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EPIDEMIOLOGY

Vitamin D threshold to prevent aromatase inhibitor-related bone loss: the B-ABLE prospective cohort study

Daniel Prieto-Alhambra · Sonia Servitja · M. Kassim Javaid · Laia Garrigós · Nigel K. Arden · Cyrus Cooper · Joan Albanell · Ignasi Tusquets · Adolfo Diez-Perez · Xavier Nogues

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Abstract Aromatase inhibitor (AI)-related bone loss is associated with increased fracture rates. Vitamin D might play a role in minimising this effect. We hypothesised that 25-hydroxy-vitamin D concentrations [25(OH)D] after 3 months supplementation might relate to bone loss after 1 year on AI therapy. We conducted a prospective cohort study from January 2006 to December 2011 of a consecutive sample of women initiating AI for early breast cancer who were ineligible for bisphosphonate therapy and stayed on treatment for 1 year ($N = 232$). Serum 25(OH)D was measured at baseline and 3 months, and lumbar spine (LS) bone mineral density at baseline and 1 year. Subjects were supplemented with daily calcium (1 g) and vitamin D_3 (800 IU) and additional oral 16,000 IU every 2 weeks if baseline 25(OH)D was <30 ng/ml. Linear regression models were fitted to adjust for potential confounders. After 1 year on AI therapy, 232 participants experienced a significant 1.68 % [95 % CI 1.15–2.20 %] bone loss at LS (0.017 g/cm^2 [0.012–0.024], $P < 0.0001$). Higher 25(OH)D at 3 months protected against LS bone loss (−0.5 % per 10 ng/ml [95 % CI −0.7 to −0.3 %], adjusted $P = 0.0001$), and those who reached levels ≥40 ng/ml had reduced bone loss by 1.70 % [95 % CI 0.4–3.0 %; adjusted $P = 0.005$] compared to those with low 25(OH)D levels (<30 ng/ml). We conclude that improved vitamin D status using supplementation is associated with attenuation of AI-associated bone loss. For this population, the current Institute of Medicine target recommendation of 20 ng/ml might be too low to ensure good bone health.

D. Prieto-Alhambra · A. Diez-Perez · X. Nogues (✉)
URFOA-IMIM, Parc de Salut Mar, Parc de Recerca Biomèdica de Barcelona, C/Dr Aiguader, 88, 2nd Floor, 08003 Barcelona, Spain
e-mail: XNogues@parcdesalutmar.cat

D. Prieto-Alhambra
e-mail: dprieto@imim.es

A. Diez-Perez
e-mail: ADiez@parcdesalutmar.cat

D. Prieto-Alhambra · M. K. Javaid · N. K. Arden · C. Cooper
Oxford NIHR Musculoskeletal Biomedical Research Unit, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Windmill Road, Headington, Oxford OX3 7LD, UK
e-mail: kassim.javaid@ndorms.ox.ac.uk

N. K. Arden
e-mail: nka@mrc.soton.ac.uk

C. Cooper
e-mail: cc@mrc.soton.ac.uk

D. Prieto-Alhambra
IDIAP Jordi Gol, Unitat de Suport a la Recerca Barcelona, Institut Català de la Salut, Av. Gran Via de les Corts Catalanes, 587 Àtic, 08007 Barcelona, Spain

D. Prieto-Alhambra · S. Servitja · J. Albanell · I. Tusquets · A. Diez-Perez · X. Nogues
Medicine Department, Universitat Autònoma de Barcelona Edificio W – UD de Medicina de la Vall d'Hebron Paseo Vall d'Hebron, 119, 08035 Barcelona, Spain
e-mail: sservitja@parcdesalutmar.cat

J. Albanell
e-mail: JAlbanell@parcdesalutmar.cat

I. Tusquets
e-mail: ITusquets@parcdesalutmar.cat

D. Prieto-Alhambra · A. Diez-Perez · X. Nogues
RETICEF (Red Temática de Investigación Cooperativa en Envejecimiento y Fragilidad), Instituto Carlos III, Madrid, Spain

