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## **Vitamin D threshold to prevent aromatase inhibitor-induced arthralgia: a prospective cohort study**

Daniel Prieto-Alhambra, M. Kassim Javaid, Sonia Servitja, Nigel K. Arden, Maria Martinez-García, Adolfo Diez-Perez, Joan Albanell, Ignasi Tusquets, Xavier Nogues

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## **TITLE PAGE**

**-Title: “Vitamin D threshold to prevent aromatase inhibitor induced arthralgia: a prospective cohort study”.**

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## **ABSTRACT**

*Purpose:* Aromatase inhibitor (AI) associated arthralgia limits adherence to therapy in breast cancer. The pathophysiology may involve vitamin D status. We wished to establish the optimal concentration of 25(OH)D that prevents or minimizes arthralgia.

*Methods:* We used a prospective cohort of 290 women starting AI in whom baseline vitamin D was measured. All received daily vitamin D3 (800 IU) with calcium. Women with baseline 25(OH)D concentration <30 ng/ml also received 16,000 IU of D3 orally every two weeks. The primary outcome was incident or worsening joint pain derived from baseline and three month VAS for joint pain. Regression models were used to analyse the association between vitamin D concentrations at 3 months and pain adjusting for age, BMI, season when the sample was drawn, aromatase inhibitor (exemestane vs letrozole/anastrozole), prior tamoxifen therapy, baseline NTX, and previous fracture.

*Results:* 90% of women had a 25(OH)D <30 ng/ml at baseline. After supplementation (daily 800 IU and additional additional 16,000 IU every two week), 50% of them still failed to reach adequate concentrations at three months. In the whole cohort, there was an increase in joint pain (mean 1.16 points SD 2.66;  $p < 0.001$ ) and the increase was significantly ( $p = 0.02$ ) attenuated in those that reached concentrations of 25(OH)D of  $\geq 40$  ng/ml, with a lower risk of incident arthralgia (OR 0.12 \*\* [0.03 to 0.40]).

*Conclusion:* A target concentration of 40ng/ml 25OHD may prevent development of AI arthralgia but higher loading doses are required to attain this level in women with deficiency at baseline.

**KEYWORDS:** Breast Neoplasms; Aromatase Inhibitors; Arthralgia; Vitamin D; Cohort Studies

## INTRODUCTION

Aromatase inhibitors (AI) are routinely used as adjuvant therapy for postmenopausal women with estrogen receptor positive breast cancer to improve survival [1]. Of the unwanted musculoskeletal effects, in addition to a recognised increased risk of osteoporosis[2] and fracture [3,4], AI use is associated with an arthralgia syndrome [5] with incidence rates of 19 –35% in clinical trials[6,1,7,4,8]. In a retrospective consecutive series, arthralgia was the commonest reason given for discontinuation [9]. There is therefore a clinical need to understand the pathophysiology of AI arthralgia as well as potential treatments to improve the clinical effectiveness of AI.

Vitamin D status has a number of potential roles in musculoskeletal health in women receiving AI therapy. Firstly, poor vitamin D status predicts fracture risk[3,10] and vitamin D therapy, in some[11] but not all [12,13] studies, reduces risk of future fracture. Secondly vitamin D has non-skeletal effects on a number of tissues including synovium, muscle and so putative roles in arthritis. In animal models, pre-treatment with 1, 25 OHD has been demonstrated to prevent the onset of inflammatory arthritis [14]. Given the potential associations with adverse outcomes it is a concern that biochemical vitamin D insufficiency as defined by the concentration of serum 25OHD <30 ng/ml is being increasingly recognized as common [15], with community rates of 67 to 87% in Barcelona, Spain [16,17]. In studies of women with breast cancer, 30% had concentrations of less than <20 ng/ml, and this was in part dependent on the season the sample was taken [18]. The prevalence of vitamin D deficiency among postmenopausal women diagnosed with early breast cancer and candidates for AI treatment in our hospital has been reported elsewhere [19]: 88.1% of them had serum 25(OH)D concentrations <30 ng/ml, and 21.2% had serum 25(OH)D <10ng/ml.

While there are trial data [20] and national guidance on the prevention of bone loss in women receiving aromatase inhibitors [21,22], similar guidance for vitamin D supplementation in the patient groups is lacking for two reasons: the lack of consensus on the target 25(OH)D concentration and validated methods for achieving this target. It is now recognized that the target level of 25(OH)D for vitamin D repletion is likely to differ for different tissues [23]. Using a prospective cohort we therefore wished to determine the target concentration of 25OHD that prevents or reduces the onset of AI associated arthralgia.

