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Protective effect of denosumab on bone in female older adults with primary hyperparathyroidism

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Complete List of Authors:	Eller-Vainicher, Cristina; Ospedale Maggiore Policlinico, Unit of Endocrinology Palmieri, Serena; Universita degli Studi di Milano, Dpt. of Clinical Sciences and Community Health Cairoli, Elisa; Ospedale Maggiore Policlinico, Unit of Endocrinology; Universita degli Studi di Milano, Dpt. of Clinical Sciences and Community Health Goggi, Giovanni; Universita degli Studi di Milano, Dpt. of Clinical Sciences and Community Health Scillitani, Alfredo; Ospedale "Casa Sollievo della Sofferenza", Endocrinology; Arosio, Maura; Ospedale Maggiore Policlinico, Unit of Endocrinology; Universita degli Studi di Milano, Dpt. of Clinical Sciences and Community Health Falchetti, Alberto; Casa di Cura Villalba SRL, Endocrinology; EndOsMet, Villa Donatello, Private Hospital, Endocrinology Chiodini, Iacopo; Ospedale Maggiore Policlinico, Unit of Endocrinology
Key Words:	denosumab, primary hyperparathyroidism, fragility fractures, bone mineral density

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Authors:[1]Cristina Eller-Vainicher*, [2]Serena Palmieri*, [1,2]Elisa Cairoli, [1]Giovanni Goggi, [3]Alfredo Scillitani, [1,2] Maura Arosio, [4]Alberto Falchetti, [1]Iacopo Chiodini.

Affiliations: [1]Unit of Endocrinology, Fondazione IRCCS Cà Granda-Ospedale Maggiore Policlinico. [2]Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy. [3]Unit of Endocrinology, 'Casa Sollievo della Sofferenza', IRCCS, San Giovanni Rotondo, Foggia, Italy. [4]Villa Alba Hospital, Villa Maria Group, Bologna, Italy; EndOsMet, Villa Donatello, Private Hospital, Florence, Italy.

*These authors equally contributed to the study

Correspondence: Iacopo Chiodini, Unit of Endocrinology and Metabolic Diseases, Padiglione Granelli, Via Francesco Sforza 35, 20122, Milan, Italy; Email: iacopo.chiodini@policlinico.mi.it

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We certify that this work is novel

This study shows for the first time that denosumab is effective in preserving bone health in female older adults with primary hyperparathyroidism.

Abstract

Background/Objectives: Denosumab (DMab) is used in primary osteoporosis (PO), while its effect in primary hyperparathyroidism (PHPT) related osteoporosis is unknown.

Design: retrospective, longitudinal study

Setting: out-patient Clinic for Osteoporosis

Participants: 25 PHPT female older adults (78.6±5.5 years) and 25 PO female subjects (age 78.8±5.2 years). The patients with PO and PHPT were matched for age, body mass index, familiar history of hip fracture, femoral bone mineral density (BMD) and personal history of fragility fractures.

Intervention: 24 months DMab therapy.

Measurements: in all subjects at baseline and after 24 months we assessed the calcium-phosphorous metabolism parameters, BMD at lumbar spine (LS), femoral neck (FN), total hip (TH) by dual by X-ray absorptiometry, the morphometric vertebral fractures by radiograph. The BMD and alkaline phosphatase total activity (ALP) changes were considered significant if higher than the least significant change (LS 2.8%, FN 5.9%, TH 4.8%, ALP -22%) and were expressed even as percentage difference between end of follow-up and baseline (Δ).

Results: After 24 months PHPT patients showed higher ΔALP (-30.6±11.3), ΔFN (5.6±4.8) and ΔTH (4.8±4.4) than PO subjects (-21.4±13.1, 2.9±4.8, 1.2±4.1, respectively, $p<0.05$ for all comparisons). A significant BMD increase was more frequent among PHPT patients (92%) than among PO ones (52%, $p<0.05$) and it was 13.4 fold more likely in PHPT patients than in PO ones ($p=0.023$), regardless of possible confounders. Two subjects among both PHPT and PO patients had an incident fracture.

Conclusions: The DMab therapy is effective in female older adults affected with PHPT related osteoporosis.

Key words: denosumab, primary hyperparathyroidism, fragility fractures, bone mineral density

Introduction

Primary hyperparathyroidism (PHPT) is characterized by hypercalcaemia and elevated or inappropriately normal levels of parathyroid hormone (PTH), due to an excessive PTH secretion, and it is caused by a solitary parathyroid adenoma (80% of cases) or multiple gland hyperplasia (10-15% of cases).[1] Although, nowadays, PHPT is frequently discovered when asymptomatic, this disease may be frequently associated with bone loss, kidney stones and reduced renal function, besides other less typical manifestations, such as gastrointestinal symptoms, muscle weakness and psychological disturbances.[2-7] The PHPT overall prevalence is estimated to be about 0.9%, and it increases with the advancing age, reaching a prevalence of 2-3% in postmenopausal women.[8, 9] Parathyroidectomy is the only definitive treatment of primary hyperparathyroidism and it leads to a reduction of the risk of fractures and of the overall mortality.[10-12]. However, in the "real life", many PHPT patients, in particular the older adults, cannot undergo surgery or do not accept it. In these patients, hydration for reducing the risk of renal stones and drugs for reducing the fracture risk are recommended.[13] However, as far as the PHPT-related bone consequences is concerned, to date, scarce and somewhat conflicting data are available on the effect of bone active drugs (mainly bisphosphonates) in these patients.[2, 13-15]

In the last years, Denosumab (DMab), a monoclonal antibody that binds the receptor activator of nuclear factor kappa-B (RANK) ligand (RANKL), has been demonstrated to inhibit osteoclasts differentiation and activity and to reduce the risk of fracture in patients with primary osteoporosis (PO). [16-18] In PHPT patients, bone mineral density (BMD) is decreased and the risk of fractures increased, due to the PTH-mediated activation of the RANK/RANKL pathway.[3-7, 14, 19] Therefore, DMab could be an effective therapy for reducing bone damage in PHPT, but to date, no studies have been performed about its possible use in PHPT patients with osteoporosis.

Therefore, the aim of the present study was to assess in a "real life" setting the efficacy of DMab on BMD and incident fractures in PHPT older adults, who could not undergo or refused the surgical approach.

Patients and Methods

Patients

In this observational retrospective study, we evaluated data of all (n=65) older adult (≥65 years) female Caucasian patients with sporadic PHPT, referred to our out-patient Clinic for Metabolic Bone Diseases since June 2013 to June 2015. The PHTP was diagnosed by the presence of hypercalcaemia and elevated or inappropriately normal PTH levels, after the exclusion of familial hypocalciuric hypercalcemia by evaluating calcium clearance/creatinine clearance ratio in the presence of normal renal function (creatinine clearance by Cockcroft–Gault equation >60 ml/min).[20] The PHPT patients were included in the study if they fulfilled the following criteria: i) the refusal or impossibility (for elevated anesthetic risk) of parathyroidectomy; ii) a BMD T-score (i.e: number of standard deviations above or below the mean for a healthy 30-year-old adult of the same sex and ethnicity) below -4.0 and/or prevalent vertebral and/or hip fragility fractures, or a BMD T-score below -3.0 together with a familiar history of vertebral or hip fragility fracture (as recommended by the Italian National Health System); iii) a 24 months DMab therapy (60 mg subcutaneously every 6 months), with a complete (100%) adherence. Patients were not included in the study in the presence of: i) other diseases or conditions, beside PHPT, known to affect bone metabolism (i.e. menopause before 45 years, thyrotoxicosis, gastrointestinal disorders, chronic renal failure, chronic hepatic disease, depression, alcoholism, obesity, eating disorders, rheumatological or haematological diseases, hypercortisolism, diabetes); ii) administration of drugs influencing bone metabolism or PTH and calcium levels (i.e. alendronate, risedronate, ibandronate, cinacalcet, glucocorticoids, teriparatide, thiazide diuretics, hormonal adiuvant therapy, lithium) during the previous 2 years and/or for more than 10 years and/or present or past therapy with neridronate or zoledronate; iii) multiple endocrine neoplasia type 1 and type 2 syndromes-related PHPT, that may have a different impact on the skeletal health;[21] iv) alcohol and/or tobacco abuse. Eventually 25 PHPT female patients have been included in the study (PHPT Group).