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Introduction

Aromatase inhibitors (AI) are routinely used in the adjuvant treatment of women with hormone receptor-positive early breast cancer [1, 2], and will become more prevalent with exemestane showing a 65 % reduction in primary prevention of breast cancer [3]. Third generation AI (anastrozole, exemestane and letrozole) have superior efficacy and better safety than tamoxifen [4, 5], both as first-line choice and when switching therapy [6, 7]. However, AI therapy has significant unwanted effects and anastrozole, exemestane and letrozole led to an increased risk of developing osteoporosis and fragility fractures in the registration trials [5, 8–10]. The mechanism for the accelerated bone loss is thought to be, at least in part, profound suppression of oestrogen synthesis; the end result is increased osteoclast activation and net bone resorption.

In addition to the effects of AI, the population of women with early breast cancer has a high prevalence of vitamin D insufficiency (as defined by serum concentration of 25-hydroxy-vitamin D [25(OH)D] <30 ng/ml): we have shown that in our community (Barcelona, Spain) the prevalence of vitamin D insufficiency among patients treated for early breast cancer is 88.1 %, with 21.2 % having severe vitamin D deficiency, defined by serum concentrations of 25(OH)D <10 ng/ml [11]. In a number of observational studies, low levels of serum [25(OH)D] are associated with an increased risk of hip fracture [12, 13]. Furthermore, in some [14] but not all [15, 16] studies, vitamin D supplementation reduces risk of future fracture and has non-skeletal effects on a number of tissues [17]. We recently published an observational study showing that a target concentration of 40 ng/ml 25(OH)D may prevent development of AI-induced arthralgia [18], a syndrome highly associated with therapy discontinuation in clinical settings. These findings conflict with the latest recommendations by the Institute of Medicine (IOM) [19], which proposed 20 ng/ml as a target threshold for bone health.

However, it is not known if vitamin D status affects the rate of bone loss in patients commencing AI therapy. Therefore, we aimed to test the hypothesis that vitamin D concentrations after 3 months of oral supplementation would be inversely related to bone loss as measured by DXA after 1 year of AI therapy in normal or osteopenic women not treated with bisphosphonates. Secondly, we studied the association between improvement in [25(OH)D] concentration at 3 months from baseline and bone loss.

Methods

Details on study design, recruitment methods, and study population have been fully explained elsewhere [11, 20] and are briefly summarised below.

Study design and participants

We conducted a prospective cohort study from January 2006 to December 2011. All postmenopausal women diagnosed with early breast cancer and candidates for AI treatment attending our outpatient Breast Cancer Unit (Barcelona, Spain) from 2006 to the end of 2010 were consecutively invited to participate in the B-ABLE cohort study and recruited after informed consent. Patients were selected for treatment with AI according to the current American Society of Clinical Oncology (ASCO) recommendations [21]. Patients with history of any bone disease, rheumatoid arthritis, metabolic or endocrine diseases, prior diagnosis of Paget's bone disease or osteomalacia, concurrent or prior treatment with bisphosphonates, oral corticosteroids, or any other bone-active drug except tamoxifen were excluded.

Patients with 25(OH)D concentration <30 ng/ml at the recruitment visit, were treated with oral calcium (1 g) and vitamin D (800 IU) supplements daily and additional oral 16,000 IU or 0.266 mg of vitamin D_3 (cholecalciferol, Hidroferol®, FAES FARMA) every 2 weeks throughout the year of study. Those with baseline vitamin D ≥30 ng/ml received only the oral calcium and vitamin D daily supplements of 800 IU/day.