## **PATIENTS AND METHODS**

Details on study design, recruitment methods and population of study have been published elsewhere [19]. We conducted a prospective cohort study from January 2006 to the end of 2009. All the postmenopausal women diagnosed with early breast cancer and candidates for AI treatment attending the outpatient Breast Cancer Unit, Hospital del Mar (Barcelona, Spain), were consecutively invited to participate in this prospective bone health programme study and recruited after informed consent (Figure 1). Patients were selected for treatment with AI according to the American Society of Clinical Oncology (ASCO) recommendations [24]. AIs were initiated within 6 weeks after surgery or after the last cycle of chemotherapy.

Those patients with 25(OH)D concentration of less than 30ng/ml at the recruitment visit, just before starting their treatment with AI, were treated with oral calcium (1g) and vitamin D (800 IU) supplements daily and additional oral 16,000 IU or 0.266 mg of Vitamin D<sub>3</sub> (cholecalciferol, Hidroferol®, FAES FARMA) every 2 weeks, for the three months of study. The few women with baseline Vitamin D concentrations ≥30 ng/ml received only the calcium and vitamin D daily supplements, and were excluded for this analysis.

Patients with history of any bone disease, rheumatoid arthritis, metabolic or endocrine diseases, prior diagnosis of Paget's bone disease or osteomalacia, concurrent or previous treatment with bisphosphonates, oral corticosteroids, or any other bone-active drugs except tamoxifen were excluded.

### **Measurements**

#### *Joint pain / arthralgia*

All the participants were asked to score the intensity of joint pain using a visual analogic scale (VAS), at baseline and 3 months later. The question associated to the

VAS reads as follows: “please, score the intensity of the pain you feel in your peripheral joints (knee, wrist, fingers / toes, elbow, shoulder, etc), excluding spine/back pain and pain at the operated area” (translated from Catalan/Spanish by the authors).

We defined absolute change in joint pain VAS score for the whole cohort as VAS score at 3 months - VAS at baseline, and identified a sub-sample with incident arthralgia for those with a baseline joint pain VAS score of 0 but a VAS of greater than 0 at 3 months follow-up.

#### *Serum concentrations of 25(OH)D*

Plasma concentrations of 25(OH)D were determined at baseline before AI treatment was started and at three months after AIs and Vitamin D supplements were initiated, 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) 3.4%, and inter-assay CV is 7.6% and the laboratory is part of the Vitamin D external quality assessment programme by the College of American Pathologists ([www.cap.org](http://www.cap.org)).

#### *Biochemical markers of bone turnover*

Urinary N-terminal cross-linked telopeptide of type-I collagen (NTX) was measured at baseline, in early morning urine samples two hours post-voiding, by chemiluminescence assay (Ortho-Clinical Diagnostics, Rochester, NY, USA).

#### *Bone mineral density (BMD) analysis*

BMD was measured at baseline at lumbar spine L2-L4, femoral neck and total hip using dual-energy X-ray densitometer QDR 4500 SL® (Hologic, Waltham, Mass, USA), following the usual protocol in our unit. In our department, the technique has

an in vivo coefficient of variation CV that ranges from 1.0% at spine to 1.65% at Femoral Neck.

### *Spine and non-vertebral fractures*

Previous non-fragility fractures, height decrease, and back pain were recorded. Lateral thoracic and lumbar spine X-rays were performed to assess vertebral fractures (as defined by Genant et al[25]) at baseline in all participants.

### *Calcium daily intake*

Dietary calcium intake was estimated using a weekly food-intake frequency questionnaire, validated for the Spanish population [26]. Previous treatment with calcium supplements was also recorded.

### **Ethics approval**

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

### **Statistical analysis**

The association between incident pain and vitamin D concentrations at 3 months was assessed using logistic regression. Four vitamin D thresholds were defined *a priori*, based on previous evidence ( $\geq 20$  [27],  $\geq 30$  [28],  $\geq 40$  [29] and  $\geq 50$  [30] ng/ml) and tested using logistic regression models adjusting for age, BMI (WHO categories), baseline vitamin D concentrations, season when the second sample was drawn, type of AI (exemestane vs letrozole/anastrozole), bisphosphonate therapy, prior tamoxifen therapy, and previous fracture/s. Similarly, multivariate linear regression models were fitted to assess the association between Vitamin D concentrations at 3 months and absolute increase in VAS scores (=3 months - baseline VAS).