During the same period of time, 25 female patients with primary osteoporosis (PO Group) were enrolled as control subjects, if they fulfilled the same inclusion and exclusion criteria used for PHPT patients. Each control subject was chosen by matching with a PHPT patient considering the following variables: body mass index (BMI), familiar history of hip fracture, femoral neck BMD and personal history of vertebral and/or hip fragility fractures. Among PHPT patients and PO subjects 13 and 15 were hypertensive, respectively and 5 and 7 were taking statins, respectively.

As per our protocols, in order to normalize vitamin D (25OHVitD) levels, all patients with 25OHVitD concentration below 30 ng/mL received cholecalciferol supplementation. An oral bolus of 100000 IU or 300000 IU of cholecalciferol was administered in patients with 25OHVitD levels between 10 and 30 ng/mL and below 10 ng/mL, respectively. Subsequently, in all non-obese patients with osteoporosis a cholecalciferol supplementation of 50000 IU monthly and 400IU daily was recommended [22] and in those with a calcium intake <1000 mg/day also an oral calcium citrate supplementation (500 mg/day or 1000 mg/day in patients with an estimated calcium intake above or below 500 mg/day, respectively) was prescribed.[23] The calcium intake, expressed as mg/day, was assessed using a validated questionnaire.[24] In particular, usual calcium intake coming from some selected calcium-rich foods (milk and dairy products) and waters was estimated by a 7-day food frequency questionnaire. The foods checked include milk, aged cheese, soft cheese, cottage cheese and yoghurt. The portion sizes were quantified by means of slices and cups. To standardize the slice weight, three cardboard samples of different sizes were used (about 100, 50 and 25 g). The number of standardized servings was assessed, each containing ~300 mg of calcium (a 250 ml cup of milk or yoghurt, a portion of about 100 g of cottage cheese, a 50 g slice of soft cheese and a 25 g slice of aged cheese).[24] An informed written consent was obtained from each patient.

Methods

In all patients, data regarding years since menopause and familiar history of vertebral and/or hip fragility fractures have been reported. In all patients the presence of clinical fragility fractures, weight, height and body mass index (BMI), and estimated duration of osteoporosis (i.e. months

since the first finding of densitometric osteoporosis and/or since the occurrence of a fragility fracture) have been recorded at the beginning and at the end of the study.

Serum and urinary samples were collected and stored at -20 °C until assayed. In all subjects, the following data were reported at the beginning and at the end of the DMab course: serum calcium, phosphorus, creatinine, alkaline phosphatase total activity (ALP), intact PTH, 25OHVitD. Total calcium was corrected for serum albumin according to the formula: (total calcium+(4.4 – albumin mg/dl) x 0.8) (reference interval 8.4–10.4 g/dl).[25] Urinary calcium and creatinine were measured in 24-h urine collections and calcium clearance was calculated in order to exclude the presence of benign hypocalciuric hypercalcaemia. Calcium, phosphorus, albumin, and creatinine in serum and urinary calcium and creatinine were measured by standard colorimetric techniques. Serum intact PTH was measured by electrochemiluminescence immunoassay (ECLIA) (reference interval = 15–65 pg/mL). Serum 25-hydroxyvitamin D concentration was measured by chemiluminescent immunoassay (reference interval: 30–100 ng/mL). Serum ALP was measured by standard colorimetric techniques (reference interval: 35–140 U/L, CV 12%). The ALP change was considered significant if it was below the negative least significant change (LSC), calculated by the formula 2.8×precision error (i.e.: -22%).[26] The difference of ALP levels between end of follow-up and baseline (Δ) was expressed as percentage of baseline values (ΔALP).

In all patients, at the beginning and at the end of the DMab course, BMD was measured by dual X-ray absorptiometry (Hologic Discovery, Software version 13.3:3, Bedford MA, USA), and expressed as Z-score, at lumbar (L1-L4) spine (LS, Z-LS, in vivo precision 1.0%), femoral neck (FN, Z-FN, in vivo precision 1.8%) and total hip (TH, Z-TH, in vivo precision 1.7%). The BMD change at LS, FN and TH was considered significant if it was above the positive LSC (LS 2.8%, FN 5.9% and TH 4.8%) and were also expressed as percentage of baseline values (ΔZ-LS, ΔZ-FN and ΔZ-TH respectively). At the same intervals, a conventional spinal radiograph in lateral and anteroposterior projection (T4–L4) was obtained in all subjects with standardized technique. Two trained physicians, who were blinded to BMD and biochemical data, independently reviewed the

radiographs, and they discussed questionable cases to agree on a diagnosis. Vertebral fractures were diagnosed on visual inspection using the semiquantitative visual assessment (SQ) previously described by Genant and colleagues.[27] and fractures assessed on lateral thoracolumbar spine radiographs were defined as of >20% in anterior, middle, or posterior vertebral height reduction: 13 vertebrae (from T4 to L4) were evaluated visually and classified as intact (SQ grade 0) or as having mild (20% to 25% compression), moderate (25% to 40% compression), or severe (>40% compression) deformity (SQ grades 1, 2, and 3, respectively). Subsequently, for each subject, the spinal deformity index (SDI) was calculated by summing the SQ grade for each vertebra (SDI = SQT4 + … + SQT12 + SQL1 + … +SQL4).[28] According to the working group of the IOF, patients were classified as inadequate responders in the presence of two or more incident fragility fractures and/or a decrease in BMD greater than the LSC.[29] A good response to DMAb was arbitrarily defined in the presence of an increase in BMD at any site greater than the LSC (without a decrease in BMD greater than LSC at any site) and in the absence of incident fragility fractures.

Statistical analysis

Statistical analysis was performed by SPSS version 21.0 statistical package (SPSS Inc, Chicago, IL). The results are expressed as mean±SD, unless differently specified. Categorical variables were compared by $\chi 2$ test or Fisher Exact test, as appropriate. Comparison of continuous variables among the different groups was performed using Student t-test or Mann-Whitney U-test, as appropriate. Bivariate associations between the biochemical parameters and the SDI were tested by either Pearson product moment correlation or Spearman correlation, as appropriate.

In the whole group of subjects, the logistic regression analysis assessed the association between the presence of a good response to DMab and the presence of PHPT, after adjusting for other possible confounding factors (such as age, BMI, familial history of hip fractures, personal history of clinical fragility fractures, daily calcium intake and previous use of bisphosphonates). P-values of less than 0.05 were considered significant.

Results

The comparison of the biochemical and clinical characteristics between PHPT Group and PO Group at baseline and at the end of the 24 months DMab therapy is reported in table 1.

At baseline PHPT patients and PO subjects were comparable in age, BMI, familiar history of fragility fracture, previous fragility fractures, SDI, mean estimated duration of osteoporosis, serum creatinine, ALP and 25OHVitD levels, Z-LS, Z-FN and Z-TH. At variance, the prevalence of subjects who had previously assumed a bisphosphonate therapy (alendronate or risedronate), serum and urinary calcium and serum PTH levels were higher, while daily calcium intake (sum of dietary calcium intake plus calcium from supplements) and serum phosphorous levels were lower in PHPT patients than in PO ones. The mean duration of previous bisphosphonate therapy and the prevalence of inadequate responders to bisphosphonate therapy was not different between PHPT patients and PO subjects (data not shown).

At the end of the 24 months DMab course, the differences in serum and urinary calcium, serum phosphorous and PTH levels between PHPT patients and PO subjects were maintained. In PHPT patients the daily calcium intake significantly increased and became comparable to that in PO subjects. Furthermore, the 25OHVitD levels significantly increased while ALP levels significantly decreased in both PHPT patients and PO subjects. As compared to PO subjects, PHPT patients showed a higher negative ΔALP, that was below the negative LSC in PHPT patients but not in PO subjects.

The prevalence of an inadequate BMD (at any site) response to DMab and the incident fragility fractures were similar in PHPT patients and PO subjects. One patients in PHPT Group and 3 subjects in PO Group (table 2) were classified as inadequate responders (on the basis of the definition reported in the Material and Methods section).[29] Two inadequate responders to DMab had been taking bisphosphonates for at least 6 years before but not during the two years before the study enrolment (PHPT patient and PO subject #3), and all three inadequate responders PO patients had a small negative ΔALP (<22% and below the LSC) during DMAb therapy (table 2).