Patients were then stratified by bone mineral density (BMD) at the lumbar spine (LS), femoral neck (FN), and total hip (TH), and assigned to the corresponding

S. Servitja · L. Garrigós · J. Albanell · I. Tusquets
Oncology Department, Parc de Salut Mar, Barcelona, Spain
e-mail: LGarrigos@parcdesalutmar.cat

S. Servitja · L. Garrigós · J. Albanell · I. Tusquets
Programa Consolider, Instituto de Salud Carlos III/FEDER, Barcelona, Spain

C. Cooper
Lifecourse Epidemiology Unit, MRC Southampton, Southampton General Hospital, Southampton SO16 6YD, UK

A. Diez-Perez · X. Nogues
Internal Medicine Department, Parc de Salut Mar, Passeig Marítim 25-29, 08003 Barcelona, Spain

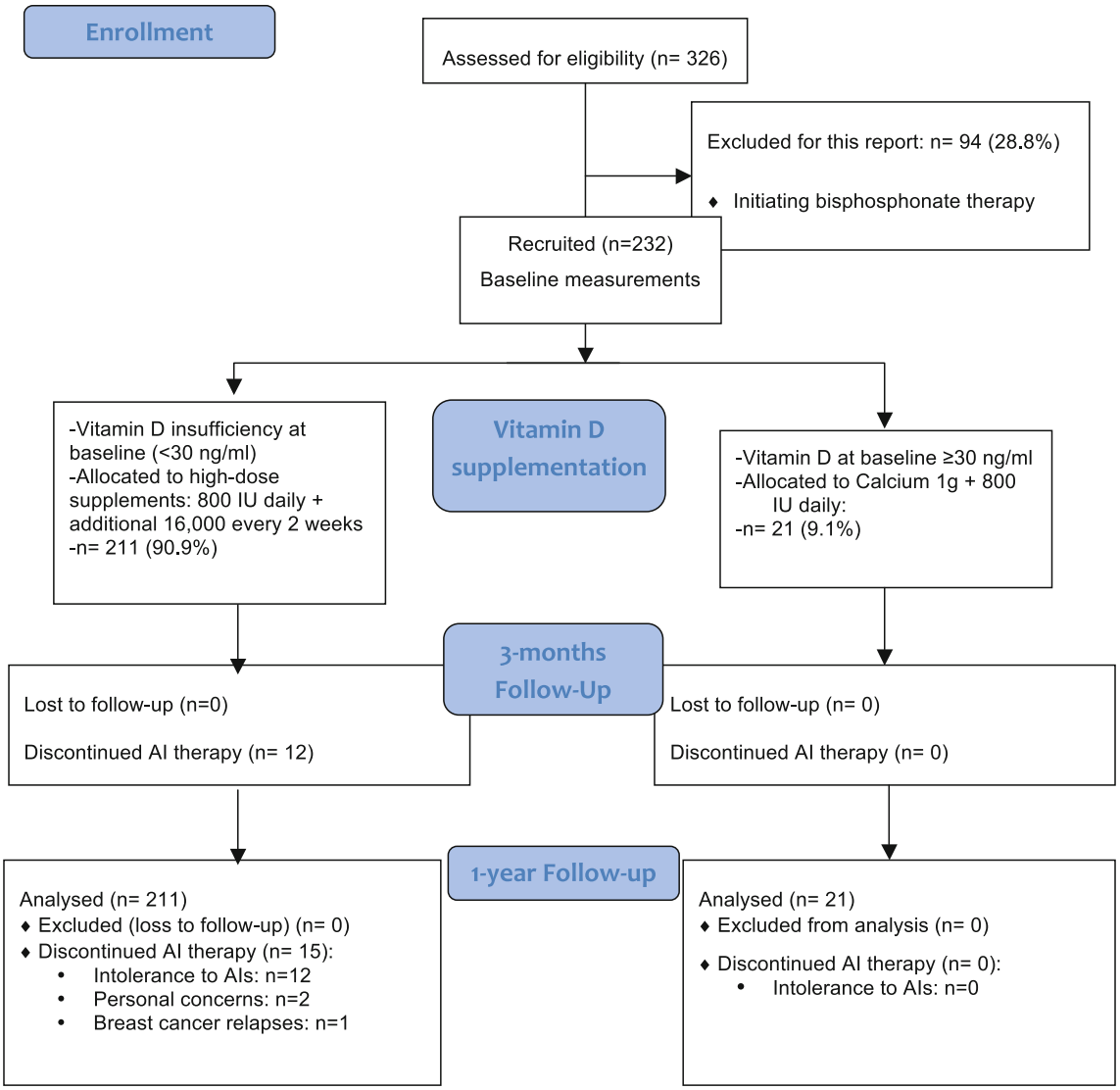

Fig. 1 Population (CONSORT) diagram

therapeutic regimen. Weekly bisphosphonate therapy (either risedronate or alendronate, randomly assigned) was provided to patients with osteoporosis [T score < -2.5] or with a T score ≤ -2.0 at any site plus 1 major risk factor or prevalent fragility fractures.

For the current analyses, we studied the population of women who, according to this therapeutic regimen, were not treated with bisphosphonates [see CONSORT diagram, Fig. 1].

Power estimation

The available sample of 232 women completing the study follow-up, ensures >85 % power to estimate a difference of 0.5 standard deviations in bone loss between the group of women achieving levels ≥40 mg/dl (about 20 % of participants according to our previous experience [18]) and those with serum levels <40 mg/dl after 3 months of supplementation.

Measurements

Serum concentrations of 25(OH)D

At baseline and at 3 months follow-up, plasma concentrations of 25(OH)D were determined using competitive immunoluminometric direct assay with direct-coated magnetic microparticles (DiaSorin Iberia SA, Madrid, Spain). The detection threshold of the tool is 4.0 ng/ml, intra-assay coefficient of variation (CV) is 3.4 %, and inter-assay CV is 7.6 %. Our laboratory is part of the vitamin D external quality assessment programme of the College of American Pathologists.

Bone mineral density

At baseline and at 1 year, BMD was measured at the LS (L1–L4), FN and TH using a dual-energy X-ray (DXA) densitometer QDR 4500 SL® (Hologic, Waltham, MA, USA), following the usual protocol in our unit. In our department, the in vivo CV of this technique ranges from 1.0 % at LS to 1.65 % at FN.