As a sensitivity analysis, we explored whether the associations between the vitamin D and joint pain differed by season of assessment at three months.

All analyses were two-tailed, and p values were considered significant when  $<0.05$ .

Statistical analyses were performed using R for Mac OS ver. 2.9.1, using the *foreign*, *car*, *Hmisc* and *mass* packages.

## **RESULTS**

### **Baseline descriptive statistics**

During the recruitment period, 290 women were assessed for eligibility. After baseline Vitamin D concentrations assessment, 260 (89.7%) of them were found to be Vitamin D deficient ( $<30$  ng/ml), 48 (18.5%) of these were severely deficient ( $<10$  ng/ml). Baseline characteristics of both women with baseline Vitamin D  $<30$ ng/ml and  $\geq 30$ ng/ml, and three-month 25(OH)D concentrations are shown in Table 1.

Among patients with baseline Vitamin D insufficiency, 146 (56.2%) patients had been treated previously with Tamoxifen, for a mean of 25.78 months (95%CI 22.88 to 28.68). 238 (91.5%) of them were receiving radiotherapy, and 161 (61.9%) were also having chemotherapy. Patients with osteoporosis or with osteopenia at any site plus one major risk factor or previous osteoporosis fragility fractures received also oral bisphosphonates: 41 (16.3%) received weekly Alendronate, 39 (15.5%) weekly Risedronate, and 1 (0.4%) monthly Ibandronate.

From baseline to 3 months follow-up, only 6 (2.3%) of these 260 women had discontinued AI treatment, due to AI intolerance.

### **AI-associated joint pain and vitamin D status at 3 months**

In the whole cohort the median VAS for joint pain increased from 3.0 (IQR 5.0) at baseline to 4.5 (IQR 5.0) at 3 months follow-up,  $p < 0.001$ . Of the 260 women, 79 had no joint pain at baseline and of these, 32 (42%) reported incident joint pain at three months. Of the 172 with prevalent joint pain at baseline, 85 (49.4%) reported had a worse VAS at three months, 45 (26.2%) no change and 42 (24.4%) got better. The bivariate and adjusted models to assess Vitamin D thresholds to achieve at 3 months to prevent worse and incident pain (in the whole, incident and prevalent cohorts) are shown in table 2. In the whole cohort, those with a higher 25OHD had a reduced increase in joint pain VAS and this was significant in the 30 and 40 ng/ml thresholds (table 2). In those without joint pain at baseline, incident joint pain was significantly less likely in patients with a three-month 25OHD of  $\geq 40$  ng/ml (adjusted  $p = 0.003$ ) [Table 3]. Among the 80 cases without pain at baseline, 25 (53.2%) with a three month 25(OH) concentrations of less than 40 ng/ml developed incident pain, compared with only 7/33 (21.2%) of those with concentrations equal or more than 40 ng/ml ( $p = 0.008$ ). Stratification by season when the 3 months sample was drawn did not change these results (see tables 2 and 3).

### **Vitamin D concentrations at baseline and at 3 months follow-up**

Figure 2 shows Vitamin D concentrations at baseline and at 3 months follow-up. Among those who were vitamin D severely deficient at baseline (25OHD  $< 10$  ng/ml), after 3 months of AI therapy and vitamin D supplementation (800 IU/day + 16,000 IU every other week), 40/48 (83.3%) were replete ( $\geq 30$  ng/ml), but only 25 (52.1%) achieved serum concentrations of  $\geq 40$  ng/ml. Among women with Vitamin D concentrations at baseline between 10 and  $< 30$  ng/ml ( $n = 212$ ), 156 (73.6%) were replete at 3 months time, but only 106 (50.0%) had plasma Vitamin D concentrations  $\geq 40$  ng/ml.

## DISCUSSION

We demonstrate that almost 90% of women commencing AI therapy have vitamin D deficiency. Also that a proportion of AI induced joint pain is vitamin D dependent, and the target threshold of 25(OH)D is 40 ng/ml to reduce the risk of incident and worsening joint pain. Further treating with a cumulative dose of 280,000 over 3 months (Vitamin D<sub>3</sub> 800 IU daily + 16,000 IU every 2 weeks) was only successful to achieve the target concentrations of Vitamin D plasma concentrations in about half of the population of study in those with concentrations of less than 30 ng/ml.