198 At variance with PO subjects, in PHPT patients the mean Z-LS, Z-FN and Z-TH increased
199 significantly between the baseline and the end of follow-up. Furthermore, as compared to PO
200 subjects, PHPT patients showed higher positive ΔZ-LS, ΔZ-FN and ΔZ-TH, even though the
201 difference in ΔZ-LS did not reach the statistical significance (p=0.06). The number of patients
202 showing a good response to DMab (as defined in the Material and Methods section), was
203 significantly higher in PHPT Group than in the PO Group and the logistic regression analysis
204 showed that the presence of a good response to DMab was independently associated with PHPT and
205 with the personal history of clinical fragility fractures regardless for age, BMI, familial history of
206 hip fracture, daily calcium intake and previous bisphosphonate therapy (table 3).
207 Finally, no correlation was found between the baseline ALP levels or ΔALP and the BMD
208 changes during the 24 months DMab therapy.

Discussion

To the best of our knowledge this is the first study investigating the effect of DMab therapy in patients with PHPT. The present data suggest that in female older adults with PHPT a course of 24 months DMAb therapy has an efficacy in increasing BMD higher than that in matched female older adults with PO and independent of the baseline fracture risk profile.

The medical therapy in PHPT patients is normally aimed to control calcium levels and to reduce the fracture risk, in patients who cannot undergo surgery or who refuse it.[15] However, in the "real life", while the majority of relatively younger subjects accept surgery as the only definitive therapy for PHPT, among the older adults the refusal of the neck exploration is more common, and therefore, alendronate has been studied as a possible alternative therapy for reducing the fracture risk in these patients. [30-35]. Overall, in PHPT patients, alendronate has been suggested to be effective in protecting from bone loss in particular at lumbar spine in both men and women and even in patients with normocalcaemic primary hyperparathyroidism.[30-35] However, the fracture risk reduction after a long-term use of alendronate in PHPT is still unknown.[17] In addition, alendronate is contraindicated in the presence of a reduced renal function, as often is the case in older adults. Therefore, alternative bone-protective agents, such as DMab, may be considered for the treatment of the PHPT induced osteoporosis in older adults.

Although, the use of DMab has been suggested as an interesting approach in PHPT because it inhibits the RANK/RANKL pathway, that is important in the catabolic actions of PTH, [3, 7, 14-19] no data were available so far in PHPT patients. The results of the present study, are, therefore, of importance since they suggest for the first time that DMab may be effectively used in female older adults with PHPT. The finding that the DMab effect in terms of ΔALP change is more evident in PHPT patients than in PO ones suggests that the DMab related reduction of bone turnover may be higher in PHPT than in PO. In PHPT, the increased bone turnover and the consequent bone damage is thought to be mainly due to the RANK/RANKL pathway.[3, 7, 14-19] On the basis of these

considerations, the present finding of a dramatic positive effect of DMab on BMD in PHPT patients could be considered somewhat expected.

The present study has some limitations. Firstly, its retrospective and not randomized design may have introduced a unknown bias. However, it must be considered that performing a prospective randomized trial with DMab in older adults at high risk of fragility fracture may raise ethical concerns. Secondly, the small sample size may have reduced the statistical power, therefore concealing a possible different effect of DMab in terms of inadequate response and/or fracture risk reduction between PHPT patients and PO subjects. Indeed, since only 1 patient in the PHPT Group (4%) but 3 patients in PO Group (12%) showed an inadequate response to DMab, and considering the known anti-fracture efficacy of DMAb in PO,[16] it is possible to hypothesize that increasing the sample size may consent reaching a statistical significance. Similarly, the small sample size did not consent to individuate the factors predictive of an inadequate response to DMab. However, two out of the 4 patients with an inadequate response to DMab had been previously treated with alendronate for 6 and 10 years, and in all inadequate responder subjects the baseline ALP levels were in the low half of the normal range (table 2). Thus, larger studies should be performed for assessing the possibility that a long-term previous bisphosphonate therapy and/or a low bone turnover may affect the efficacy of DMab in both PHPT and PO. Finally, we have no information about the BMD changes after DMab therapy at wrist, since DXA at this site is not routinely performed in PO patients.

The strengths of the present study are related to its "real life" setting and, therefore, the present results may be more easily transferable in the daily clinical practice. Moreover, the precise matching of PHPT patients and PO subjects consented us to importantly reduce the risk of biases related to the individual fracture risk profile at baseline. Finally, thanks to this design, the finding that the number of subjects experiencing an incident fragility fracture was equal between PHPT patients and PO subjects strongly suggests that the DMab anti-fracture effect, which is clearly demonstrated in PO,[16] is present even in PHPT.

In conclusion, the present study shows for the first time that in female older adults with PHPT DMAb is extremely efficacious in increasing BMD. Further larger studies might confirm the efficacy of the DMab therapy in PHPT patients even in term of fracture risk reduction. Finally, further studies are needed for evaluating the protective effect of DMab on PHPT-related osteoporosis in comparison with other osteoporosis therapies, which have already been tested in PHPT patients.

Acknowledgments

Authors' roles:

Cristina Eller-Vainicher: study design and conduct, data analysis and interpretation, drafting manuscript, approving final version of manuscript.

Serena Palmieri: study design, data analysis and interpretation, approving final version of manuscript.

Elisa Cairoli: study design and conduct, data analysis and interpretation, approving final version of manuscript.

Giovanni Goggi: study conduct, data analysis, drafting manuscript, approving final version of manuscript.

Alfredo Scillitani: study design, data analysis and interpretation, revision of the manuscript, approving final version of manuscript.

Maura Arosio: study design, data analysis and interpretation, revision of the manuscript, approving final version of manuscript.

Alberto Falchetti: study design, data analysis and interpretation, revision of the manuscript, approving final version of manuscript

Iacopo Chiodini: Study design, data analysis and interpretation, drafting manuscript, approving final version of manuscript.

Iacopo Chiodini takes responsibility for the integrity of the data analysis.

Conflict of Interest:

The authors state that they have no conflicts of interest

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Figure 1

Title: Bone mineral density changes before and after 24 months denosumab therapy in patients with primary hyperparathyroidism (PHPT) and subjects with primary osteoporosis (PO)

Legend: ΔZ-LS, ΔZ-FN and ΔZ-TH: difference in bone mineral density between end of follow-up and baseline (as percentage of baseline value) at spine (L1-L4), femoral neck and total hip, respectively.

385 **Table 1.** Clinical and biochemical characteristics of PHPT patients and of PO subjects at baseline
386 and the end of the follow-up.
387
388