Percentage BMD loss was estimated according to the following formula: (baseline BMD − BMD at 1 year follow-up)/baseline BMD.

Calcium daily intake

Dietary calcium intake was estimated using a validated weekly food-intake frequency questionnaire [22]. Calcium supplements use was also recorded.

Covariates

For potential further adjustments in multivariate models, we collected information at baseline on the following: age, years since menopause, body mass index (BMI), smoking (current/ex/never), prior tamoxifen therapy, AI used (exemestane/letrozole), chemotherapy and radiotherapy. Season when blood samples were drawn was also registered.

Ethics approval

The study protocol was approved by the corresponding ethics committee (Hospital del Mar's Human Research Ethics Committee) and written informed consent was obtained from all participants.

Statistical analysis

We used paired *T* tests to assess changes in BMD at the three sites measured. The association between BMD loss at LS and vitamin D concentrations at 3 months was assessed using linear regression. Multivariate models were fitted to adjust for the season when serum samples were drawn, age, years since menopause, prior tamoxifen use, BMI, dietary calcium intake, and type of AI used (exemestane vs letrozole). Models for absolute bone loss were further adjusted for baseline BMD. As baseline vitamin D had been used to determine whether the participants should be supplemented with 800 IU daily or an additional 16,000 IU every other week, we did not adjust the whole cohort analyses for baseline 25(OH)D. However, we did so in the multivariate models for the population receiving high-dose vitamin D supplementation. Secondly, similar linear regression models were used to assess the existing relationship between vitamin D increment after 3 months of supplementation and BMD loss at 1 year (both absolute and bone loss rates).

All analyses were two-tailed, and p values were considered significant when <0.05. Statistical analyses were performed using Stata for Mac version 10 and R for Mac version 2.9.1, using the *foreign*, *car*, *Hmisc*, *sciplot*, *Design* and *mass* packages.

