline at all time points evaluated up to 24 months later.
Galvano A. et al., 2023 [31]	Observational retrospective clinical study	50 osteopenic women with early BC on AI	Denosumab 60 mg/6m vs Alendronate 70 mg/w: significant T-score improvement at the lumbar spine level (-1.92 vs -1.52, p=0.03), with a comparable contribution from alendronate (-1.60 vs -1.45, p=0.07) and denosumab (-2.26 vs -1.58, p=0.07). As for the femoral region, neither alendronate nor denosumab were able to produce any statistically relevant effect.
Casabella A. et al., 2024 [32]	Retrospective cohort study	60 postmenopausal women with HR+ non-metastatic BC on AI	Denosumab 60 mg/6m (n=30) vs alendronate 70 mg/week (n=30, historical cohort): improvements in trabecular bone score (TBS) at the lumbar spine (OR 0.263, p = 0.0048). The body composition analysis showed a significant increase in muscle tissue expressed by LM (OR 2.054, p = 0.0021) and FFM (OR 2.049, p = 0.0022) in the denosumab group. In addiction there was a positive trend for RSMI values (OR = 0.418, p = 0.0392) in denosumab-treated patients. There was no significant improvement at BMD T-scores between two groups.
Zhang Y. et al., 2025 [33]	Retrospective observational study	121 postmenopausal women with HR+ BC on AI with treatment-	Denosumab 60 mg/6m (n=57) vs alendronate 70 mg/week (n=64): following the intervention period, an independent samples t-test demonstrated significantly greater improvement in lumbar spine BMD T-score (P = 0.003), in femoral neck BMD

		induced osteoporosis	T-score (*P* = 0.005), in total body BMD T-score (p=0.026) in the denosumab group compared to the alendronate group. Higher response rate with denosumab (91.2% vs 82.8%; *p*<0.05). In the alendronate group, post-treatment thoracic compression fractures occurred in 3 cases and lumbar compression fractures in 6 cases (total fracture rate: 12.5%). The denosumab group reported 1 thoracic and 3 lumbar compression fractures (total fracture rate: 7.017%). Fisher's exact test demonstrated a significantly lower fracture incidence in the denosumab group (P < 0.05)

Table 2. Effects of Denosumab on Bone Health in Non-Metastatic Breast Cancer Patients on Aromatase Inhibitors

5. Denosumab and muscle health

The RANK/RANKL signalling pathway plays a pivotal role in the pathogenesis of osteoporosis. RANKL (receptor activator of nuclear factor-κB ligand), produced primarily by osteoblasts and stromal cells, binds to its receptor RANK on the surface of osteoclast precursors, promoting their differentiation, activation, and survival.[34] This interaction leads to increased osteoclast-mediated bone resorption. In postmenopausal women, estrogen deficiency upregulates RANKL expression and downregulates its decoy receptor osteoprotegerin (OPG), resulting in an imbalance between bone resorption and formation and thereby contributing to bone loss and increased fracture risk.[35]

RANK has been also identified in skeletal muscle tissue, where its activation by RANKL initiates pro-inflammatory signalling cascades that impair myogenic differentiation and stimulate proteolytic pathways such as the ubiquitin–proteasome system, ultimately contributing to muscle degradation. [36] Although the mechanistic links between muscle and bone remain an evolving area of investigation, they represent a promising target for pharmacologic intervention.

In a recent preclinical study using a murine model of osteosarcopenia—generated through deletion of the peroxisome proliferator-activated receptor β transcription factor—RANKL

blockade was shown to reverse muscle deficits. Treatment led to improvements in muscle volume and contractile strength, along with upregulation of myosin I and II gene expression and suppression of the anti-myogenic and pro-inflammatory factor myostatin.[37] The same group also analysed the effects of denosumab in a clinical setting: group of postmenopausal women treated for osteoporosis for an average duration of 3 years, as compared with similar women without treatment or who received bisphosphonates. While both treatments improved BMD, only denosumab increased appendicular lean mass and handgrip strength; this change was strongly related with lumbar spine BMD changes.[37] Previously, Lefkowitz et al. reported a case report describing dramatic reversal of symptoms and increased functionality in a 66 years old female with osteoporosis and facioscapulohumeral muscular dystrophy after injections of denosumab.[38]

Phu's group performed a prospective comparative study evaluating a total of 79 community-dwelling older adults (≥65 years) at risk of falls and fractures, assigned to receive either denosumab or zoledronic acid. [39] Denosumab was associated with meaningful short-term improvements in mobility, dynamic balance, and confidence in older adults at risk of falls.

In 2020, Chotiyarnwong and colleagues, prompted by the growing interest in denosumab as a drug potentially exerting effects beyond bone metabolism, including possible effects on muscle function, investigated the lower incidence of falls observed in the denosumab arm of the pivotal Fracture Reduction Evaluation of Denosumab in Osteoporosis Every 6 Months (FREEDOM) trial. In this large placebo-controlled study of postmenopausal women with osteoporosis, falls reported as adverse events during the 3-year follow-up occurred in 4.5% of women treated with denosumab, compared with 5.7% of those receiving placebo (log-rank $p = 0.02$). This finding led the authors to further explore the hypothesis that denosumab might reduce fall risk. To test this hypothesis, they conducted an exploratory pooled analysis of all placebo-controlled trials of denosumab performed in conditions associated with bone loss and contributing to its FDA approval for these indications. The analysis included five studies involving postmenopausal women with osteoporosis, postmenopausal women with low bone mass, men with osteoporosis, women with non-metastatic breast cancer receiving adjuvant aromatase inhibitors, and men with non-metastatic prostate cancer undergoing androgen deprivation therapy. The final pooled population comprised 10,036 subjects. At least one fall was reported in 5.8% of participants in the pooled placebo group (289/5006) and in 4.6% of those in the pooled denosumab group (231/5030). Kaplan-Meier estimates showed a cumulative incidence of falls of 6.5% in the placebo group versus 5.2% in the denosumab group, corresponding to a hazard ratio of 0.79 (95% CI 0.66-0.93; $p = 0.0061$). A greater reduction in fall risk was observed in subjects younger than

75 years than in those aged 75 years or older (interaction p = 0.012). Specifically, denosumab treatment was associated with a 35% reduction in fall risk in subjects aged under 75 years (HR 0.65, 95% CI 0.52-0.82), whereas no clear reduction was observed in those aged 75 years or older (HR 1.01, 95% CI 0.78-1.31), although a possible early effect on fall risk was suggested. Overall, these findings supported the hypothesis that denosumab may have beneficial effects not only on bone health but also on functional outcomes related to muscle performance and fall risk.[40]

Miedany et al. conducted a longitudinal multicenter, controlled, prospective study to assess a potential dual effect of Denosumab on both bone and muscle in comparison to other anti-resorptive agents: after five years of treatment, the denosumab group exhibited a marked reduction in fall risk score (–1.9; 95% CI: –2.8 to –0.7; p = 0.001), along with significant gains in physical performance measures. Specifically, grip strength improved by +4.3 kg (p = 0.01), Timed Up and Go (TUG) test times decreased by 1.5 seconds (95% CI: –2.2 to –0.1; p = 0.001), and gait speed increased by 0.1 m/s (95% CI: 0.03–0.2; p = 0.001). There was also a significant worsening of the falls risk score, grip strength, TUG, and gait speed ($P = 0.001$) one year after stopping denosumab. [41] Pizzonia's group conducted a study involving 41 elderly women with femoral fractures, 26 of whom received alendronate and 15 were treated with denosumab. [42] At one-year follow-up, a trend toward improvement in RSMI was observed in the denosumab group, along with gains in hand grip strength in both groups, although interpretation was limited by a substantial amount of missing data.

Rupp's retrospective, propensity score-matched cohort study included 150 osteopenic or osteoporotic patients receiving basic (60), BP (30) or Denosumab (60) therapy.[43] A significantly greater improvement in grip strength was observed in both the denosumab group ($p < 0.001$) and the bisphosphonate group ($p = 0.001$) compared to the vitamin D group. Furthermore, the denosumab group demonstrated a significantly larger increase in lower-limb strength, as measured by the chair rising test, compared to the bisphosphonate group.

In 2024, Casabella et al. published a study comparing 30 elderly women with non-metastatic breast cancer receiving adjuvant endocrine therapy and treated with denosumab to a historical cohort of 30 similar patients treated with oral bisphosphonates.[32] The results demonstrated a significant improvement not only in trabecular bone score, but also RSMI and overall body composition in the denosumab group.

Oliveri et al. showed a correlation between a muscle mass measured by calf circumference and a positive gain in BMD in postmenopausal women at high fracture risk receiving denosumab.[44]

In a double-blind, placebo-controlled randomized trial conducted within a long-term care setting, Haeri et al. did not confirm a beneficial effect of denosumab on muscle health. Among the 201 older adults analysed, no statistically significant differences were observed between the denosumab and placebo groups in appendicular lean mass, lower extremity lean mass, chair stand performance, Short Physical Performance Battery (SPPB), or gait speed. However, a trend toward improvement was noted in women at 18 and 24 months of treatment in chair stand performance and hand grip strength.[45]

In a Chinese cohort with primary osteoporosis, denosumab significantly improved muscle strength and functional performance over 12 months, without significant gains in muscle mass. [46]Mendelian randomization further demonstrated a negative causal relationship between RANKL expression and both appendicular lean mass and grip strength, highlighting RANKL as a potential molecular target in the pathogenesis of sarcopenia. A randomized, double-blind, double-dummy clinical trial is currently underway in Hong Kong, enrolling individuals over the age of 65 diagnosed with osteosarcopenia.[47] The study aims to evaluate the therapeutic effects of denosumab on muscle strength, muscle mass, and physical performance, as well as on clinical outcomes including incidence of falls, fractures, and mortality.

Overall, the emerging evidence hints that denosumab may have beneficial effects on muscle, potentially improving or preserving muscle mass and strength in populations with osteoporosis or osteosarcopenia.[48] However, the data are heterogeneous. Some trials show only neutral effects on muscle outcomes (especially in very old or frail individuals), and one systematic review concluded the evidence is heterogeneous and inconclusive despite promising trends. Some analysis provides intriguing signals suggesting a potential reduction in falls but these findings derive from post hoc and exploratory analyses not specifically designed to assess muscle outcomes. Therefore, although denosumab may exert indirect or pleiotropic effects on muscle performance, current data are insufficient to establish a definitive causal relationship.