	PHPT patients n=25		PO patients n=25	
	Baseline	**24 months**	**Baseline**	**24 months**
Age (years)	78.6±5.5 (67–85)	80.7±5.6 (69–87)	78.8±5.2 (69–86)	80.7±5.2 (71–88)
BMI (Kg/m^2)	24.3±2.7 (19.8–30.0)	24.3±2.6 (19.9–30.0)	24.3±2.5 (19.1–29.7)	24.7±2.7 (19.5–31.2)
Familial history of hip fx	8 (32)	-	7 (28)	-
Prevalent fragility fx[1]	24 (96)	-	25 (100)	-
Spinal Deformity Index	3.7±2.1 (0–9)	3.8±2.2 (0–9)	4.0±2.1 (1–9)	4.2±2.4 (1–11)
Previous bisphosphonates therapy[2]	13° (52.0)	-	21 (84.0)	-
Osteoporosis duration[3] (months)	72.3±42.3 (2–120)	-	68.2±45.1 (0–120)	-
Daily calcium intake (mg/day)	752±264° (300–1200)	972±79[#] (800–1200)	987±220 (671–1600)	1040±224 (700–1600)
Calcium (mg/dL)	10.8±0.9^ (10–13.2)	10.5±0.7* (9.7–12.0)	9.8±0.3 (9.1–10.4)	9.6±0.5 (8.6–10.6)
PTH (pg/mL)	117.5±42.3^ (74.1–239.2)	138.7±48.9* (63.4–258.4)	42.9±11.1 (29.0–66.1)	44.7±14.4 (17.7–67.0)
Phosphorous (mg/dL)	2.7±0.3^ (2.1–3.2)	2.6±0.4* (1.7–3.7)	3.5±0.4 (2.8–4.1)	3.4±0.4 (2.7–4.4)
Creatinine (mg/dL)	0.8±0.2 (0.5–1.2)	0.8±0.2 (0.5–1.3)	0.8±0.2 (0.6–1.3)	0.8±0.2 (0.5–1.3)
ALP (U/L)	76.8±15.4 (45.0–104.0)	52.9±12.4[#] (35.7–85.2)	69.1±21.1 (38.7–125.2)	54.3±16.9[¶] (32–94.0)
ΔALP	-	-30.6±11.3[§] (0.0–47.2)	-	-21.4±13.1 (0.0–47.1)
Urinary calcium (mg/kg/d)	3.7±1.4^ (1.6–6.5)	3.8±1.3* (2.3–7.7)	2.2±1.2 (0.5–4.7)	2.2±0.7 (0.5–3.5)
25-Hydroxyvitamin D (ng/ml)	33.5±9.4 (20.1–56.1)	43.7±7.4[#] (34.0–67.1)	38.0±9.1 (20.0–57.0)	45.7±6.8[¶] (33.1–58.9)
Z-LS (Z-score)	-0.73±0.91 (-2.5–1.0)	-0.02±1.00† (-1.4–2.7)	-0.72±0.93 (-2.7–1.0)	-0.40±1.00 (-2.9–2.4)
Z-FN (Z-score)	-0.89±0.64 (-2.0–0.4)	-0.54±0.58† (-1.9–0.7)	-0.88±0.61 (-2.1–0.30)	-0.53±0.78 (-2.9–2.4)
Z-TH (Z-score)	-0.58±0.51 (-1.7–0.2)	-0.20±0.53†[§] (-1.1–0.9)	-0.84±0.75 (-2.0–1.0)	-0.62±0.64 (-2.0–0.6)
ΔZ-LS	-	6.2±4.5¬ (-1.1–15.8)	-	3.5±5.5 (-13.4–15.9)

ΔZ-FN	-	5.6±4.8[§] (-4.3–17.6)	-	2.9±4.8 (-4.7–17.4)
ΔZ-TH	-	4.8±4.4[§] (-1.4–17.6)	-	1.2±4.1 (-13.2–5.9)
Incident fragility fractures[1]	-	2 (8.0)		2 (8.0)
Inadequate responders to DMab	-	1 (4.0)	-	3 (12.0)
Inadequate BMD response to DMab	-	0 (0.0)	-	2 (8.0)
Good response to DMab	-	23[§] (92)	-	13 (52)

Data are mean±SD or absolute number with range or percentage in parentheses, respectively.

°p<0.001 and ^p<0.0001 vs PO patients at baseline

¯p=0.06, [§]p<0.05 and *p<0.0001 vs PO patients at the end of the follow-up (24 months)

†p<0.05 and [#]p<0.0001 vs PHPT patients at baseline

[¶]p<0.005 vs PO patients at baseline

PHPT: primary hyperparathyroidism. PO: primary osteoporosis. BMI: Body mass index; Fx: fracture. ALP: alkaline phosphatase total activity; ΔALP: difference in ALP between end of follow-up and baseline (as percentage of baseline value). Z-LS, Z-FN and Z-TH: bone mineral density measured by Dual X-ray Energy Absorptiometry and expressed as Z-score at spine (L1-L4), femoral neck and total hip, respectively. ΔZ-LS, ΔZ-FN and ΔZ-TH: difference in bone mineral density between end of follow-up and baseline (as percentage of baseline value) at spine (L1-L4), femoral neck and total hip, respectively.

Inadequate responders: presence of at least one of the following criteria: (i) two or more incident fragility fractures and (ii) a decrease in BMD greater than the least significant change (LSC, reference #26). Inadequate BMD response to DMAb: a decrease in BMD at any site greater than the LSC. Good response to DMAb: increase in BMD at any site greater than the LSC (without a decrease in BMD greater than LSC at any site) and the absence of incident fragility fractures.

[1]Vertebral or hip fragility fracture.

[2]All previously treated patients assumed alendronate or risedronate until 2 years before the study entry and for no more than 10 years.

[3]The duration of the osteoporosis has been estimated since the first diagnosis of reduced BMD and/or since the occurrence of the first fragility fracture

Table 2. Clinical and biochemical characteristics of PHPT patients and of PO subjects with an inadequate response to DMab

	PHPT Patient	**PO Patient #1**	**PO Patient #2**	**PO Patient #3**
Age at baseline (years)	75	78	84	75
BMI (Kg/m^2)	23.3	24.9	23.2	24.2
Familial history of hip fx	no	no	no	no
Comorbidities	DL	DL, AH	DL, AH	AH
Prevalent clinical fragility fx	hip	vertebral	no	vertebral
Prevalent morphometric fx	yes	yes	yes	yes
Spinal Deformity Index at baseline	5	3	1	8
Previous bisph therapy (months)	120	no	9	72
Previous inadequate response to bisph	yes	-	yes	no
Calcium intake during DMab (mg/day)	900	900	1200	1100
Calcium at baseline (mg/dL)	10.4	10.1	9.7	9.8
PTH at baseline (pg/mL)	128	47	29	39
Phosphorous at baseline (mg/dL)	2.6	3.6	3.5	3.5
Creatinine at baseline (mg/dL)	0.6	0.8	0.7	0.8
ALP at baseline (U/L)	62.4	52	50.9	55.1
ΔALP	-38	-12	-22	-18
Urinary calcium (mg/kg/d)	2.2	1.3	2.3	0.5
25-Hydroxyvitamin D (ng/ml)	26.5	37	36.1	44.3
Z-LS at baseline (Z-score)	-1.7	0.0	-0.8	-2.7
Z-FN at baseline (Z-score)	-2.0	0.3	-0.8	-2.1
Z-TH at baseline (Z-score)	-1.4	1.0	-0.9	-1.8
ΔZ-LS	4.3	-13.4	1.4	-5.0
ΔZ-FN	1.8	0.0	-4.7	-1.8
ΔZ-TH	1.4	-13.2	-4.2	0.0
Incident fragility fracture (type)	vertebral	no	vertebral	vertebral, wrist
Inadequate BMD response to DMab	no	yes	no	yes

PHPT: primary hyperparathyroidism. PO: primary osteoporosis. BMI: Body mass index; Fx: fracture. Bisph: bisphosphonates. DL: dyslipidemia. AH: arterial hypertension. ALP: alkaline phosphatase total activity; ΔALP: difference in ALP between end of follow-up and baseline (as percentage of baseline value). Z-LS, Z-FN and Z-TH: bone mineral density measured by Dual X-ray Energy Absorptiometry and expressed as Z-score at spine (L1-L4), femoral neck and total hip, respectively. ΔZ-LS, ΔZ-FN and ΔZ-TH: difference in bone mineral density between end of follow-up and baseline (as percentage of baseline value) at spine (L1-L4), femoral neck and total hip, respectively.

Inadequate responders: presence of at least one of the following criteria: (i) two or more incident fragility fractures and (ii) a decrease in BMD greater than the least significant change (reference #26). Inadequate BMD response to DMAb: a decrease in BMD at any site greater than the LSC.

Table 3: Factors independently associated with a good BMD response to DMab in the whole group of subjects assessed by Logistic Regression analysis

	OR	95%CI	P
Age (1 year decrease)	1.18	0.98 – 1.41	0.830
BMI (1 Kg/m^2 increase)	1.13	0.81 – 1.59	0.471
Familiar history of hip fracture (no)	3.45	0.51 – 23.26	0.205
Personal history of clinical fragility fracture (no)	1.04	1.39 – 76.9	0.023
Daily calcium intake (1 mg increase)	1.00	0.99 – 1.01	0.266
Previous bisphosphonate therapy (yes)	1.90	0.27 – 13.5	0.520
Primary hyperparathyroidism (yes)	13.44	1.43 – 125.9	0.023

BMI: Body mass index.

Good BMD response to DMAb: increase in BMD at any site greater than the LSC (without a decrease in BMD greater than LSC at any site) and absence of incident fragility fractures

Title: Protective effect of denosumab on bone in female older adults with primary hyperparathyroidism.