Results

Of the 326 women recruited between January 2006 and December 2010, 324 (99.4 %) have completed a year of follow-up. After risk fracture assessment, 94 (28.8 %) were initiated on bisphosphonates per protocol (see "Study design and participants"). The remaining 232 (71.2 %) were only given calcium and vitamin D supplements, constituting the population of this study (see Flowchart in Fig. 1). Baseline characteristics of this population are presented in Table 1.

Only 21 (9.0 %) participants had baseline 25(OH)D ≥30 ng/ml, and so were treated with calcium 1,000 mg and 800 IU of vitamin D_3 per day; the remaining 211 (90.9 %) had

Table 1 Baseline characteristics

	Mean (SD) or *N* (%) are shown
N	232
Age (years)	60.9 (9.0)
Age of menopause onset (years)	49.3 (4.6)
Body mass index (kg/m^2)	29.9 (5.3)
Current smokers	42 (18.1 %)
Dietary calcium intake (mg/day)	822.6 (313.7)
Prior HRT	28 (13.3 %)
Aromatase inhibitor used	
Exemestane	87 (37.5 %)
Letrozole	145 (62.5 %)
Prior tamoxifen therapy	116 (50.0 %)
Prior chemotherapy	142 (61.2 %)
Prior radiotherapy	213 (91.8 %)
Bone mineral density (g/cm^2)	
Lumbar spine	0.958 (0.111)
Total hip	0.899 (0.093)
Femoral neck	0.752 (0.091)
Prevalent fracture at baseline	9 (3.9 %)
Baseline 25(OH)D (ng/ml)	
<10	41 (17.7 %)
10−<30	170 (73.3 %)
≥30	21 (9.0 %)

Table 2 BMD at baseline and at 1 year follow-up

	Baseline BMD (g/cm^2) Mean ± SD	1-year follow-up BMD (g/cm^2) Mean ± SD	Mean BMD change (g/cm^2) [95 % CI]	Mean %BMD change [95 % CI]
Total hip	0.899 ± 0.093	0.891 ± 0.096	−0.008**	−0.72 %
			[−0.002 to −0.012]	[−1.19 to −0.02]
Femoral neck	0.752 ± 0.091	0.740 ± 0.106	−0.012**	−1.49 %
			[−0.019 to −0.004]	[−2.44 to −0.55]
Lumbar spine	0.958 ± 0.111	0.941 ± 0.114	−0.017***	−1.68 %
			[−0.024 to −0.012]	[−2.20 to −1.15]

Significance (paired *T* test) for a difference: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

different degrees of vitamin D insufficiency, and were additionally prescribed 16,000 IU of vitamin D_3 orally every 2 weeks. After 3 months of supplementation, mean (standard deviation, SD) 25(OH)D concentrations were 42.0 (22.4) ng/ml; 67 (28.9 %) women remained at <30 ng/ml, 60 (25.9 %) had levels between 30 and <40 ng/ml, and 105 (45.2 %) achieved 25(OH)D ≥40 ng/ml.

After 1 year on AI therapy, participants had a significant bone loss at the 3 sites measured (see Table 2).

Among women with baseline vitamin D deficiency, there was no significant association between baseline vitamin D concentrations and BMD loss (adjusted $P = 0.16$). However, vitamin D concentrations after 3 months of supplementation were inversely associated with bone loss at LS: for each 10 ng/ml increase in serum [25(OH)D] there was a 0.5 % [95 % CI 0.26–0.75; $P < 0.001$] reduction in bone loss at LS, equivalent to 0.005 g/cm^2 [95 % CI 0.002–0.007], or 0.13 SD. This remained significant after adjustment for season, BMI, calcium intake, AI used (exemestane vs letrozole), age, years since menopause and baseline BMD ($P < 0.001$) (see Table 3). Among those with baseline vitamin D insufficiency, BMD loss was also significantly reduced, by 0.5 % over the year per each 10 ng/ml increase in 25(OH)D concentrations at 3 months [95 % CI 0.2–0.7; $P < 0.001$], and still significant after multivariate adjustment for the same covariates plus baseline vitamin D ($P < 0.001$).