6. Adherence to antiresorptive therapy

Despite the existence of multiple international guidelines for initiating osteoporosis treatment, a substantial proportion of postmenopausal women who meet clinical criteria do not start therapy. According to reports from the International Osteoporosis Foundation (IOF), the average

treatment gap in Europe in 2019 was 71%, leaving approximately 15 million eligible women untreated. [49]

An earlier survey conducted by the International Osteoporosis Foundation (IOF) in 2000 across 11 countries identified several key factors associated with poor adherence to osteoporosis treatment: denial of personal fracture risk among postmenopausal women, lack of patient–physician communication regarding osteoporosis, and limited access to diagnostic services and appropriate care.[50] In Italy, the reported treatment gap was 59% in 2010, increasing to 71% by 2019.[51] Even when osteoporosis therapy is prescribed by a physician, a proportion of patients never initiate treatment. For instance, a survey reported that approximately 40% of women who had been prescribed anti-osteoporotic therapy did not start it within the following two years.[52]

The reasons for failure to initiate therapy are multifactorial. According to the aforementioned survey, the most commonly reported barriers included fear of side effects (77%), cost-related concerns (34%), and a history of gastrointestinal disorders (25%), particularly in the context of initiating oral bisphosphonate therapy. Additionally, many patients cited a lack of perceived need for treatment, reflecting a general underestimation of personal fracture risk.[52] Regarding the latter, qualitative research has shown that many women underestimate their personal fracture risk or the potential consequences of osteoporosis. Since osteoporosis is an asymptomatic condition until a fracture occurs, patients often do not perceive an immediate benefit from treatment and may struggle to engage with the idea of starting a preventive therapy.[53]

Another survey conducted by Lindsay et al. identified fear of side effects as the most commonly reported barrier to initiating osteoporosis therapy.[54] Wozniak et al. conducted interviews with 21 patients who had sustained upper limb fractures. The participants commonly cited underestimation of osteoporosis-related risk and a misjudgement of the risk–benefit profile of treatment as key reasons for not initiating therapy.[55]

Swart KMA et al. interviewed patients who did not initiate bisphosphonate therapy and identified the main reasons as insufficient medical advice, negative attitudes toward medication use—including concerns about side effects—and limited awareness of the disease and its consequences.[56]

System-level barriers also contribute to under-prescription: a proportion of eligible patients are never offered therapy by their healthcare providers. Additionally, poor coordination between hospital-based care and primary care exacerbates under-treatment. Indeed, an IOF report highlighted that approximately 80% of individuals at high fracture risk—such as those with

previous fractures—are “neither identified nor treated” for osteoporosis in routine clinical practice.[49] Among other system-related barriers are limited access to DXA scanning and long wait times for specialist consultations.

More specifically, in the context of breast cancer, real-world data show that many patients receiving aromatase inhibitors do not receive appropriate prophylaxis for bone health. For instance, a 2019 U.S. analysis found that among women over the age of 75 with breast cancer receiving AI therapy, only 15 out of 38 eligible patients (39%) were actually started on an antiresorptive agent.[57] A multicenter Croatian study showed that fewer than one-third of patients underwent baseline DXA screening; consequently, only 12% of the cohort initiated anti-osteoporotic therapy.[58]

It has also been demonstrated that prioritizing bone health improves treatment compliance. In a single-center Swiss study, active implementation of bone protection guidelines resulted in approximately 75% adherence to antiresorptive therapy.[59]

In the oncologic setting, many of the same reasons for not initiating therapy persist, with some specific nuances. These include the addition of yet another medication to an already complex treatment regimen, challenges in multidisciplinary coordination, and practical barriers such as the need for a dental evaluation prior to initiating antiresorptive therapy.[57]

The low rates of therapy initiation in eligible patients highlight a need for better awareness and coordinated efforts to ensure that patients who meet clinical criteria for denosumab actually receive it.

7. Objectives of study

Given this background, the present study aims at examining the impact of denosumab on bone and muscle health in elderly women with early breast cancer receiving aromatase inhibitors. The impact on bone is assessed by measuring the change in BMD at least one year after the initiation of therapy, while the impact on muscle is evaluated through changes in handgrip strength (HG) and Timed Up and Go (TUG) test performance at one and two years following the start of treatment.

The secondary outcomes are to assess its potential effects on other geriatric domains of CGA, including frailty assessed by Frailty Index (FI) at one and two years following the start of the treatment; to analyse treatment adherence rates and the main causes of non-compliance.

Materials and methods

1. Design, setting, and participants

A single-center retrospective observational study was conducted involving patients attending the Oncogeriatrics Outpatient Clinic at IRCCS San Martino Hospital (Genoa) within the “Breast and Bone” project between April 2021 and September 2024. The “Breast and Bone” project was established through a collaboration between oncologists and geriatricians, driven by the need to monitor and manage bone health in older women with breast cancer undergoing treatment with aromatase inhibitors. The initiative involves referring all women aged over 65 with non-metastatic, hormone receptor–positive breast cancer receiving adjuvant aromatase inhibitor therapy for geriatric evaluation and management.

Inclusion criteria were women aged ≥65 years, with non-metastatic breast cancer under aromatase inhibitor therapy, followed for at least one year by our clinic, and who provided informed consent. Patients were excluded if they were younger than 65 years, had another type of breast cancer, had metastatic cancer, or did not refer to our clinic for at least three visits (one year follow up), either due to personal choice or because of complications or referral to other specialists for complex clinical management, or those who were prescribed an antiresorptive therapy other than denosumab.

Collected data included general medical history and osteoporosis risk factors such as family history of major osteoporotic fractures, prior fragility fractures, age at menarche and menopause, adequate calcium intake, presence of comorbidities known to increase fracture risk (such as chronic obstructive pulmonary disease, diabetes mellitus, rheumatoid arthritis, etc.), and current or prior corticosteroid therapy.

Furthermore, tumour characteristics and data on ongoing and planned therapies were collected.

As for geriatric evaluation, all patients received Comprehensive Geriatric Assessment (CGA) and frailty assessment according to accumulation deficits model, based on 40-items Frailty Index

(FI).[60, 61] Muscle function was evaluated using handgrip strength (HG) and Timed Up and Go (TUG [62]) tests. Additionally, adherence data and reasons for non-adherence were recorded.
The CGA included the following domains and respective assessment tools: cognitive status (Mini Mental State Examination, MMSE [63], or Montreal Cognitive Assessment, MOCA[64], converted to MMSE-equivalent according to Lawton et al [65]), psychological status (Geriatric Depression scale, GDS 15 items [66]), functional status (Instrumental Activities of Daily Living, IADL [67] and Barthel Index [68]), postural stability and risk of falls (Tinetti Scale [69]), nutritional status (Mini Nutritional Assessment [70], social vulnerability (Gijon Scale [71]), physical burden of illness (Cumulative Illness Rating Scale, CIRS: Illness Severity Index-SI, and Co-morbidity Index-CI [72]). Pain at rest was assessed using the Numeric Rating Scale (NRS), both at rest and during ambulation [73]. The final recorded value corresponded to the higher of the two scores.

Polypharmacy was also collected. The EuroQol-5D was used to assess the quality of life.[74] On the basis of the FI assessment, a score of ≤ 0.08 defined patients as fit; a score of ≥ 0.25 as frail and a score between 0.08 and 0.25 defined patients as pre-frail.

All the participants received an oral supplementation of cholecalciferol based on the serum levels.

At each follow up visit, the following data were recorded: occurrence of new fractures, biochemical markers of bone turnover, therapy-related adverse events.

Participants were divided into two subgroups: “Denosumab” group - patients who had initiated and continued denosumab treatment for at least one year, and” No Denosumab” group- patients who had never initiated denosumab or had received only one injection. For both groups, we analysed BMD assessed by DXA at two different points—one prior to therapy initiation (T0) and another after at least one year of follow-up (T1). DXA BMD was measured and expressed as T-score at lumbar spine and femur (total and neck).

The study received approval from the institutional ethics committee of the participating hospital, and written informed consent was obtained from all participants or their legal representatives.

2. Measures

Bone densitometry with the quantitative analysis of the BMD was performed to assess osteoporosis in all patients. Subjects were classified as osteopenic (T-score = − 1.0 to − 2.5 SD) or osteoporotic (T-score < −2.5 SD).

Grip strength, an indicator of upper extremity muscle strength, was evaluated at baseline and follow up visits in the dominant hand using a standard dynamometer (JAMAR Technologies, Hatfield, PA). The assessment was conducted with the upper arm adducted and the elbow flexed at a 90-degree angle.

The EWGSOP2 criteria assess muscle strength using grip strength, with thresholds set at <16 kg for women.[75]

Timed up and go is a simple, common assessment of functional mobility and fall risk, especially for older adults, involving timing how long it takes to stand up from a chair, walk three meters, turn around, walk back, and sit down.[62]

3. Outcomes

The primary objective of the study was to assess the impact of at least one year of denosumab therapy on muscle and bone health. Muscle function was evaluated by comparing strength and physical performance indices, while bone health was assessed by comparing bone mineral density values prior to the initiation of antiresorptive treatment and at one and two years of follow-up. Although follow-up visits were conducted every six months, our analysis focused on comparisons between the visit at which denosumab therapy was first recommended (T0) and those occurring approximately one year and ≥2 years after treatment initiation.

Secondary outcomes included evaluating the potential impact of denosumab therapy on domains of CGA and the trajectory of frailty over time, and on laboratory parameters such as renal function, calcium, phosphorus, vitamin D, alkaline phosphatase, parathormone and bone turnover marker (Bone Alkaline Phosphatase-BALP, and Beta CrossLaps-CTX) where available. Additionally, the study aimed to assess treatment adherence and identify main reasons for non-compliance.

4. Statistical analysis

Statistical analyses were designed to evaluate whether longitudinal changes differed between patients treated with denosumab and untreated controls. At baseline, demographic, clinical, oncological, and geriatric characteristics were compared between groups to assess their initial comparability and to exclude major imbalances that could bias subsequent analyses.

For the densitometric evaluation, BMD was assessed using T-scores at the lumbar spine, femoral neck, and total hip. For each patient, two consecutive DXA scans performed after the index date (defined as treatment initiation for the “Denosumab” group and a corresponding timepoint for controls) were considered.

For each skeletal site, change in bone density (ΔT-score) was calculated as the difference between the second and the first measurement. To account for differences in follow-up duration across patients, these changes were also normalized for the time interval between scans and expressed as annualized variations. Therefore, both absolute (non-annualized) and annualized delta values were analysed for densitometric outcomes.

The null hypothesis assumed no significant change in bone density over time, corresponding to a mean difference of zero. The alternative hypothesis posited a significant modification in T-scores following initiation of treatment. Statistical testing was performed using a paired t-test, assuming normality of the distribution of differences, and confirmed with the non-parametric Wilcoxon signed-rank test, in order to determine whether bone density trajectories differed between groups and could be attributed to treatment rather than natural variation over time. To support clinical interpretation, confidence intervals for the mean (parametric analysis) or the median (non-parametric analysis) were calculated, and the inclusion or exclusion of zero within these intervals was used to determine the presence or absence of a statistically significant change over time. For CGA variables, changes over time were calculated as the difference between follow-up and baseline values. The variables analysed included cognitive function (MMSE), functional status (Barthel Index and IADL), mood (GDS), balance and gait (Tinetti test), physical performance (Timed Up and Go and handgrip strength), nutritional status (MNA), sarcopenia screening (SARC-F), social vulnerability (Gijón scale), quality of life (EuroQol), pain (NRS), and frailty (40-item Frailty Index). These outcomes were assessed at both 1-year and 2-year follow-up. Between-group comparisons were performed using the Wilcoxon rank-sum test.