Estrogen deficiency is thought to cause arthritis through effects locally on joint tissues directly and via inflammatory pathways such as IL6 [5]. Another mechanism may involve estrogen effects on central and peripheral nociception<sup>5</sup>.

The mechanism of AI arthralgia seems also to involve an inflammatory component as evidenced by a increased risk of wrist effusions in those on AI with arthralgia (50% prevalence) compared with those without (25% prevalence) [31,32].

The role of vitamin D deficiency in AI induced arthralgia is controversial [33], with some studies demonstrating a positive association between vitamin D deficiency and symptoms [34]. The mechanism for this association is unclear, It has been proposed[18] that the AI induced reduction in estrogen may unmask subclinical osteomalacia through the loss of estrogen mediated activation of 1alpha hydroxylase [35], the vitamin D receptor[36,37] and/or vitamin D binding protein. In addition, AI use may attenuate vitamin D metabolism through competition with the hepatic CYP3A4 system [18]. Conversely, the promoter region of the aromatase gene is also activated by vitamin D in a number of tissues [38] hence higher concentrations of vitamin D may attenuate local estrogen deficiency [39].

Vitamin D has potent immuno-modulator effects [40], affecting both innate, as evidenced by production of cathelicidin [41] , and adaptive immunity, through effects

on T lymphocyte proliferation and function [42]. From animal studies, pre-treatment with 1,25OHD has been shown to prevent the development of both collagen and Lyme induced arthritis [14].

Vitamin D may also modulate central and peripheral pain processing[43] and increases the expression of specific aromatase transcripts in glial tissue, suggesting rescue of neural tissue from AI effects [44]. However, to date clinical trials have not demonstrated an improvement in pain with high dose D2 therapy [45].

In a previous study of women of different ethnicities starting letrozole, there was a prevalence of vitamin D deficiency of 18/60 and with only 13/60 with concentrations > 100nM [18]. In this study, which measured 25(OH)D using liquid chromatography with mass spectroscopy (LC/MS) , the threshold was approximately 165 nM (equivalent to 66 ng/ml) using disability from joint pain as the outcome.

Vitamin D status has been shown to be associated with other drug-induced immune phenomena such as the acute inflammatory response after therapy with zoledronate [46]. Patients with lower concentrations of 25(OH)D had a higher risk of an acute inflammatory response after therapy with zoledronate. Interestingly, this study also detected a threshold of 25(OH)D of 100 nM, using pyrexia or CRP as the functional outcome.

There is little controlled evidence base for the management of AI arthralgia [47]. The most effective management option is AI discontinuation with prompt resolution of symptoms. Therapeutic options include the use of non-steroidal anti-inflammatory drugs, cyclo-oxygenase-2 inhibitors, antidepressants and gabapentin, each associated with significant unwanted effects. While concerns regarding the safety of vitamin D therapy have been raised [48,49]. However, the gastrointestinal and renal unwanted effects were limited to the supplements also containing calcium and activated vitamin D preparations only, with no toxicity with high dose parenteral

vitamin D preparations. While there is a need for longer term studies of possible unwanted effects of vitamin D therapy, as the aim of therapy is to treat insufficiency/ deficiency to replace vitamin D concentrations to those seen in healthy individuals rather than raise to supra-normal concentrations, we believe the risk of harm is low.