In addition, the 105 patients (45.3 % of the total study population) who achieved a vitamin D ≥40 ng/ml threshold at 3 months had less BMD loss at LS than those who reached lower concentrations (<30 ng/ml): 1.7 % [95 % CI 0.4–3.0; $P = 0.010$], equivalent to 0.017 g/cm^2 (0.44 SD) [see Fig. 2]. This remained significant after multivariate adjustment ($P = 0.007$) [Table 3]. This association was also seen in those with baseline 25(OH)D < 30 ng/ml, after adjustment for baseline concentrations ($P = 0.03$) [Table 3].

Table 3 Vitamin D status at 3 months and relative (%) BMD change at lumbar spine

	Crude beta coefficient [95 % CI]; *P* value		Multivariate adjusted beta [95 % CI]; *P* value	
	Whole cohort ($n = 232$)	High-dose vitamin D supplements ($n = 211$)	Whole cohort ($n = 232$)[a]	High-dose vitamin D supplements ($n = 211$)[b]
Vitamin D serum levels at 3 months (per 10 ng/ml increase)	−0.5 % [−0.7 to −0.3]; $P < 0.001$	−0.5 % [−0.7 to −0.2]; $P < 0.001$	−0.5 % [−0.7 to −0.3]; $P < 0.001$	−0.5 % [−0.7 to −0.3]; $P < 0.001$
Vitamin D threshold at 3 months				
<30 ng/ml ($n = 67$)	REF	REF	REF	REF
30 to <40 ($n = 60$)	−0.5 % [−2.0 to 1.0]; $P = 0.51$	−0.2 % [−1.6 to 1.3]; $P = 0.84$	−0.3 % [−1.7 to 1.1]; $P = 0.67$	−0.1 % [−1.6 to 1.3]; $P = 0.86$
≥40 ng/ml ($n = 105$)	−1.7 % [−3.0 to −0.4]; $P = 0.010$	−1.6 % [−2.9 to −0.2]; $P = 0.022$	−1.7 % [−2.9 to −0.5]; $P = 0.007$	−1.4 % [−2.8 to −0.1]; $P = 0.030$
P for trend	0.008	0.015	0.005	0.023
Vitamin D increment at 3 m (each 10 ng/ml increase)	−0.6 % [−0.8 to −0.4] $P < 0.001$	−0.5 % $P < 0.001$ $P < 0.001$	−0.5 % [−0.8 to −0.3] $P < 0.001$	−0.5 % [−0.8 to −0.2] $P < 0.001$

[a] Adjusted for: season when the sample was drawn, BMI, prior tamoxifen use, calcium intake, aromatase inhibitor therapy (exemestane vs letrozol), age, and years since menopause

[b] Further adjusted for baseline vitamin D

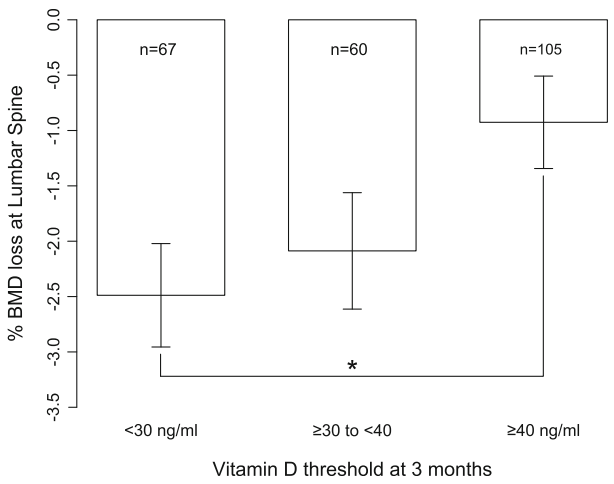

Fig. 2 Vitamin D concentrations at 3 months and %BMD loss at lumbar spine

Vitamin D increments after 3 months of supplements (defined as 25(OH)D at 3 months − 25(OH)D at baseline) in the whole population were also protective for LS bone loss: for each 10 ng/ml increase in serum 25(OH)D concentrations, bone loss was significantly reduced, by 0.6 % [95 % CI 0.4–0.8 %; $P < 0.001$] (equivalent to 0.005 g/cm^2 [95 % CI 0.003–0.007], 0.13 SD) [Fig. 3]. This remained significant in multivariate adjusted models ($P < 0.001$) and when we repeated the analyses only for those with baseline vitamin D insufficiency at baseline [Table 3].