For laboratory variables, changes were similarly calculated as the difference between follow-up and baseline values. The parameters evaluated included renal function (eGFR), calcium and phosphorus levels, vitamin D, parathyroid hormone (PTH), and bone turnover markers (including BALP and CTX when available). These variables were analysed at both 1-year and 2-year follow-up. Between-group comparisons were conducted using the Wilcoxon rank-sum test.

Within-group analyses were also performed across all domains to support interpretation, assessing whether changes over time differed from zero using paired tests (paired t-test and Wilcoxon signed-rank test).

Results

1. Baseline characteristics

Between April 2021 and November 2025, 220 patients were evaluated within the “Breast and Bone” project. Of these, 120 met inclusion criteria of our study (figure 2); 80 in the “Denosumab” group and 40 in the “No Denosumab” group, with average age of 76.9 years (SD 5.05). The two groups were substantially comparable, except for a higher prevalence of prior fragility fractures and percentage of smokers, observed in the “Denosumab” group, and higher prevalence of CKD in the “No Denosumab” group. The most prevalent tumour subtype was luminal B, with a similar distribution of stage I and stage II disease across both groups. From a geriatric standpoint as well, the two groups showed no major differences at baseline, except for a higher GDS scores in “No Denosumab” group, still within normal limits, namely in the absence of depression. In both cohorts, the median frailty index was approximately 0.15, corresponding to the pre-frail category. In the “Denosumab” group, 20% of patients were classified as fit, 69% as pre-frail, and 11% as frail. In the “No denosumab” group, 15% were fit, 55% pre-frail, and 30% frail.

In terms of mean/median values, all scales remained within normal limits.

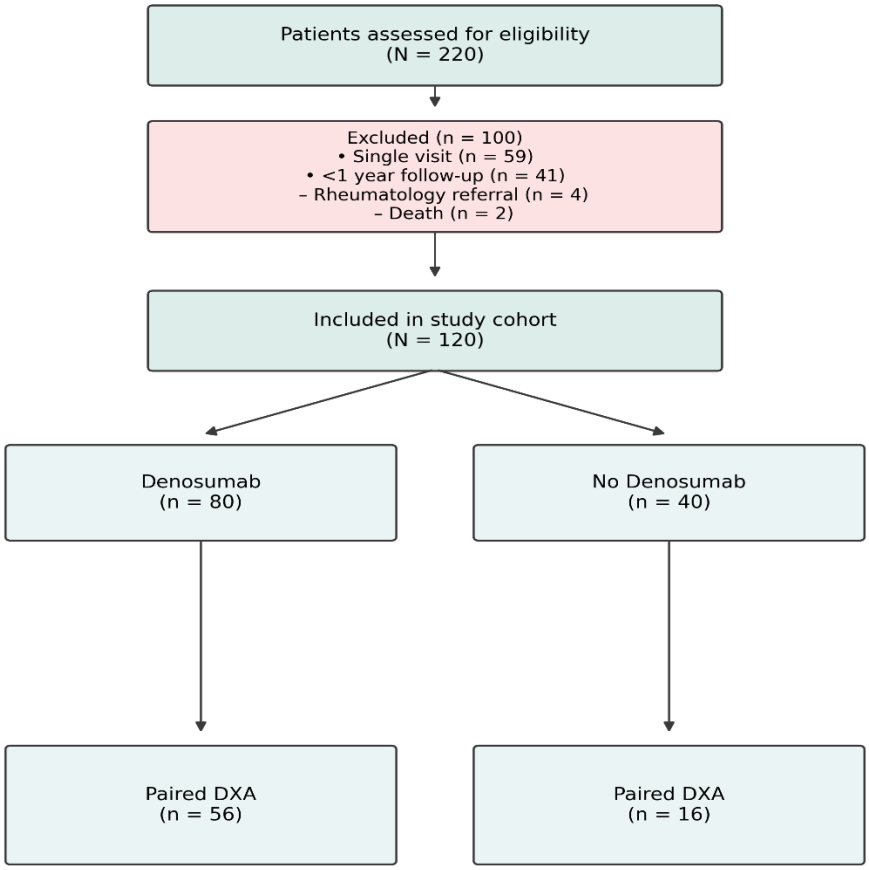

Figure 2. Flow diagram of patient selection and inclusion in the study.

	Denosumab [N=80]	**No Denosumab [N=40]**	**pValue**
Age [mean (SD), range]	76.9 (5.04), 67-88	76.9 (5.21) 65-87	0.859
Educational level [media (IQR)]	8 (8)	10 (9.5)	0.449
Living situation [N (%)]			0.430
With someone	47 (59.5%)	27 (67.5%)	
Alone	32 (40.5%)	13 (32.5%)	
BMI [mean (SD)]	24.7 (4.02)	25.3 (3.68)	0.401

Smoking [N (%)]			0.045
Former smoker	22 (27.8%)	5 (12.5%)	
Current smoker	6 (7.6%)	8 (20%)	
PY [median (IQR)]	23 (35)	25 (27)	0.869
Alcohol consumption [N (%)]			0.240
<3 Units per day	28 (35.4%)	9 (22.5%)	
≥ 3 Units per day	2 (2.5%)	-	
Adequate calcium intake [N (%)]	70 (89.7%)	33 (91.7%)	1
Previous fragility fractures [N (%)]	31 (38.7%)	6 (15%)	0.011
Femur	10 (12.5%)	1 (2.5%)	0.097
Vertebrae	9 (11.3%)	3 (7.5%)	0.749
Wrist	5 (6.3%)	1 (2.5%)	0.662
Humerus	3 (3.8%)	3 (7.5%)	0.399
Pelvic	0 (0%)	0 (0%)	-
Other sites	10 (12.5%)	1 (2.5%)	0.097
Family history of fractures [N (%)]	16 (20.5%)	8 (21.1%)	1
Risk factors for osteoporosis [N (%)]			
	1 (1.3%)	1 (2.5%)	1
Rheumatoid/psoriatic	1 (1.3%)	1 (2.5%)	1
arthritis	5 (6.3%)	5 (12.5%)	0.298
Autoimmune disease	5 (6.3%)	1 (2.5%)	0.662
Diabetes mellitus			
Chronic obstructive	3 (3.8%)	7 (17.5%)	0.015
pulmonary disease (COPD)	2 (2.5%)	1 (2.5%)	1
Chronic kidney disease	2 (2.5%)	3 (7.5%)	0.332
(CKD)			
Mobility impairment			
Dementia			
CIRS [median (IQR)]			
Severity index	1.69 (0.37)	1.65 (0.45)	0.725
Comorbidity index	3 (1.25)	3 (3)	0.696
Stage [N (%)]			0.107
I	36 (47.4%)	17 (45.9%)	
II	23 (30.3%)	17 (45.9%)	

III	17 (22.3%)	3 (8.1%)	
Molecular subtype [N (%)]			1
Luminal A	26 (32.5%)	12 (30.8%)	
Luminal B	54 (67.5%)	27 (69.2%)	
Previous oncologic treatments [N (%)]	75 (93.8%)	34 (85%)	0.177
Surgery	8 (10%)	5 (12.5%)	0.758
Neoadjuvant chemotherapy	11 (13.8%)	8 (20%)	0.430
Adjuvant chemotherapy	40 (50%)	17 (42.5%)	0.561
Radiotherapy			
Planned oncologic therapies [N (%)]			
Surgery	5 (6.3%)	5 (12.5%)	0.298
Adjuvant chemotherapy	7 (8.8%)	7 (17.5%)	0.226
Radiotherapy	20 (25%)	9 (22.5%)	0.824
Immunotherapy	1 (1.3%)	1 (2.5%)	1

Table 3. Baseline clinical characteristics

	Denosumab	**No Denosumab**	**pValue**
N° recent falls [N (%)]			0.626
0	59 (76.6%)	34 (87.2%)	
1	12 (15.6%)	3 (7.7%)	
Al least 2	6 (7.8)	2 (5.1%)	
N° drugs [median (IQR)]	4 (2)	4 (3)	0.720
ACB score [median (IQR)]	0 (1)	0 (1)	0.994
MMSE [median (IQR)]	27 (2.75)	27.5 (1.9)	0.265
Barthel Index [median (IQR)]	95 (5)	97.5 (10)	0.590
IADL (lost) [median (IQR)]	0 (0.25)	0 (1)	0.364
GDS (N= 75) [median (IQR)]	2 (4)	5 (5)	0.046
Tinetti (N= 78) [median (IQR)]	27 (4)	26 (4.5)	0.443
Hand grip (N= 75) [median (IQR)]	20 (6)	20 (5.6)	0.268
TUG (N= 74) [median (IQR)]	8 (2)	8 (5)	0.937
MNA (N= 70) [median (IQR)]	25.5 (3.9)	25 (2.5)	0.337
Gijon (N= 77) [median (IQR)]	9 (4)	10 (4)	0.315

EQ-5D (N= 58) [median (IQR)]	0.796 (0.123)	0.752 (0.219)	0.083
NRS [median (IQR)]	2.5 (5)	2 (7)	0.702
FI-40 item (N= 77) [median (IQR)]	0.15 (0.11)	0.14 (0.18)	0.352

Table 4. Baseline CGA characteristics

2. Impact of Denosumab on bone health.

With regard to fragility fractures, as reported in Table 3, the “Denosumab” and “No Denosumab” groups differed significantly at baseline. Specifically, in the “Denosumab” group, 31 patients (39%) had a history of prior fragility fractures, mainly involving the femur and vertebrae, whereas in the “No Denosumab” group, only 6 patients (15%) had experienced fragility fractures. According to the classical definition of osteoporosis (World Health Organization (WHO) criteria: T-score below −2.5), in the “Denosumab” group (n=56 patients with paired DXA scans), 30 patients (54%) were diagnosed with osteoporosis, while 26 (46%) were classified as having osteopenia. Following treatment with Denosumab, 7 patients showed sufficient improvement to be reclassified into the osteopenia category, whereas 2 patients with osteopenia progressed to osteoporosis (figure 3).