Authors:[1]Cristina Eller-Vainicher*, [2]Serena Palmieri*, [1,2]Elisa Cairoli, [1]Giovanni Goggi, [3]Alfredo Scillitani, [1,2] Maura Arosio, [4]Alberto Falchetti, [1]Iacopo Chiodini.

Affiliations: [1]Unit of Endocrinology, Fondazione IRCCS Cà Granda-Ospedale Maggiore Policlinico. [2]Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy. [3]Unit of Endocrinology, 'Casa Sollievo della Sofferenza', IRCCS, San Giovanni Rotondo, Foggia, Italy. [4]Villa Alba Hospital, Villa Maria Group, Bologna, Italy; EndOsMet, Villa Donatello, Private Hospital, Florence, Italy.

*These authors equally contributed to the study

Correspondence: Iacopo Chiodini, Unit of Endocrinology and Metabolic Diseases, Padiglione Granelli, Via Francesco Sforza 35, 20122, Milan, Italy; Email: iacopo.chiodini@policlinico.mi.it

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We certify that this work is novel

This study shows for the first time that denosumab is effective in preserving bone health in female older adults with primary hyperparathyroidism.

Abstract

Background/Objectives: Denosumab (DMab) is used in primary osteoporosis (PO), while its effect in primary hyperparathyroidism (PHPT) related osteoporosis is unknown.

Design: retrospective, longitudinal study

Setting: out-patient Clinic for Osteoporosis

Participants: 25 PHPT female older adults (78.6±5.5 years) and 25 PO female subjects (age 78.8±5.2 years). The patients with PO and PHPT were matched for age, body mass index, familiar history of hip fracture, femoral bone mineral density (BMD) and personal history of fragility fractures.

Intervention: 24 months DMab therapy.

Measurements: in all subjects at baseline and after 24 months we assessed the calcium-phosphorous metabolism parameters, BMD at lumbar spine (LS), femoral neck (FN), total hip (TH) by dual by X-ray absorptiometry, the morphometric vertebral fractures by radiograph. The BMD and alkaline phosphatase total activity (ALP) changes were considered significant if higher than the least significant change (LS 2.8%, FN 5.9%, TH 4.8%, ALP -22%) and were expressed even as percentage difference between end of follow-up and baseline (Δ).

Results: After 24 months PHPT patients showed higher ΔALP (-30.6±11.3), ΔFN (5.6±4.8) and ΔTH (4.8±4.4) than PO subjects (-21.4±13.1, 2.9±4.8, 1.2±4.1, respectively, $p<0.05$ for all comparisons). A significant BMD increase was more frequent among PHPT patients (92%) than among PO ones (52%, $p<0.05$) and it was 13.4 fold more likely in PHPT patients than in PO ones ($p=0.023$), regardless of possible confounders. Two subjects among both PHPT and PO patients had an incident fracture.

Conclusions: The DMab therapy is effective in female older adults affected with PHPT related osteoporosis.

Key words: denosumab, primary hyperparathyroidism, fragility fractures, bone mineral density

Introduction

Primary hyperparathyroidism (PHPT) is characterized by hypercalcaemia and elevated or inappropriately normal levels of parathyroid hormone (PTH), due to an excessive PTH secretion, and it is caused by a solitary parathyroid adenoma (80% of cases) or multiple gland hyperplasia (10-15% of cases).[1] Although, nowadays, PHPT is frequently discovered when asymptomatic, this disease may be frequently associated with bone loss, kidney stones and reduced renal function, besides other less typical manifestations, such as gastrointestinal symptoms, muscle weakness and psychological disturbances.[2-7] The PHPT overall prevalence is estimated to be about 0.9%, and it increases with the advancing age, reaching a prevalence of 2-3% in postmenopausal women.[8, 9] Parathyroidectomy is the only definitive treatment of primary hyperparathyroidism and it leads to a reduction of the risk of fractures and of the overall mortality.[10-12]. However, in the "real life", many PHPT patients, in particular the older adults, cannot undergo surgery or do not accept it. In these patients, hydration for reducing the risk of renal stones and drugs for reducing the fracture risk are recommended.[13] However, as far as the PHPT-related bone consequences is concerned, to date, scarce and somewhat conflicting data are available on the effect of bone active drugs (mainly bisphosphonates) in these patients.[2, 13-15]

In the last years, Denosumab (DMab), a monoclonal antibody that binds the receptor activator of nuclear factor kappa-B (RANK) ligand (RANKL), has been demonstrated to inhibit osteoclasts differentiation and activity and to reduce the risk of fracture in patients with primary osteoporosis (PO). [16-18] In PHPT patients, bone mineral density (BMD) is decreased and the risk of fractures increased, due to the PTH-mediated activation of the RANK/RANKL pathway.[3-7, 14, 19] Therefore, DMab could be an effective therapy for reducing bone damage in PHPT, but to date, no studies have been performed about its possible use in PHPT patients with osteoporosis.

Therefore, the aim of the present study was to assess in a "real life" setting the efficacy of DMab on BMD and incident fractures in PHPT older adults, who could not undergo or refused the surgical approach.

Patients and Methods

Patients

In this observational retrospective study, we evaluated data of all (n=65) older adult (≥65 years) female Caucasian patients with sporadic PHPT, referred to our out-patient Clinic for Metabolic Bone Diseases since June 2013 to June 2015. The PHTP was diagnosed by the presence of hypercalcaemia and elevated or inappropriately normal PTH levels, after the exclusion of familial hypocalciuric hypercalcemia by evaluating calcium clearance/creatinine clearance ratio in the presence of normal renal function (creatinine clearance by Cockcroft–Gault equation >60 ml/min).[20] The PHPT patients were included in the study if they fulfilled the following criteria: i) the refusal or impossibility (for elevated anesthetic risk) of parathyroidectomy; ii) a BMD T-score (i.e: number of standard deviations above or below the mean for a healthy 30-year-old adult of the same sex and ethnicity) below -4.0 and/or prevalent vertebral and/or hip fragility fractures, or a BMD T-score below -3.0 together with a familiar history of vertebral or hip fragility fracture (as recommended by the Italian National Health System); iii) a 24 months DMab therapy (60 mg subcutaneously every 6 months), with a complete (100%) adherence. Patients were not included in the study in the presence of: i) other diseases or conditions, beside PHPT, known to affect bone metabolism (i.e. menopause before 45 years, thyrotoxicosis, gastrointestinal disorders, chronic renal failure, chronic hepatic disease, depression, alcoholism, obesity, eating disorders, rheumatological or haematological diseases, hypercortisolism, diabetes); ii) administration of drugs influencing bone metabolism or PTH and calcium levels (i.e. alendronate, risedronate, ibandronate, cinacalcet, glucocorticoids, teriparatide, thiazide diuretics, hormonal adiuvant therapy, lithium) during the previous 2 years and/or for more than 10 years and/or present or past therapy with neridronate or zoledronate; iii) multiple endocrine neoplasia type 1 and type 2 syndromes-related PHPT, that may have a different impact on the skeletal health;[21] iv) alcohol and/or tobacco abuse. Eventually 25 PHPT female patients have been included in the study (PHPT Group).

During the same period of time, 25 female patients with primary osteoporosis (PO Group) were enrolled as control subjects, if they fulfilled the same inclusion and exclusion criteria used for PHPT patients. Each control subject was chosen by matching with a PHPT patient considering the following variables: body mass index (BMI), familiar history of hip fracture, femoral neck BMD and personal history of vertebral and/or hip fragility fractures. Among PHPT patients and PO subjects 13 and 15 were hypertensive, respectively and 5 and 7 were taking statins, respectively.