Discussion

As expected, in patients on AI therapy for a year, we found significant bone loss at the three sites measured: TH, FN and LS.

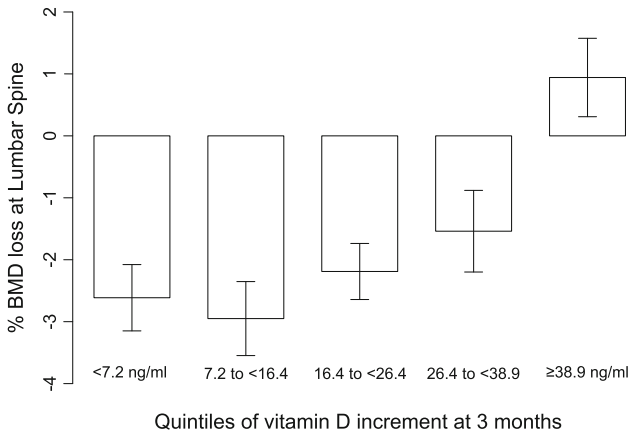

Fig. 3 Vitamin D serum levels increments (quintiles) at 3 months and %BMD loss at lumbar spine

Baseline vitamin D concentrations were not significantly related to bone loss. By contrast, vitamin D after 3 months of supplementation was inversely correlated to LS bone loss at 1 year follow-up, independently of baseline 25(OH)D concentrations and of initial BMD. In addition, patients who reached a threshold of 25(OH)D ≥40 ng/ml at 3 months had a significant reduction of 1.7 % (almost half standard deviation according to our data) in bone loss rates compared to those who stayed at vitamin D serum levels <30 ng/ml.

Vitamin D increments at 3 months were also inversely correlated to bone loss rates at LS: each 10 ng/ml increase in vitamin D translated into a 0.6 % bone loss reduction.

Most of the big clinical trials have evaluated bone loss rates as a main side effect of AI therapy, and almost all of them reported significant bone loss at LS and hip. Rates of bone density change after 1 year of AI treatment ranged from −1.66 % [23] to −7.40 % [24], with wide variation in between depending on baseline characteristics of the patients studied. At least two studies have reported significant bone loss among patients switching from tamoxifen to AI therapy: Hines et al. [23] reported a 1.66 % bone loss rate at LS in patients after 1 year on Letrozole, and Coleman et al. [25] reported a slightly higher bone loss rate in patients who switched to exemestane and were on it for a year: −2.70 %BMD reduction at LS. The average bone loss rate observed in our population was in the lower range (−1.68 % at LS), which could be due to several reasons, such as a longer time since menopause at baseline or previous tamoxifen use.

Extensive data is available on the efficacy of bisphosphonates [26–28] and denosumab [29] to prevent bone loss and fractures in patients with low bone mass or with clinical risk factors for fracture, and clinical guidelines have been published on whom to treat with anti-resorptive agents [30]. These reports recommend that patients at low risk for fractures should be supplemented with calcium and vitamin D, although the dosage recommended (calcium 1 g/day and vitamin D 400 to 800 IU daily) is probably too low to attain adequate levels in those with vitamin D deficiency at baseline: almost 30 % of participants in this study did not reach a concentration of 30 ng/ml at 3 months of much higher dose supplementation (16,000 IU every 2 weeks and 800 IU daily). In addition, the possibility that calcium supplements might be related to an increase in cardiovascular events has raised safety concerns about their use [31]. Hence, high-dose vitamin D supplements, not accompanied by calcium, might be more useful in these patients to achieve the target levels of 40 ng/ml.