Using instead an operational definition of osteoporosis—based on the T-score cut-offs recommended by societies such as the International Osteoporosis Foundation and the European Society for Medical Oncology to guide treatment initiation in this patient population (i.e., women with breast cancer receiving aromatase inhibitors; T-score ≤ −2)—41 patients (73%) in the “Denosumab” group were classified as osteoporotic at baseline, while 15 (27%) were osteopenic. Following treatment with Denosumab, 6 patients shifted from osteoporosis to osteopenia, whereas 5 patients transitioned from osteopenia to osteoporosis according to this operational definition (figure 4).

In the “No Denosumab” group (n = 16 with paired DXA scans), at baseline, according to the classical definition of osteoporosis, 5 patients (31%) were classified as osteoporotic and 7 (43%) as osteopenic. At follow-up, 3 patients progressed from osteopenia to osteoporosis, and 4 patients with normal bone health at baseline developed osteopenia.

According to the operational definition, in the “No Denosumab” group, 8 patients (50%) were classified as osteoporotic and 4 (25%) as osteopenic at baseline. At follow-up, 2 patients progressed from osteopenia to osteoporosis, 3 patients with normal bone status developed

osteopenia, and 1 patient with normal bone status developed osteoporosis, while 2 patients improved from osteoporosis to osteopenia.

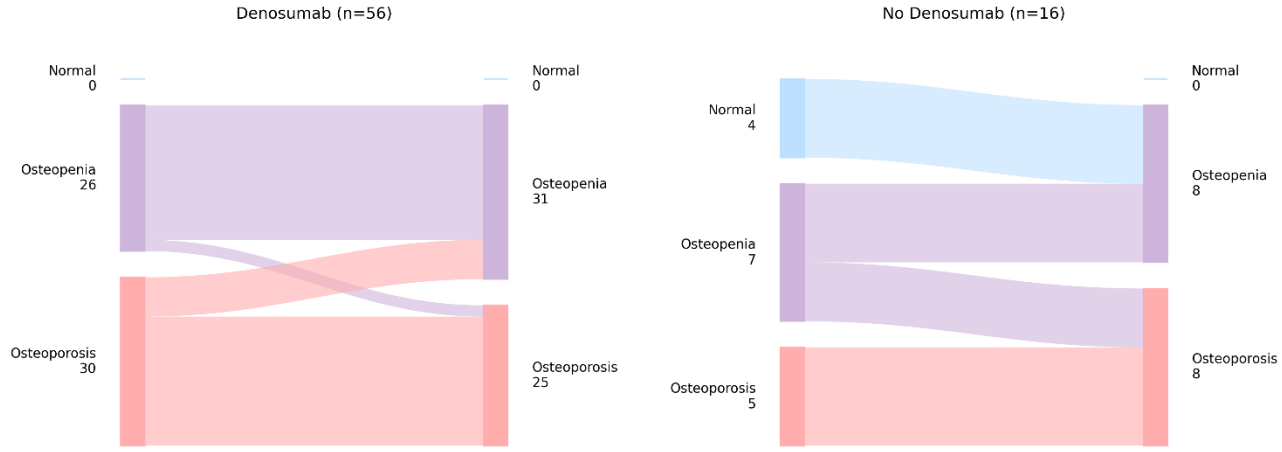

Figure 3. Transition of bone health categories between baseline and follow-up in the "Denosumab" and "No Denosumab" groups, using the WHO definition of osteoporosis (T-score ≤ −2.5), osteopenia (−2.5 < T-score < −1), and normal bone mineral density (T-score ≥ −1).

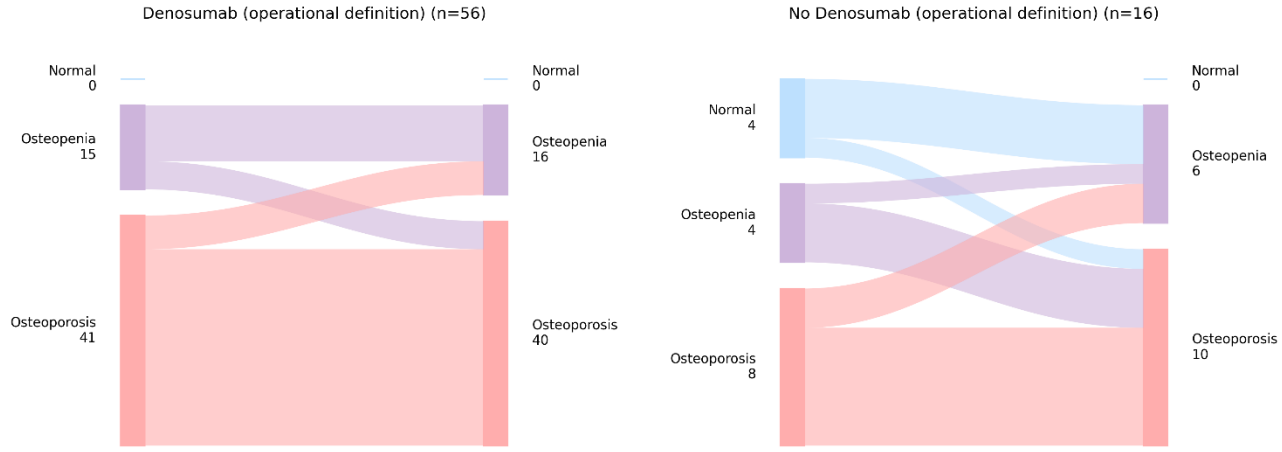

Figure 4. Transition of bone health categories between baseline and follow-up in the "Denosumab" and "No Denosumab" groups, using an operational definition of osteoporosis (T-score ≤ −2), osteopenia (−2 < T-score < −1), and normal bone mineral density (T-score ≥ −1).

In the “Denosumab” group, a significant increase in BMD T-score was observed between baseline and follow-up at lumbar spine: mean +0.38, 95% CI 0.21–0.55 (normalized +0.19, CI 0.11–0.28) and total hip: mean +0.33, 95% CI 0.18–0.49 (normalized +0.19, CI 0.11–0.28). Less significant increase was registered at femoral neck: mean +0.08, 95% CI -0.03-0.20 (normalized +0.05, 95% CI -0.01-0.12) (figures 5-10).

In the “No Denosumab” group, a non-significant trend toward BMD decline was detected at lumbar spine: mean -0.13, 95% CI -0.53-0.28 (normalized 0.04, 95% CI -0.23-0.31) and femoral neck: mean -0.34, 95% CI -0.63-(-0.06) (normalized –0.18, 95 % CI -0.34-(-0.01)). At the level of the total hip, the results were highly heterogeneous, with wide confidence intervals (mean: -0.01, 95% CI -0.68-0.66, normalized 0.13, 95% CI -0.36-0.62).

Furthermore, in a subgroup analysis of patients who underwent DXA assessment after at least two years of denosumab therapy (n = 21), a significant improvement in lumbar spine BMD was confirmed (+0.54; 95% CI 0.39–0.69), with a normalized increase of +0.20 (95% CI 0.14–0.26), while a positive trend was observed at the femoral site.

The between-group differences in densitometric changes were statistically significant across all DXA sites (see Supplementary materials).

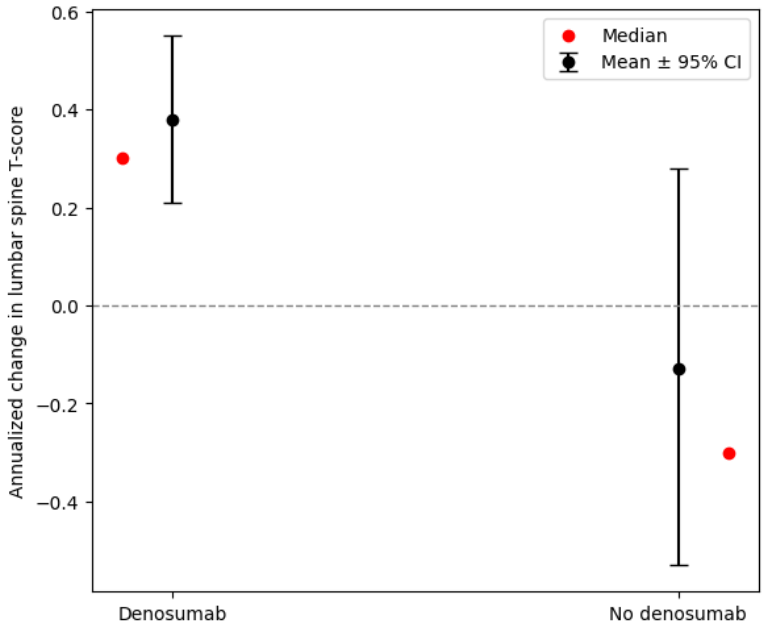

Figure 5. Annualized median and mean (with 95% CI) variations in lumbar spine T-score between two groups, “Denosumab” and “No Denosumab”.

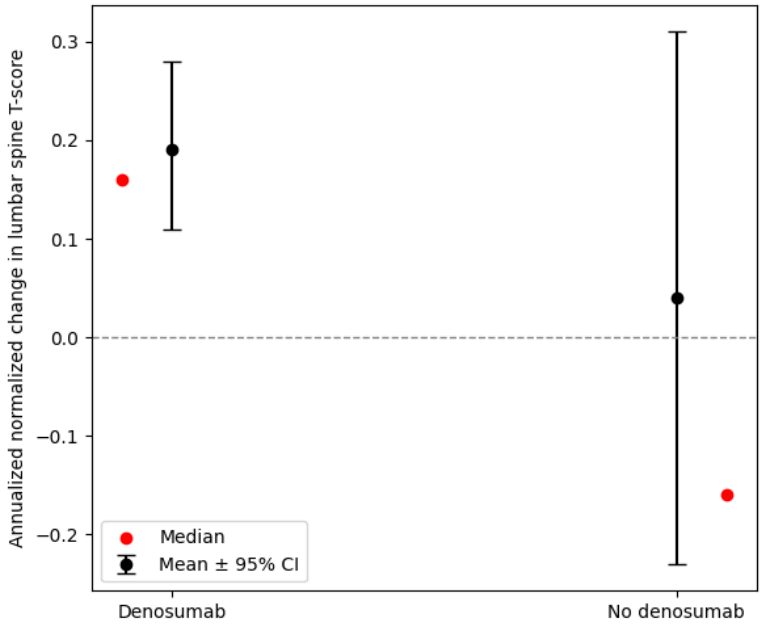

Figure 6. Annualized normalized median and mean (with 95% CI) variations in T-score between two groups, "Denosumab" and "No Denosumab".