As per our protocols, in order to normalize vitamin D (25OHVitD) levels, all patients with 25OHVitD concentration below 30 ng/mL received cholecalciferol supplementation. An oral bolus of 100000 IU or 300000 IU of cholecalciferol was administered in patients with 25OHVitD levels between 10 and 30 ng/mL and below 10 ng/mL, respectively. Subsequently, in all non-obese patients with osteoporosis a cholecalciferol supplementation of 50000 IU monthly and 400IU daily was recommended [22] and in those with a calcium intake <1000 mg/day also an oral calcium citrate supplementation (500 mg/day or 1000 mg/day in patients with an estimated calcium intake above or below 500 mg/day, respectively) was prescribed.[23] The calcium intake, expressed as mg/day, was assessed using a validated questionnaire.[24] In particular, usual calcium intake coming from some selected calcium-rich foods (milk and dairy products) and waters was estimated by a 7-day food frequency questionnaire. The foods checked include milk, aged cheese, soft cheese, cottage cheese and yoghurt. The portion sizes were quantified by means of slices and cups. To standardize the slice weight, three cardboard samples of different sizes were used (about 100, 50 and 25 g). The number of standardized servings was assessed, each containing ~300 mg of calcium (a 250 ml cup of milk or yoghurt, a portion of about 100 g of cottage cheese, a 50 g slice of soft cheese and a 25 g slice of aged cheese).[24] An informed written consent was obtained from each patient.

Methods

In all patients, data regarding years since menopause and familiar history of vertebral and/or hip fragility fractures have been reported. In all patients the presence of clinical fragility fractures, weight, height and body mass index (BMI), and estimated duration of osteoporosis (i.e. months

since the first finding of densitometric osteoporosis and/or since the occurrence of a fragility fracture) have been recorded at the beginning and at the end of the study.

Serum and urinary samples were collected and stored at -20 °C until assayed. In all subjects, the following data were reported at the beginning and at the end of the DMab course: serum calcium, phosphorus, creatinine, alkaline phosphatase total activity (ALP), intact PTH, 25OHVitD. Total calcium was corrected for serum albumin according to the formula: (total calcium+(4.4 – albumin mg/dl) x 0.8) (reference interval 8.4–10.4 g/dl).[25] Urinary calcium and creatinine were measured in 24-h urine collections and calcium clearance was calculated in order to exclude the presence of benign hypocalciuric hypercalcaemia. Calcium, phosphorus, albumin, and creatinine in serum and urinary calcium and creatinine were measured by standard colorimetric techniques. Serum intact PTH was measured by electrochemiluminescence immunoassay (ECLIA) (reference interval = 15–65 pg/mL). Serum 25-hydroxyvitamin D concentration was measured by chemiluminescent immunoassay (reference interval: 30–100 ng/mL). Serum ALP was measured by standard colorimetric techniques (reference interval: 35–140 U/L, CV 12%). The ALP change was considered significant if it was below the negative least significant change (LSC), calculated by the formula 2.8×precision error (i.e.: -22%).[26] The difference of ALP levels between end of follow-up and baseline (Δ) was expressed as percentage of baseline values (ΔALP).

In all patients, at the beginning and at the end of the DMab course, BMD was measured by dual X-ray absorptiometry (Hologic Discovery, Software version 13.3:3, Bedford MA, USA), and expressed as Z-score, at lumbar (L1-L4) spine (LS, Z-LS, in vivo precision 1.0%), femoral neck (FN, Z-FN, in vivo precision 1.8%) and total hip (TH, Z-TH, in vivo precision 1.7%). The BMD change at LS, FN and TH was considered significant if it was above the positive LSC (LS 2.8%, FN 5.9% and TH 4.8%) and were also expressed as percentage of baseline values (ΔZ-LS, ΔZ-FN and ΔZ-TH respectively). At the same intervals, a conventional spinal radiograph in lateral and anteroposterior projection (T4–L4) was obtained in all subjects with standardized technique. Two trained physicians, who were blinded to BMD and biochemical data, independently reviewed the

radiographs, and they discussed questionable cases to agree on a diagnosis. Vertebral fractures were diagnosed on visual inspection using the semiquantitative visual assessment (SQ) previously described by Genant and colleagues.[27] and fractures assessed on lateral thoracolumbar spine radiographs were defined as of >20% in anterior, middle, or posterior vertebral height reduction: 13 vertebrae (from T4 to L4) were evaluated visually and classified as intact (SQ grade 0) or as having mild (20% to 25% compression), moderate (25% to 40% compression), or severe (>40% compression) deformity (SQ grades 1, 2, and 3, respectively). Subsequently, for each subject, the spinal deformity index (SDI) was calculated by summing the SQ grade for each vertebra (SDI = SQT4 + … + SQT12 + SQL1 + … +SQL4).[28] According to the working group of the IOF, patients were classified as inadequate responders in the presence of two or more incident fragility fractures and/or a decrease in BMD greater than the LSC.[29] A good response to DMAb was arbitrarily defined in the presence of an increase in BMD at any site greater than the LSC (without a decrease in BMD greater than LSC at any site) and in the absence of incident fragility fractures.

Statistical analysis

Statistical analysis was performed by SPSS version 21.0 statistical package (SPSS Inc, Chicago, IL). The results are expressed as mean±SD, unless differently specified. Categorical variables were compared by $\chi 2$ test or Fisher Exact test, as appropriate. Comparison of continuous variables among the different groups was performed using Student t-test or Mann-Whitney U-test, as appropriate. Bivariate associations between the biochemical parameters and the SDI were tested by either Pearson product moment correlation or Spearman correlation, as appropriate.

In the whole group of subjects, the logistic regression analysis assessed the association between the presence of a good response to DMab and the presence of PHPT, after adjusting for other possible confounding factors (such as age, BMI, familial history of hip fractures, personal history of clinical fragility fractures, daily calcium intake and previous use of bisphosphonates). P-values of less than 0.05 were considered significant.

Results

The comparison of the biochemical and clinical characteristics between PHPT Group and PO Group at baseline and at the end of the 24 months DMab therapy is reported in table 1.

At baseline PHPT patients and PO subjects were comparable in age, BMI, familiar history of fragility fracture, previous fragility fractures, SDI, mean estimated duration of osteoporosis, serum creatinine, ALP and 25OHVitD levels, Z-LS, Z-FN and Z-TH. At variance, the prevalence of subjects who had previously assumed a bisphosphonate therapy (alendronate or risedronate), serum and urinary calcium and serum PTH levels were higher, while daily calcium intake (sum of dietary calcium intake plus calcium from supplements) and serum phosphorous levels were lower in PHPT patients than in PO ones. The mean duration of previous bisphosphonate therapy and the prevalence of inadequate responders to bisphosphonate therapy was not different between PHPT patients and PO subjects (data not shown).

At the end of the 24 months DMab course, the differences in serum and urinary calcium, serum phosphorous and PTH levels between PHPT patients and PO subjects were maintained. In PHPT patients the daily calcium intake significantly increased and became comparable to that in PO subjects. Furthermore, the 25OHVitD levels significantly increased while ALP levels significantly decreased in both PHPT patients and PO subjects. As compared to PO subjects, PHPT patients showed a higher negative ΔALP, that was below the negative LSC in PHPT patients but not in PO subjects.

The prevalence of an inadequate BMD (at any site) response to DMab and the incident fragility fractures were similar in PHPT patients and PO subjects. One patients in PHPT Group and 3 subjects in PO Group (table 2) were classified as inadequate responders (on the basis of the definition reported in the Material and Methods section).[29] Two inadequate responders to DMab had been taking bisphosphonates for at least 6 years before but not during the two years before the study enrolment (PHPT patient and PO subject #3), and all three inadequate responders PO patients had a small negative ΔALP (<22% and below the LSC) during DMAb therapy (table 2).

198 At variance with PO subjects, in PHPT patients the mean Z-LS, Z-FN and Z-TH increased
199 significantly between the baseline and the end of follow-up. Furthermore, as compared to PO
200 subjects, PHPT patients showed higher positive ΔZ-LS, ΔZ-FN and ΔZ-TH, even though the
201 difference in ΔZ-LS did not reach the statistical significance (p=0.06). The number of patients
202 showing a good response to DMab (as defined in the Material and Methods section), was
203 significantly higher in PHPT Group than in the PO Group and the logistic regression analysis
204 showed that the presence of a good response to DMab was independently associated with PHPT and
205 with the personal history of clinical fragility fractures regardless for age, BMI, familial history of
206 hip fracture, daily calcium intake and previous bisphosphonate therapy (table 3).
207 Finally, no correlation was found between the baseline ALP levels or ΔALP and the BMD
208 changes during the 24 months DMab therapy.