Consistent with our data, one recent small pilot trial including 60 participants has shown a borderline-significant protective effect ($P = 0.06$) of high-dose vitamin D supplementation on AI-induced bone loss [32], but these

results require confirmation in bigger studies. We report here that vitamin D repletion can have a protective effect on bone loss among low-risk patients who did not require bisphosphonate therapy. In addition, we show that a threshold of ≥40 ng/ml after 3 months of supplementation can be a reasonable target, as our data appear to show a relationship to a significant decrease in bone loss rate, compared to those who remained at insufficient (below 30 ng/ml) levels, and almost 50 % of patients receiving our supplementation protocol achieved that threshold. Both the supplement dosage and the threshold suggested here are clearly higher than those proposed by the last IOM report, which advised a recommended dietary allowance (RDA) of 600 to 800 IU of vitamin D, and a 20 ng/ml target 25(OH)D concentration. These conflicting results provide a rationale for an individualised vitamin D supplementation regimen depending on patient characteristics and antecedents. Therefore, at least for this population of women on AI treatment, our data suggest that 25(OH)D levels of 40 ng/ml might be a more reasonable therapeutic target. Interestingly enough, 40 ng/ml is the same threshold found to prevent AI-induced arthralgia in our previous work [18]. The combined benefit of bone loss attenuation and decreased AI-associated arthralgia strengthens the case for this higher level as the optimal threshold.

Furthermore, we also found in our analyses that vitamin D increments (defined as the difference in vitamin D concentrations between 3 months and baseline) were inversely related to bone loss. This supports the hypothesis that Vitamin D repletion can play a protective role against AI-induced bone loss. The fact that baseline vitamin D concentrations did not predict bone loss, and that vitamin D increments predicted it independently of baseline levels, suggests that the achieved levels at 3 months are a clinically important measurement in deciding whether a higher dose supplementation should be prescribed or not. However, these are novel findings, which need replication in further studies.

Congruent with our results, vitamin D status has been related to BMD [33], and most of the trials and available meta-analyses have shown that vitamin D supplementation is protective for fractures [34, 35]. Besides, vitamin D can have other beneficial effects on bone health, as some trials have reported that it can protect from falls [17]. Nevertheless, some concerns have been recently raised in a clinical trial, where elderly patients given 500,000 IU vitamin D_3 once yearly were at higher falls risk than those treated with placebo [36]; however, almost half of patients were probably vitamin D replete with a minority (<5 %) deficient and so while of concern these findings can not be generalised to patients with vitamin D deficiency or insufficiency. Moreover, the administration regime with very high peak levels after each dose, might also contribute to this paradoxical effect.

Strengths and limitations

Our study has several limitations. As this is an observational study, causality for the described association between vitamin D concentrations and bone loss cannot be ensured. Thus, we cannot exclude confounding such as higher vitamin D being a surrogate of higher outdoors activity, which could lead to a reduced bone loss. However, the biological plausibility and the strength of the association observed support our results. A randomised clinical trial is, however, required to confirm them.

After this first year of follow-up, we have not enough statistical power to address the most important outcome in this context: the potential preventive effect of vitamin D on the occurrence of incident fractures. In our data, only five new fractures have been observed so far.

Our data were collected in a clinical setting, not in a randomised clinical trial, and patients were recruited consecutively, which make them more likely to be representative of the population treated with AI in actual practise. Thus, one can assume that the external validity of our results is high.

Conclusions

Our results suggest that Vitamin D higher concentrations after 3 months of supplementation are protective for AI-induced bone loss. A target threshold of ≥40 ng/ml, far above the 20 ng/ml target suggested by the last IOM report, could be recommended for these patients in order to protect them from bone loss. However, a randomised clinical trial is warranted to confirm these results.

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Conflict of interest The authors have no conflicts of interest to declare.

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