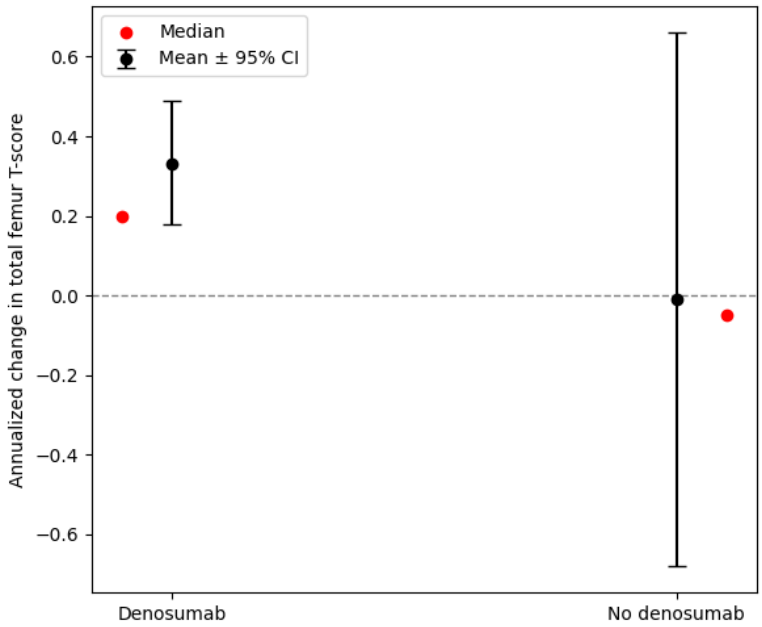

Figure 7. Annualized median and mean (with 95% CI) variations in total hip T-score between two groups, "Denosumab" and "No Denosumab".

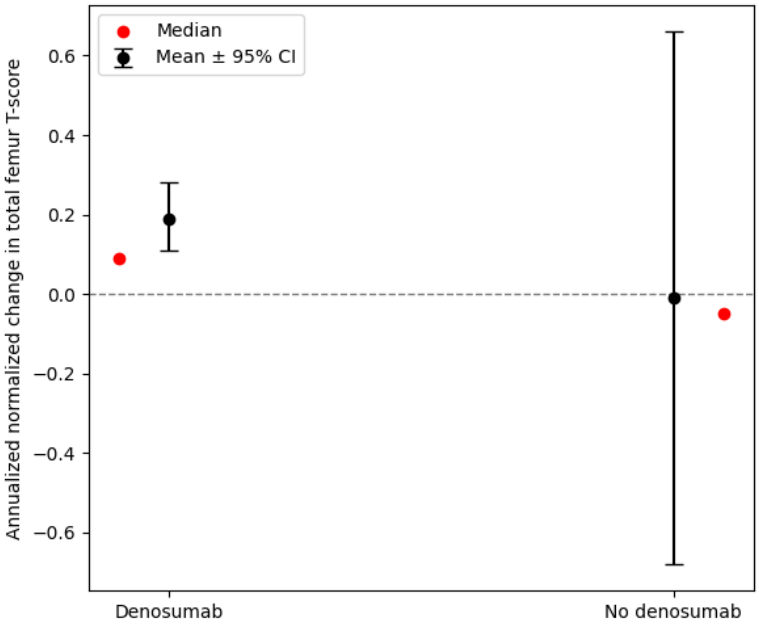

Figure 8. Annualized normalized median and mean (with 95% CI) variations in total hip T-score between two groups, "Denosumab" and "No Denosumab".

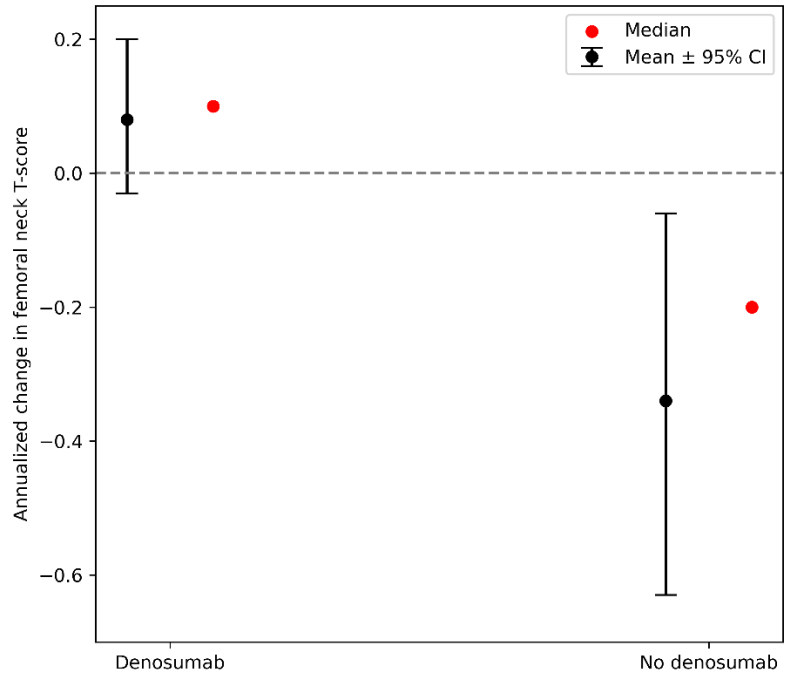

Figure 9. Annualized median and mean (with 95% CI) variations in femoral neck T-score between two groups, "Denosumab" and "No Denosumab".

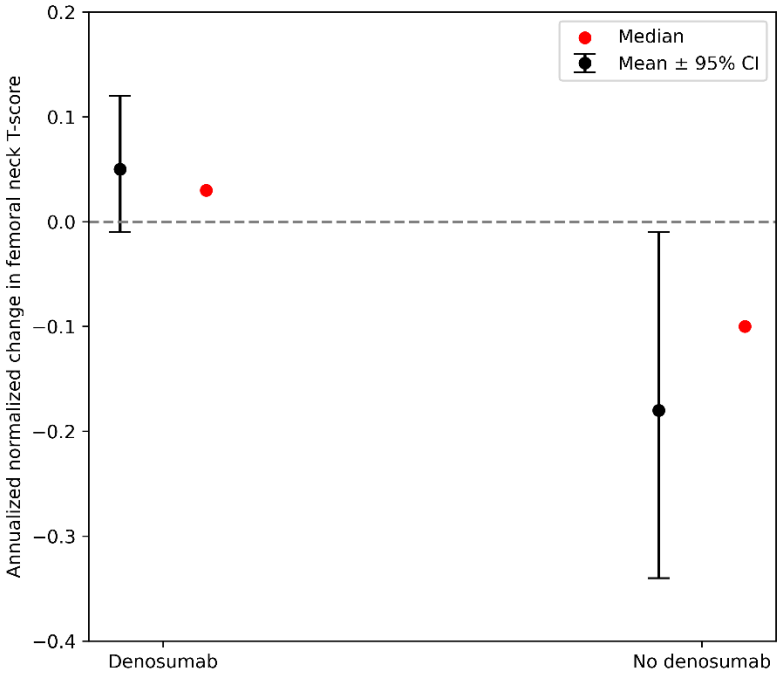

Figure 10. Annualized normalized median and mean (with 95% CI) variations in femoral neck T-score between two groups, "Denosumab" and "No Denosumab".

3. Impact of Denosumab on muscle health.

Only 16 patients in "Denosumab" group (n=80) had handgrip strength values suggestive of sarcopenia at baseline, of whom only 1 patient also presented with pathological TUG score; another patient had a pathological TUG, while handgrip strength was above the threshold. No significant improvement was observed in the Denosumab group at either 1 or 2 years in these two variables, both overall (see table 7 and 9) and when stratifying by established cut-offs (HG <16 kg; TUG ≥20 seconds).

In particular, 5 patients with suspected sarcopenia at baseline showed an improvement in handgrip strength after one year of denosumab therapy, reaching the "≥16 kg" cut-off; the remaining 11 continued to have handgrip strength values below the cut-off. Conversely, 13 patients with normal handgrip strength at baseline declined to below the cut-off after one year of therapy.

In the “No Denosumab” group, 11 patients had HG <16 kg at baseline, of whom 4 also had a pathological TUG. No significant changes were observed in the “No Denosumab” group at either 1 or 2 years for these two variables, both overall (see Tables 7 and 9) and when stratified according to established cut-offs. Specifically, 8 patients remained with pathological HG after one year of follow-up, 3 improved (HG ≥16 kg), and 2 patients who were normal at baseline developed suspected sarcopenia.

Because of the very small number of patients with pathological baseline TUG values according to sarcopenia guidelines, we performed a sub-analysis using TUG >10 s, a threshold commonly used in frailty studies as a marker of abnormal gait and included in our Frailty Index. This sub-analysis showed no significant difference in improvement between the two groups among patients with pathological baseline TUG values.

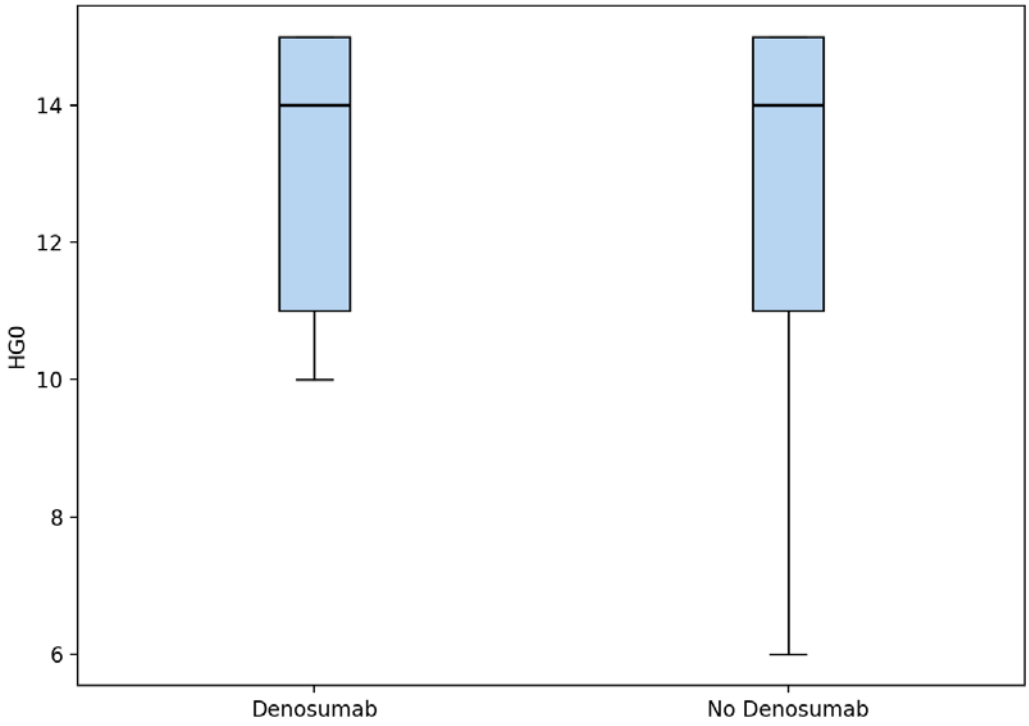

Figure 11. Comparison of baseline handgrip strength (HG) in patients suspected of sarcopenia between the "Denosumab" group and the "No Denosumab" group.