Discussion

To the best of our knowledge this is the first study investigating the effect of DMab therapy in patients with PHPT. The present data suggest that in female older adults with PHPT a course of 24 months DMAb therapy has an efficacy in increasing BMD higher than that in matched female older adults with PO and independent of the baseline fracture risk profile.

The medical therapy in PHPT patients is normally aimed to control calcium levels and to reduce the fracture risk, in patients who cannot undergo surgery or who refuse it.[15] However, in the "real life", while the majority of relatively younger subjects accept surgery as the only definitive therapy for PHPT, among the older adults the refusal of the neck exploration is more common, and therefore, alendronate has been studied as a possible alternative therapy for reducing the fracture risk in these patients. [30-35]. Overall, in PHPT patients, alendronate has been suggested to be effective in protecting from bone loss in particular at lumbar spine in both men and women and even in patients with normocalcaemic primary hyperparathyroidism.[30-35] However, the fracture risk reduction after a long-term use of alendronate in PHPT is still unknown.[17] In addition, alendronate is contraindicated in the presence of a reduced renal function, as often is the case in older adults. Therefore, alternative bone-protective agents, such as DMab, may be considered for the treatment of the PHPT induced osteoporosis in older adults.

Although, the use of DMab has been suggested as an interesting approach in PHPT because it inhibits the RANK/RANKL pathway, that is important in the catabolic actions of PTH, [3, 7, 14-19] no data were available so far in PHPT patients. The results of the present study, are, therefore, of importance since they suggest for the first time that DMab may be effectively used in female older adults with PHPT. The finding that the DMab effect in terms of ΔALP change is more evident in PHPT patients than in PO ones suggests that the DMab related reduction of bone turnover may be higher in PHPT than in PO. In PHPT, the increased bone turnover and the consequent bone damage is thought to be mainly due to the RANK/RANKL pathway.[3, 7, 14-19] On the basis of these

considerations, the present finding of a dramatic positive effect of DMab on BMD in PHPT patients could be considered somewhat expected.

The present study has some limitations. Firstly, its retrospective and not randomized design may have introduced a unknown bias. However, it must be considered that performing a prospective randomized trial with DMab in older adults at high risk of fragility fracture may raise ethical concerns. Secondly, the small sample size may have reduced the statistical power, therefore concealing a possible different effect of DMab in terms of inadequate response and/or fracture risk reduction between PHPT patients and PO subjects. Indeed, since only 1 patient in the PHPT Group (4%) but 3 patients in PO Group (12%) showed an inadequate response to DMab, and considering the known anti-fracture efficacy of DMAb in PO,[16] it is possible to hypothesize that increasing the sample size may consent reaching a statistical significance. Similarly, the small sample size did not consent to individuate the factors predictive of an inadequate response to DMab. However, two out of the 4 patients with an inadequate response to DMab had been previously treated with alendronate for 6 and 10 years, and in all inadequate responder subjects the baseline ALP levels were in the low half of the normal range (table 2). Thus, larger studies should be performed for assessing the possibility that a long-term previous bisphosphonate therapy and/or a low bone turnover may affect the efficacy of DMab in both PHPT and PO. Finally, we have no information about the BMD changes after DMab therapy at wrist, since DXA at this site is not routinely performed in PO patients.

The strengths of the present study are related to its "real life" setting and, therefore, the present results may be more easily transferable in the daily clinical practice. Moreover, the precise matching of PHPT patients and PO subjects consented us to importantly reduce the risk of biases related to the individual fracture risk profile at baseline. Finally, thanks to this design, the finding that the number of subjects experiencing an incident fragility fracture was equal between PHPT patients and PO subjects strongly suggests that the DMab anti-fracture effect, which is clearly demonstrated in PO,[16] is present even in PHPT.

In conclusion, the present study shows for the first time that in female older adults with PHPT DMAb is extremely efficacious in increasing BMD. Further larger studies might confirm the efficacy of the DMab therapy in PHPT patients even in term of fracture risk reduction. Finally, further studies are needed for evaluating the protective effect of DMab on PHPT-related osteoporosis in comparison with other osteoporosis therapies, which have already been tested in PHPT patients.

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Authors' roles:

Cristina Eller-Vainicher: study design and conduct, data analysis and interpretation, drafting manuscript, approving final version of manuscript.

Serena Palmieri: study design, data analysis and interpretation, approving final version of manuscript.

Elisa Cairoli: study design and conduct, data analysis and interpretation, approving final version of manuscript.

Giovanni Goggi: study conduct, data analysis, drafting manuscript, approving final version of manuscript.

Alfredo Scillitani: study design, data analysis and interpretation, revision of the manuscript, approving final version of manuscript.

Maura Arosio: study design, data analysis and interpretation, revision of the manuscript, approving final version of manuscript.

Alberto Falchetti: study design, data analysis and interpretation, revision of the manuscript, approving final version of manuscript

Iacopo Chiodini: Study design, data analysis and interpretation, drafting manuscript, approving final version of manuscript.

Iacopo Chiodini takes responsibility for the integrity of the data analysis.

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The authors state that they have no conflicts of interest

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Figure 1

Title: Bone mineral density changes before and after 24 months denosumab therapy in patients with primary hyperparathyroidism (PHPT) and subjects with primary osteoporosis (PO)

Legend: ΔZ-LS, ΔZ-FN and ΔZ-TH: difference in bone mineral density between end of follow-up and baseline (as percentage of baseline value) at spine (L1-L4), femoral neck and total hip, respectively.

385 **Table 1.** Clinical and biochemical characteristics of PHPT patients and of PO subjects at baseline
386 and the end of the follow-up.
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	PHPT patients n=25		PO patients n=25	
	Baseline	**24 months**	**Baseline**	**24 months**
Age (years)	78.6±5.5 (67–85)	80.7±5.6 (69–87)	78.8±5.2 (69–86)	80.7±5.2 (71–88)
BMI (Kg/m^2)	24.3±2.7 (19.8–30.0)	24.3±2.6 (19.9–30.0)	24.3±2.5 (19.1–29.7)	24.7±2.7 (19.5–31.2)
Familial history of hip fx	8 (32)	-	7 (28)	-
Prevalent fragility fx[1]	24 (96)	-	25 (100)	-
Spinal Deformity Index	3.7±2.1 (0–9)	3.8±2.2 (0–9)	4.0±2.1 (1–9)	4.2±2.4 (1–11)
Previous bisphosphonates therapy[2]	13° (52.0)	-	21 (84.0)	-
Osteoporosis duration[3] (months)	72.3±42.3 (2–120)	-	68.2±45.1 (0–120)	-
Daily calcium intake (mg/day)	752±264° (300–1200)	972±79[#] (800–1200)	987±220 (671–1600)	1040±224 (700–1600)
Calcium (mg/dL)	10.8±0.9^ (10–13.2)	10.5±0.7* (9.7–12.0)	9.8±0.3 (9.1–10.4)	9.6±0.5 (8.6–10.6)
PTH (pg/mL)	117.5±42.3^ (74.1–239.2)	138.7±48.9* (63.4–258.4)	42.9±11.1 (29.0–66.1)	44.7±14.4 (17.7–67.0)
Phosphorous (mg/dL)	2.7±0.3^ (2.1–3.2)	2.6±0.4* (1.7–3.7)	3.5±0.4 (2.8–4.1)	3.4±0.4 (2.7–4.4)
Creatinine (mg/dL)	0.8±0.2 (0.5–1.2)	0.8±0.2 (0.5–1.3)	0.8±0.2 (0.6–1.3)	0.8±0.2 (0.5–1.3)
ALP (U/L)	76.8±15.4 (45.0–104.0)	52.9±12.4[#] (35.7–85.2)	69.1±21.1 (38.7–125.2)	54.3±16.9[¶] (32–94.0)
ΔALP	-	-30.6±11.3[§] (0.0–47.2)	-	-21.4±13.1 (0.0–47.1)
Urinary calcium (mg/kg/d)	3.7±1.4^ (1.6–6.5)	3.8±1.3* (2.3–7.7)	2.2±1.2 (0.5–4.7)	2.2±0.7 (0.5–3.5)
25-Hydroxyvitamin D (ng/ml)	33.5±9.4 (20.1–56.1)	43.7±7.4[#] (34.0–67.1)	38.0±9.1 (20.0–57.0)	45.7±6.8[¶] (33.1–58.9)
Z-LS (Z-score)	-0.73±0.91 (-2.5–1.0)	-0.02±1.00† (-1.4–2.7)	-0.72±0.93 (-2.7–1.0)	-0.40±1.00 (-2.9–2.4)
Z-FN (Z-score)	-0.89±0.64 (-2.0–0.4)	-0.54±0.58† (-1.9–0.7)	-0.88±0.61 (-2.1–0.30)	-0.53±0.78 (-2.9–2.4)
Z-TH (Z-score)	-0.58±0.51 (-1.7–0.2)	-0.20±0.53†[§] (-1.1–0.9)	-0.84±0.75 (-2.0–1.0)	-0.62±0.64 (-2.0–0.6)
ΔZ-LS	-	6.2±4.5[¬] (-1.1–15.8)	-	3.5±5.5 (-13.4–15.9)