Group	n	Mean	Median	IQR	Min	Max
“Denosumab”	16	13.06	14.00	4.00	10	15
“No Denosumab”	11	12.55	14.00	4.00	6	15

Table 5. Mean and median changes in handgrip strength (HG) in the two groups, “Denosumab” and “No denosumab”. SD: standard deviation, SE: standard error.

Finally, when bone and muscle health parameters were considered together, in the Denosumab group (n = 56 patients with paired DXA scans), 9 patients presented at baseline with both a BMD T-score ≤ -2 and HG <16 kg. After one year of denosumab therapy, 2 of these patients still had impaired BMD but no longer showed pathological HG values, whereas the remaining patients were unchanged (as category). In addition, 6 patients with osteoporosis at baseline also developed suspected sarcopenia, while 2 patients progressed from osteopenia to osteoporosis and concomitant suspected sarcopenia.

In the “No Denosumab” group (n = 16 patients with paired DXA scans), only one patient presented at baseline with both a BMD T-score ≤ -2 and HG <16 kg, and she remained in the same category after one year of follow-up. Moreover, 2 patients transitioned from osteopenia to osteoporosis with concomitant suspected sarcopenia.

To further reduce bias, we performed a sensitivity analysis by excluding from the control group patients who had received only a single administration of denosumab or had been treated with denosumab for less than one year, as these could potentially confound results due to treatment effects or rebound after discontinuation. The difference in the change in femoral neck T-score remained statistically significant. For further details, see the Supplementary Material.

4. CGA and frailty.

At one- and two-year follow-up, no significant differences were observed between the groups receiving denosumab and those not treated. A trend was noted in IADL and Gijón scores; however, it was not clinically meaningful due to the wide confidence intervals.

	Denosumab (0=no, 1=yes)	N	Median	IQR
Delta_MMSE	0	27	0.0000	3.0000
	1	71	0.1000	3.0000
Delta_Barthel	0	25	0	5.0000
	1	73	0	5.0000
Delta_IADL	0	26	0.5000	1.0000
	1	73	0	0.0000
Delta_GDS	0	13	0	3.0000
	1	49	0	2.0000
Delta_Tinetti	0	26	-2.0000	3.5000
	1	72	0.0000	2.0000
Delta_HG	0	22	-0.5000	3.7500
	1	68	-1.0000	5.2500
Delta_TUG	0	23	1	3.0000
	1	66	1.0000	4.0000
Delta_MNA	0	22	0.0000	2.2500
	1	63	0.0000	2.7500
Delta_Gijon	0	25	0	2.0000
	1	70	1.0000	2.0000
Delta_EQ-5D	0	22	-0.0520	0.2095
	1	54	0.0000	0.1643
Delta_NRS	0	26	0.0000	0.0000
	1	72	0.0000	3.2500
Delta_FI	0	24	0.0250	0.1375
	1	69	0.0000	0.0800

Table 6. Median and Interquartile range (IQR) of CGA variations between two groups at one year follow up.

		Statistics	P
Delta_MMSE	U di Mann-Whitney	911	0.954

Delta_Barthel	U di Mann-Whitney	826	0.456
Delta_IADL	U di Mann-Whitney	738	0.045
Delta_GDS	U di Mann-Whitney	255	0.545
Delta_Tinetti	U di Mann-Whitney	830	0.390
Delta_HG	U di Mann-Whitney	663	0.483
Delta_TUG	U di Mann-Whitney	702	0.917
Delta_MNA	U di Mann-Whitney	684	0.843
Delta_Gijon	U di Mann-Whitney	615	0.032
Delta_EQ-5D	U di Mann-Whitney	538	0.451
Delta_NRS	U di Mann-Whitney	819	0.431
Delta_FI	U di Mann-Whitney	736	0.369

Table 7. Independent samples t-test to compare CGA changes between the two groups at one year follow up. Note. $H_a\ \mu\ 0 \neq \mu\ 1$

	Denosumab (0=no, 1=yes)	**N**	**Median**	**IQR**
Delta_MMSE	0	27	0.0000	3.1500
	1	68	0.0000	3.0000
Delta_Barthel	0	25	0	10.0000
	1	73	0	5.0000
Delta_IADL	0	26	0.0000	1.0000
	1	73	0	0.0000
Delta_GDS	0	12	-1.5000	3.2500
	1	48	0.0000	3.0000
Delta_Tinetti	0	26	-2.0000	4.7500
	1	72	-1.0000	3.0000

Delta_HG	0	22	-0.5000	4.7500
	1	67	-2.0000	7.0000
Delta_TUG	0	23	1	3.0000
	1	62	1.0000	5.0000
Delta_MNA	0	22	0.0000	1.7500
	1	64	0.0000	4.5000
Delta_Gijon	0	25	0	2.0000
	1	69	1	2.0000
Delta_EQ-5D	0	22	-0.0520	0.1533
	1	55	0.0000	0.1565
Delta_NRS	0	26	0.0000	0.0000
	1	70	0.0000	3.0000
Delta_FI	0	24	0.0350	0.1275
	1	70	0.0100	0.0975

Table 8. Median and Interquartile range (IQR) of CGA variations between two groups at two year follow up.

		Statistics	**P**
Delta_MMSE	U di Mann-Whitney	911	0.954
Delta_Barthel	U di Mann-Whitney	826	0.456
Delta_IADL	U di Mann-Whitney	738	0.045
Delta_GDS	U di Mann-Whitney	255	0.545
Delta_Tinetti	U di Mann-Whitney	830	0.390
Delta_HG	U di Mann-Whitney	663	0.483
Delta_TUG	U di Mann-Whitney	702	0.917

		Statistics	P
Delta_MNA	U di Mann-Whitney	684	0.843
Delta_Gijon	U di Mann-Whitney	615	0.032
Delta_EQ-5D	U di Mann-Whitney	538	0.451
Delta_NRS	U di Mann-Whitney	819	0.431
Delta_FI	U di Mann-Whitney	736	0.369

Table 9. Independent samples t-test for CGA variables at two-year follow up. $H_a\ \mu\ 0 \neq \mu\ 1$

5. Laboratory parameters

Only a limited number of patients underwent repeated assessments of bone turnover biomarkers. For the remaining bone metabolism parameters, no statistically significant differences were observed at one- or two-year follow-up, nor between the two groups. For further details, see Supplementary Materials.

6. Adherence analysis

Among the 40 patients in the “No Denosumab” group, 27 (22.5% of total) never initiated treatment due to: ongoing or planned dental procedures / dentist’s recommendation (7), fear or refusal of new therapies (19), hospitalization or worsening general condition (1). Of the remaining 13 who received only one injection, delays between indication and initiation were attributed to dental issues (3) or patient choice (8); 2 discontinued after the first dose upon dentist’s advice. No major adverse events were reported

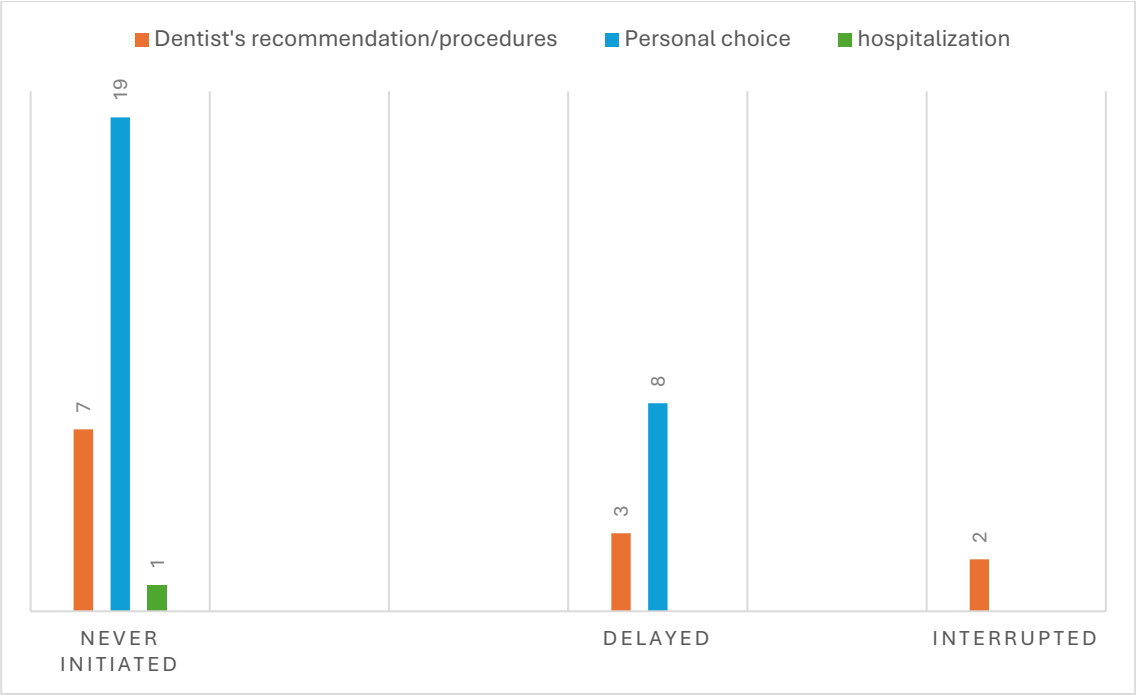

Figure 12. Main causes of non-adherence to denosumab therapy.

No new fractures were reported during the follow-up period in our study. Moreover, no major adverse events were observed in the “Denosumab” group.

Discussion

This retrospective study investigated the potential impact of denosumab on muscle strength and physical performance in older women with hormone receptor–positive breast cancer receiving adjuvant aromatase inhibitor therapy. Although treatment with denosumab for at least one year was associated with a clear improvement in bone mineral density, no statistically significant differences were observed between groups in measures of muscle strength or performance. Similarly, no significant changes were detected in frailty indices or other domains of comprehensive geriatric assessment over time.

In our study, at baseline, the “Denosumab” and “No Denosumab” groups were broadly comparable, except for a few differences outlined below. In particular, the “Denosumab” group showed a higher prevalence of prior fragility fractures (38.7 % vs 15%, “Denosumab” vs “No Denosumab”, respectively), identifying a population at intrinsically higher skeletal risk than the

control group. As a consequence, favorable effects observed in the “Denosumab” group may be underestimated, because the treated patients started from a more compromised clinical profile; also, it may have influenced, at least in part, treatment adherence, potentially leading to earlier initiation of therapy. Another significant difference was the higher prevalence of smoking in the “Denosumab” group. Smoking is a well-established risk factor for osteoporosis and may have further worsened the baseline skeletal profile of these patients. Conversely, poorer renal function was observed in the “No Denosumab” group. This factor, too, may have influenced treatment adherence, given the higher likelihood of adverse effects in patients with renal impairment, as well as the substantial burden of concomitant medications that such patients typically receive.