ΔZ-FN	-	5.6±4.8[§] (-4.3–17.6)	-	2.9±4.8 (-4.7–17.4)
ΔZ-TH	-	4.8±4.4[§] (-1.4–17.6)	-	1.2±4.1 (-13.2–5.9)
Incident fragility fractures[1]	-	2 (8.0)		2 (8.0)
Inadequate responders to DMab	-	1 (4.0)	-	3 (12.0)
Inadequate BMD response to DMab	-	0 (0.0)	-	2 (8.0)
Good response to DMab	-	23[§] (92)	-	13 (52)

Data are mean±SD or absolute number with range or percentage in parentheses, respectively.

°p<0.001 and ^p<0.0001 vs PO patients at baseline

¯p=0.06, [§]p<0.05 and *p<0.0001 vs PO patients at the end of the follow-up (24 months)

†p<0.05 and [#]p<0.0001 vs PHPT patients at baseline

[¶]p<0.005 vs PO patients at baseline

PHPT: primary hyperparathyroidism. PO: primary osteoporosis. BMI: Body mass index; Fx: fracture. ALP: alkaline phosphatase total activity; ΔALP: difference in ALP between end of follow-up and baseline (as percentage of baseline value). Z-LS, Z-FN and Z-TH: bone mineral density measured by Dual X-ray Energy Absorptiometry and expressed as Z-score at spine (L1-L4), femoral neck and total hip, respectively. ΔZ-LS, ΔZ-FN and ΔZ-TH: difference in bone mineral density between end of follow-up and baseline (as percentage of baseline value) at spine (L1-L4), femoral neck and total hip, respectively.

Inadequate responders: presence of at least one of the following criteria: (i) two or more incident fragility fractures and (ii) a decrease in BMD greater than the least significant change (LSC, reference #26). Inadequate BMD response to DMAb: a decrease in BMD at any site greater than the LSC. Good response to DMAb: increase in BMD at any site greater than the LSC (without a decrease in BMD greater than LSC at any site) and the absence of incident fragility fractures.

[1]Vertebral or hip fragility fracture.

[2]All previously treated patients assumed alendronate or risedronate until 2 years before the study entry and for no more than 10 years.

[3]The duration of the osteoporosis has been estimated since the first diagnosis of reduced BMD and/or since the occurrence of the first fragility fracture

Table 2. Clinical and biochemical characteristics of PHPT patients and of PO subjects with an inadequate response to DMab

	PHPT Patient	**PO Patient #1**	**PO Patient #2**	**PO Patient #3**
Age at baseline (years)	75	78	84	75
BMI (Kg/m^2)	23.3	24.9	23.2	24.2
Familial history of hip fx	no	no	no	no
Comorbidities	DL	DL, AH	DL, AH	AH
Prevalent clinical fragility fx	hip	vertebral	no	vertebral
Prevalent morphometric fx	yes	yes	yes	yes
Spinal Deformity Index at baseline	5	3	1	8
Previous bisph therapy (months)	120	no	9	72
Previous inadequate response to bisph	yes	-	yes	no
Calcium intake during DMab (mg/day)	900	900	1200	1100
Calcium at baseline (mg/dL)	10.4	10.1	9.7	9.8
PTH at baseline (pg/mL)	128	47	29	39
Phosphorous at baseline (mg/dL)	2.6	3.6	3.5	3.5
Creatinine at baseline (mg/dL)	0.6	0.8	0.7	0.8
ALP at baseline (U/L)	62.4	52	50.9	55.1
ΔALP	-38	-12	-22	-18
Urinary calcium (mg/kg/d)	2.2	1.3	2.3	0.5
25-Hydroxyvitamin D (ng/ml)	26.5	37	36.1	44.3
Z-LS at baseline (Z-score)	-1.7	0.0	-0.8	-2.7
Z-FN at baseline (Z-score)	-2.0	0.3	-0.8	-2.1
Z-TH at baseline (Z-score)	-1.4	1.0	-0.9	-1.8
ΔZ-LS	4.3	-13.4	1.4	-5.0
ΔZ-FN	1.8	0.0	-4.7	-1.8
ΔZ-TH	1.4	-13.2	-4.2	0.0
Incident fragility fracture (type)	vertebral	no	vertebral	vertebral, wrist
Inadequate BMD response to DMab	no	yes	no	yes

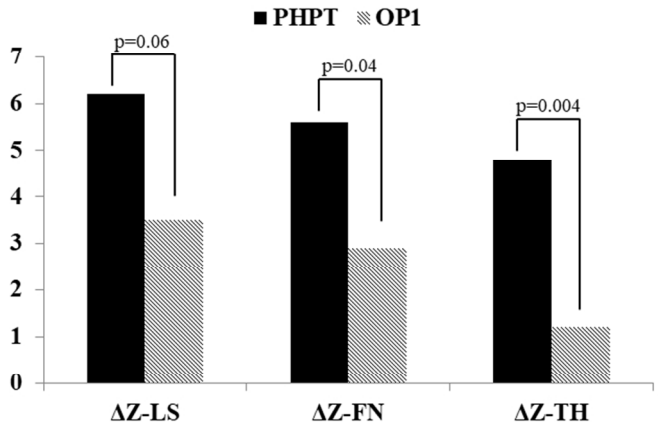
PHPT: primary hyperparathyroidism. PO: primary osteoporosis. BMI: Body mass index; Fx: fracture. Bisph: bisphosphonates. DL: dyslipidemia. AH: arterial hypertension. ALP: alkaline phosphatase total activity; ΔALP: difference in ALP between end of follow-up and baseline (as percentage of baseline value). Z-LS, Z-FN and Z-TH: bone mineral density measured by Dual X-ray Energy Absorptiometry and expressed as Z-score at spine (L1-L4), femoral neck and total hip, respectively. ΔZ-LS, ΔZ-FN and ΔZ-TH: difference in bone mineral density between end of follow-up and baseline (as percentage of baseline value) at spine (L1-L4), femoral neck and total hip, respectively.

Inadequate responders: presence of at least one of the following criteria: (i) two or more incident fragility fractures and (ii) a decrease in BMD greater than the least significant change (reference #26). Inadequate BMD response to DMAb: a decrease in BMD at any site greater than the LSC.

426 **Table 3**: Factors independently associated with a good BMD response to DMab in the whole group
427 of subjects assessed by Logistic Regression analysis

	OR	95%CI	P
Age (1 year decrease)	1.18	0.98 – 1.41	0.830
BMI (1 Kg/m^2 increase)	1.13	0.81 – 1.59	0.471
Familiar history of hip fracture (no)	3.45	0.51 – 23.26	0.205
Personal history of clinical fragility fracture (no)	1.04	1.39 – 76.9	0.023
Daily calcium intake (1 mg increase)	1.00	0.99 – 1.01	0.266
Previous bisphosphonate therapy (yes)	1.90	0.27 – 13.5	0.520
Primary hyperparathyroidism (yes)	13.44	1.43 – 125.9	0.023

428 BMI: Body mass index.
429 Good BMD response to DMAb: increase in BMD at any site greater than the LSC (without a
430 decrease in BMD greater than LSC at any site) and absence of incident fragility fractures



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Legend: ΔZ-LS, ΔZ-FN and ΔZ-TH: difference in bone mineral density between end of follow-up and baseline (as percentage of baseline value) at spine (L1-L4), femoral neck and total hip, respectively

254x190mm (96 x 96 DPI)