Our study confirms previously established evidence in the literature demonstrating that denosumab is associated with an absolute increase in BMD. A 2025 network meta-analysis of 17 RCTs in breast cancer patients on AIs (n=6,900) confirmed that denosumab provides the largest spine BMD increases among bone-protective therapies. [76] Denosumab was associated with an estimated +5.6% gain in LS BMD at 12 months and +8.0% at 24 months. As for T-score changes, for example Zhang et al. in his retrospective study reported increase of +0.28 ($p<0.001$) in LS, + 0.14 in femoral neck after 1 year of denosumab in postmenopausal women with early BC under AI treatment. [33] Galvano et al. in their prospective cohort in early BC under AI women found a trend of increasing T-score in LS (+0.68 but $p<0.07$).[31] Denosumab therefore proves to be effective also in this specific real-world population of older women with early-stage breast cancer receiving aromatase inhibitor therapy.

In our study, no fracture events were observed in either study arm. However, the available literature reports that among women receiving aromatase inhibitors without osteoprotective therapy, fracture rates are approximately 9.6% at 3 years (large multicenter randomized controlled trial ABCSG-18 [22]) and about 2.93% per year (ATAC trial [77]). A reduced, yet still present, incidence of fractures has also been described under denosumab therapy (approximately 5% after 3 years in the ABCSG-18 trial). The absence of fracture events in our cohort may be explained by several factors, including a relatively short follow-up duration and a limited sample size, particularly in the untreated group. Moreover, vertebral fractures are increasingly recognized as frequently silent and therefore potentially underdiagnosed. Radiographic studies indicate that a significant proportion of vertebral fractures are not documented in routine clinical reports, with one cohort showing that ~66.8% of vertebral fractures identified on imaging were initially unrecognized.[78]

Regarding the impact on muscle, our study did not show any statistically significant differences in handgrip strength or Timed Up and Go performance in either group. This finding may be explained by multiple factors, but it may also be consistent with existing evidence in the literature, which suggests that the effects of denosumab on skeletal muscle remain uncertain. With regard to the former, majority of the study population was classified as fit or pre-frail, with only a small proportion meeting criterion for probable sarcopenia at baseline, thereby limiting the potential for clinically detectable improvement. Moreover, most available studies primarily rely on quantitative measures of muscle mass, which were not available in our study, whereas strength and performance indices may be more strongly influenced by external and contextual factors.

Several studies, including recent ones, have raised uncertainties regarding the direct effect of denosumab on sarcopenia. Haeri et al. conducted a 2-year, double-blind, placebo-controlled randomized trial in older adults residing in long-term care facilities and found no significant differences between the denosumab and placebo groups in appendicular lean mass, lower extremity lean mass, handgrip strength, or Short Physical Performance Battery scores. However, a non-significant trend toward improvement in handgrip strength and physical performance was observed in women receiving denosumab at 24 months. Notably, the study population consisted of individuals with established osteoporosis, whereas in our cohort only approximately one-quarter of women in the denosumab group and one-seventh in the no-denosumab group were osteoporotic at baseline.

Ramchand S.K. and colleagues performed a prespecified secondary analysis of a 12-month randomized, double-blind, placebo-controlled trial involving 68 premenopausal women with breast cancer who were starting ovarian function suppression combined with aromatase inhibitor therapy. [79] Participants were randomized to receive denosumab 60 mg or placebo at baseline and after 6 months. The evaluated outcomes included, among the others, total and regional fat and lean mass measured by DXA. After 12 months, compared with placebo, denosumab treatment prevented the increase in both android (−266 [95% CI, −453 – (−79)], $P = 0.02$) and gynoid fat mass (−452 g [95% CI, −783 –(−122)], $P = 0.03$). No significant treatment effect was observed on measures of total or regional lean mass, showing once again no clear impact on lean mass or metabolic parameters.

Miedany et al. conducted a prospective longitudinal study demonstrating significant effects of denosumab on muscle function, including marked improvements in handgrip strength (+4.3 kg, $p = 0.01$), Timed Up and Go performance (–1.5 s; 95% CI –2.2 to –0.1; $p = 0.001$), and gait speed

(+0.1 m/s; 95% CI 0.03–0.2; p = 0.001). [41] However, these results were reported after five years of treatment, a follow-up duration substantially longer than that of our study, potentially suggesting that the effects of denosumab on muscle function may emerge only over the long term. Suzuki et al. reported that handgrip strength increased only during the first two months of denosumab therapy, followed by a decline at 12 months.[80] In the study by Pizzonia et al., a trend toward improvement in handgrip strength was observed in both treatment groups; however, the study population consisted of patients with major fractures (hip fractures), in whom handgrip strength was likely underestimated during hospitalization due to poor general clinical conditions.[42] Other studies have suggested an effect of denosumab on physical performance and muscle strength, although their predominantly retrospective design partially limits the strength and generalizability of these findings.[43]

Overall, the available evidence on the effects of denosumab on muscle strength and physical performance remains heterogeneous and inconclusive, with results strongly influenced by study design, duration of follow-up, baseline clinical characteristics of the population, and the methods used to assess muscle outcomes.

Nonetheless, the findings reinforce the importance of integrating geriatric expertise in the multidisciplinary care of older oncology patients. [81]The proactive involvement of geriatricians, particularly in the prescription and monitoring of supportive therapies such as antiresorptive agents, represents a valuable strategy to maintain skeletal health and mitigate the long-term complications of cancer treatments. Denosumab demonstrated a favourable safety and efficacy profile in this older cohort, supporting its use for fracture prevention in appropriately selected patients.

Despite the absence of significant differences in frailty trajectories and geriatric assessment scales at one and two years—likely due to a predominantly fit or vulnerable baseline phenotype and the multifactorial nature of frailty in older women with breast cancer, which is unlikely to be substantially modified by a single intervention such as denosumab—our study is, to our knowledge, the first to investigate the impact of denosumab on osteosarcopenia using a comprehensive geriatric assessment, providing a detailed characterization of this specific patient cohort while confirming its robust benefits on bone health.

Our study also investigated adherence to denosumab therapy and the reasons for non-initiation, delay, or discontinuation. The findings were consistent with the existing literature, although somewhat more favorable. While the survey by Yu et al. reported a non-initiation rate of approximately 40% within two years of prescription, in our cohort about 23% of patients never

started denosumab.[52] The main determinant of poor adherence was likewise concordant with previous studies and was primarily driven by patient refusal due to fear of potential side effects. These findings further support the potential role of a structured geriatric assessment and counseling in improving patient education, addressing concerns, and ultimately enhancing adherence to supportive therapies in older oncologic patients. [55]

Our study has several limitations. It is a retrospective, single-center analysis with a relatively short follow-up (1 and 2 years), which may have limited the ability to capture long-term effects that have been reported in other studies. In our study, not all patients had paired densitometric data available for comparison; therefore, the limited sample size represents a significant limitation of the final statistical analysis. In addition, muscle assessment was restricted to measures of strength and physical performance, without quantitative evaluation of muscle mass, which may be less influenced by external factors and could provide a more objective assessment of sarcopenia.

In conclusion, our study confirms the effectiveness of denosumab in preserving bone health, a key aspect in preventing prognostically relevant events such as fractures in older women with breast cancer. Conversely, its effects on skeletal muscle remain inconclusive, reflecting the complex and multifactorial nature of sarcopenia in this population and highlighting the need for further dedicated studies to explore therapeutic strategies for osteosarcopenia. In this setting, the involvement of the geriatrician within a comprehensive, multidimensional, and multidisciplinary framework is essential for appropriate risk stratification and for the optimal management of supportive, non-oncologic therapies such as denosumab. Future prospective studies with larger sample sizes and comprehensive muscle assessments—including both quantitative and functional parameters—are needed to clarify the potential role of denosumab in the management of osteosarcopenia in older adults with cancer.

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Supplementary

		Statistics	P
Delta_lumbar spine	U di Mann-Whitney	175	0.002
Delta_femoral neck	U di Mann-Whitney	159	0.010
Delta_total femur	U di Mann-Whitney	137	0.092
Delta_norm_lumbar spine	U di Mann-Whitney	186	0.004
Delta_norm_femoral neck	U di Mann-Whitney	155	0.008
Delta_norm total femur	U di Mann-Whitney	125	0.049

Table 1. Independent samples t-test to compare BMD changes between the two groups. Note. $H_a\ \mu_0 \neq \mu_1$

		Statistics	P
Delta_eGFR	U di Mann-Whitney	118.50	0.826
Delta_Calcium	U di Mann-Whitney	121.50	0.770
Delta_Phosphorum	U di Mann-Whitney	3.50	1.000
Delta_Vitamin D	U di Mann-Whitney	138.50	0.275
Delta_PTH	U di Mann-Whitney	52.50	0.347

Table 2. Independent samples t-test to compare laboratory parameters changes at two year follow up between the two groups. Note. $H_a\ \mu_0 \neq \mu_1$

	Denosumab (0=no, 1=yes)	N	Mean	Median	SD	SE
Delta_eGFR	0	10	-10.3130	-1.550	19.765	6.250
	1	25	-7.608	-4.000	18.88	3.776
Delta_Calcium	0	9	-0.0478	-0.200	0.308	0.103

	Denosumab (0=no, 1=yes)	N	Mean	Median	SD	SE
	1	29	-0.612	-0.200	3.04	0.564
Delta_Phosphorum	0	1	-0.1000	-0.100	NaN	NaN
	1	7	-7.886	-0.100	20.87	7.887
Delta_Vitamin D	0	10	7.7400	10.950	12.631	3.994
	1	36	-7.323	4.400	62.43	10.404
Delta_PTH	0	7	12.1857	12.000	31.825	12.029
	1	20	18.070	16.400	36.40	8.139

Table 3. Mean, median, Standard deviation (SD) and standard error (SE) of laboratory variations between two groups at two year follow up.

		Statistics	P
Delta_eGFR	U di Mann-Whitney	86.00	0.331
Delta_Calcium	U di Mann-Whitney	148.00	0.739
Delta_Phosphorum	U di Mann-Whitney	6.50	0.306
Delta_Vitamin D	U di Mann-Whitney	151.00	0.416
Delta_PTH	U di Mann-Whitney	62.50	0.354
Delta_BALP	U di Mann-Whitney	1.00	0.800
Delta_CTX	U di Mann-Whitney	5.00	0.857

Table 4. Independent samples t-test to compare laboratory parameters changes at one year follow up between the two groups. Note. $H_a\ \mu_0 \neq \mu_1$

	Denosumab (0=no, 1=yes)	N	Mean	Median a	SD	SE
Delta_eGFR	0	11	-7.785	0.000	20.153	6.0764
	1	20	-10.230	-5.450	19.311	4.318
Delta_Calcium	0	11	-0.121	-0.200	0.327	0.0986
	1	29	-0.437	-0.260	1.794	0.333
Delta_Phosphorum	0	3	0.167	0.100	0.306	0.1764
	1	8	-0.162	-0.150	0.496	0.175
Delta_Vitamin D	0	11	6.518	3.000	12.202	3.6789
	1	33	-8.720	1.000	65.040	11.322
Delta_PTH	0	9	15.300	12.000	29.816	9.9388
	1	18	17.350	23.400	35.625	8.397
Delta_BALP	0	1	-3.700	-3.700	NaN	NaN
	1	4	-6.300	-6.450	5.178	2.589
Delta_CTX	0	2	-0.275	-0.275	0.143	0.1010
	1	6	-72.283	-0.345	176.225	71.943

Table 5. Mean, median, Standard deviation (SD) and standard error (SE) of laboratory variations between two groups at one year follow up.

Sensitivity analyses performed after excluding patients who received only a single administration of denosumab

							95% CI	
		Statistics	**Df**	**P**	**Mean difference**	**SE difference**	**Inferior**	**Superior**
Delta_lumbar spine	t di Student	-1.9019	63.0	0.062	-0.46000	0.2419	-0.943	0.0233
Delta_femoral neck	t di Student	-2.6854	56.0	0.010	-0.43400	0.1616	-0.758	-0.1103
Delta_total hip	t di Student	-1.2303 [a]	52.0	0.224	-0.34833	0.2831	-0.916	0.2198
Delta_lumbar spine normalized	t di Student	-0.6952 [a]	63.0	0.489	-0.10010	0.1440	-0.388	0.1876
Delta_femoral neck normalized	t di Student	-2.4221	56.0	0.019	-0.21294	0.0879	-0.389	-0.0368
Delta_total hip normalized	t di Student	0.0535 [a]	52.0	0.958	0.00964	0.1803	-0.352	0.3715

Table 6. Independent samples t-test to compare BMD T-score changes at one year follow up between the two groups. Note. $H_a\ \mu_0 \neq \mu_1$

	Denosumab (1=yes, 0=no)	**N**	**Mean**	**Median**	**SD**	**SE**
Delta_lumbar spine	0	10	-0.0800	-0.3500	1.040	0.3289

	Denosumab (1=yes, 0=no)	N	Mean	Median	SD	SE
Delta_femoral neck	1	55	0.3800	0.3000	0.630	0.0850
	0	8	-0.3500	-0.3000	0.428	0.1512
Delta_total hip	1	50	0.0840	0.1000	0.424	0.0600
	0	7	-0.0143	-0.1000	1.378	0.5207
Delta_lumbar spine normalized	1	47	0.3340	0.2000	0.552	0.0805
	0	10	0.0928	-0.1926	0.787	0.2488
Delta_femoral neck normalized	1	55	0.1929	0.1610	0.319	0.0430
	0	8	-0.1582	-0.1349	0.200	0.0706
Delta_total hip normalized	1	50	0.0547	0.0279	0.235	0.0332
	0	7	0.2023	-0.0792	1.008	0.3811
	1	47	0.1927	0.0913	0.302	0.0441

Table 7. Mean, median, Standard deviation (SD) and standard error (SE) of BMD T-score variations between two groups at one year follow up.

							95% CI	
		Statistics	df	P	Mean difference	SE difference	Inferior	Superior
delta_HG	t di Student	-0.977	79.0	0.332	-1.3230	1.3544	-4.0188	1.3728
delta_TUG	t di Student	1.248	77.0	0.216	1.4930	1.1959	-0.8884	3.8744

							95% CI	
		Statistics	**df**	**P**	**Mean difference**	**SE difference**	**Inferior**	**Superior**
delta_FI	t di Student	0.740	82.0	0.461	0.0218	0.0294	-0.0367	0.0803

Table 8. Independent samples t-test to compare muscle function and FI changes at one year follow up between the two groups. Note. $H_a\ \mu_0 \neq \mu_1$

	Denosumab (1=yes, 0=no)	**N**	**Mean**	**Median**	**SD**	**SE**
delta_HG	0	13	-2.1538	-2.0000	3.8481	1.0673
	1	68	-0.8309	-1.00	4.577	0.5551
delta_TUG	0	13	2.5385	3.0000	5.3637	1.4876
	1	66	1.0455	1.00	3.618	0.4453
delta_FI	0	15	0.0447	0.0300	0.0864	0.0223
	1	69	0.0229	0.00	0.106	0.0128

Table 9. Mean, median, Standard deviation (SD) and standard error (SE) of variations muscle function and FI between two groups at one year follow up.

							95% CI	
		Statistics	**df**	**P**	**Mean Difference**	**SE Difference**	**Inferior**	**Superior**
delta_HG	t di Stu	-0.605	78.0	0.547	-0.75488	1.2471	-3.2377	1.7280
delta_TU	t di Stu	0.243	73.0	0.808	0.31638	1.2996	-2.2736	2.9064

							95% CI	
		Statistics	**df**	**P**	**Mean Difference**	**SE Difference**	**Inferior**	**Superior**
delta_FI	t di Stu	0.287	83.0	0.775	0.00890	0.0310	-0.0528	0.0707

Table 10. Independent samples t-test to compare muscle function and FI changes at two-year follow up between the two groups. Note. $H_a\ \mu_0 \neq \mu_1$

	Denosumab (1=yes, 0=no)	**N**	**Mean**	**Median**	**SD**	**SE**
delta_HG	0	13	-2.0385	-2.0000	3.7775	1.0477
	1	67	-1.2836	-2.0000	4.174	0.5099
delta_TUG	0	13	2.4615	2.0000	5.3169	1.4746
	1	62	2.1452	1.0000	4.020	0.5105
delta_FI	0	15	0.0493	0.0400	0.0808	0.0209
	1	70	0.0404	0.0100	0.114	0.0136

Table 10. Mean, median, Standard deviation (SD) and standard error (SE) of variations muscle function and FI between two groups at two-year follow up.

							95% CI	
		Statistics	**df**	**p**	**Mean differenc**	**SE difference**	**Inferior**	**Superior**
Delta_eGFR	t di Studei	1.256	23.00	0.222	11.350	9.035	-7.341	30.04
Delta_Calcium	t di Studei	0.229	33.00	0.820	0.170	0.742	-1.340	1.68
Delta_Phosphorum	t di Studei	1.057	9.00	0.318	0.329	0.312	-0.376	1.03
Delta_Vitamin D	t di Studei	0.448	36.00	0.657	13.220	29.511	-46.631	73.07
Delta_PTH	t di Studei	0.319	20.00	0.753	6.575	20.611	-36.420	49.57
Delta_ALP	t di Studei	NaN [a]						
Delta_BALP	t di Studei	NaN [a]						
Delta_CTX	t di Studei	0.378	5.00	0.721	71.907	NaN	-417.389	561.20

Table 11. Independent samples t-test to compare laboratory parameters variations at one year follow up between the two groups. Note. $H_a\ \mu_0 \neq \mu_1$

	Denosumab (1=yes, 0=no)	**N**	**Mean**	**Median**	**SD**	**SE**
Delta_eGFR	0	5	1.120	3.000	10.304	4.6081
	1	20	-10.230	-5.450	19.311	4.318
Delta_Calcium	0	6	-0.267	-0.250	0.234	0.0955
	1	29	-0.437	-0.260	1.794	0.333
Delta_Phosphorum	0	3	0.167	0.100	0.306	0.1764
	1	8	-0.162	-0.150	0.496	0.175
Delta_Vitamin D	0	5	4.500	0.700	13.876	6.2056
	1	33	-8.720	1.000	65.040	11.322
Delta_PTH	0	4	23.925	26.200	45.575	22.7877
	1	18	17.350	23.400	35.625	8.397
Delta_ALP	0	0	NaN	NaN	NaN	NaN
	1	4	-26.325	-15.000	36.444	18.222
Delta_BALP	0	0	NaN	NaN	NaN	NaN

	Denosumab (1=yes, 0=no)	N	Mean	Median	SD	SE
Delta_CTX	1	4	-6.300	-6.450	5.178	2.589
	0	1	-0.376	-0.376	NaN	NaN
	1	6	-72.283	-0.345	176.225	71.943

Table 11. Mean, median, Standard deviation (SD) and standard error (SE) of laboratory variations between two groups at one year follow up.

							95% CI	
		Statistics	df	p	Mean differen	SE difference	Inferior	Superior
Delta_eGFR	t di Stude	0.339	26.00	0.737	3.808	11.24	-19.29	26.91
Delta_Calcium	t di Stude	0.284	31.00	0.779	0.437	1.54	-2.70	3.58
Delta_Phosphorum	t di Stude	0.349	6.00	0.739	7.786	NaN	-46.80	62.37
Delta_Vitamin D	t di Stude	0.371	37.00	0.712	13.590	36.58	-60.54	87.72
Delta_PTH	t di Stude	0.123	20.00	0.903	3.580	29.03	-56.98	64.14
Delta_ALP	t di Stude	NaN [a]						
Delta_BALP	t di Stude	NaN [a]						
Delta_CTX	t di Stude	0.447	3.00	0.685	107.930	NaN	-659.89	875.75

Table 12. Independent samples t-test to compare laboratory parameters variations at two-year follow up between the two groups. Note. $H_a\ \mu_0 \neq \mu_1$

	Denosumab (1=yes, 0=no)	N	Mean	Median	SD	SE
Delta_eGFR	0	3	-3.800	2.000	10.923	6.307
	1	25	-7.608	-4.000	18.88	3.776
Delta_Calcium	0	4	-0.175	-0.200	0.206	0.103
	1	29	-0.612	-0.200	3.04	0.564
Delta_Phosphorum	0	1	-0.100	-0.100	NaN	NaN

	Denosumab (1=yes, 0=no)	N	Mean	Median	SD	SE
	1	7	-7.886	-0.100	20.87	7.887
Delta_Vitamin D	0	3	6.267	-3.700	19.289	11.136
	1	36	-7.323	4.400	62.43	10.404
Delta_PTH	0	2	21.650	21.650	74.034	52.350
	1	20	18.070	16.400	36.40	8.139
Delta_ALP	0	0	NaN	NaN	NaN	NaN
	1	2	-118.650	-118.650	55.65	39.350
Delta_BALP	0	0	NaN	NaN	NaN	NaN
	1	3	-4.267	-5.400	3.92	2.266
Delta_CTX	0	1	-0.376	-0.376	NaN	NaN
	1	4	-108.306	-0.592	215.80	107.898

Table 13. Mean, median, Standard deviation (SD) and standard error (SE) of laboratory variations between two groups at two-year follow up.