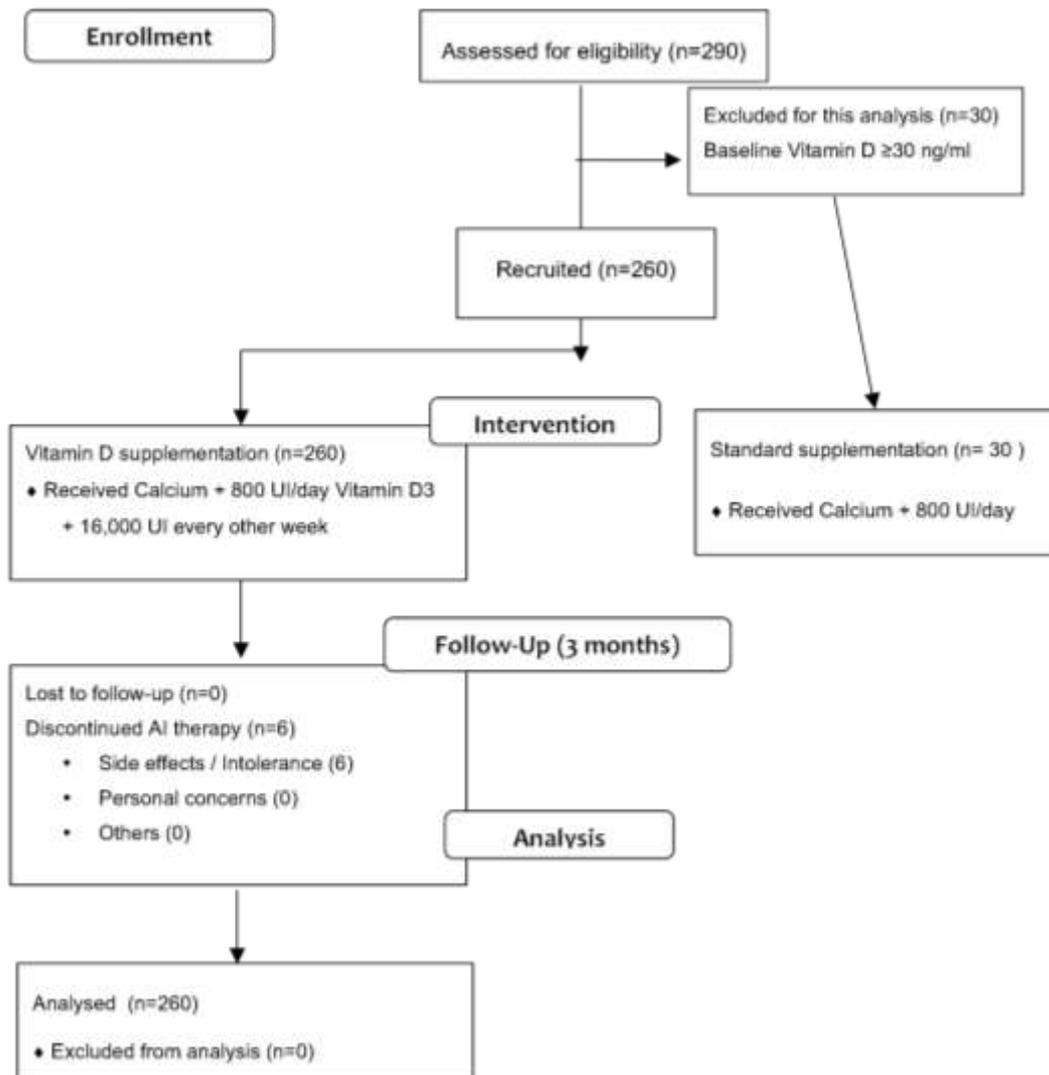
Despite a cumulative loading of 168,000 IU, a clinically significant proportion of women failed to attain adequate vitamin D status within 3 months. The poor efficacy of this regimen is supported by other studies in non-AI users which have demonstrated that initial loading of approximately 300 – 500,000 IU [50-52] with monthly 50,000 [53] is required, though a recent randomized clinical trial has raised some concerns, showing an increase in falls rate among patients having 500,000 IU in yearly basis, when compared to placebo [54].

Our study has several limitations. This is an observational study hence causality cannot be confirmed. As with many observational studies of vitamin D, we cannot exclude confounding such that increased aromatase pain leading to a reduced outdoor activity, UV-B exposure and circulating vitamin D. Based on the effect size differences demonstrated, and assuming 50% efficacy to achieve Vitamin D concentrations  $\geq 40$  ng/ml, we estimate that a trial of approximately 129 women in each arm with 25OHD concentrations of less than 40 ng/ml would be required to demonstrate causality. Another potential limitation is the current assumption that circulating 25(OH)D concentrations are a measure of functional vitamin D status. Osteomalacia, the best characterized skeletal consequence of vitamin D deficiency has now been shown to be present in patients with circulating concentrations of up to 30 ng/ml [55]. The other potential limitation is lack of information on compliance with Vitamin D supplements.. This might bias our findings in that those less likely to comply may also report more pain and so would have lower concentrations of vitamin D. However, given this is a pragmatic study the findings for the effectiveness of the dosing regimen used to increase serum 25(OH)D are valid.

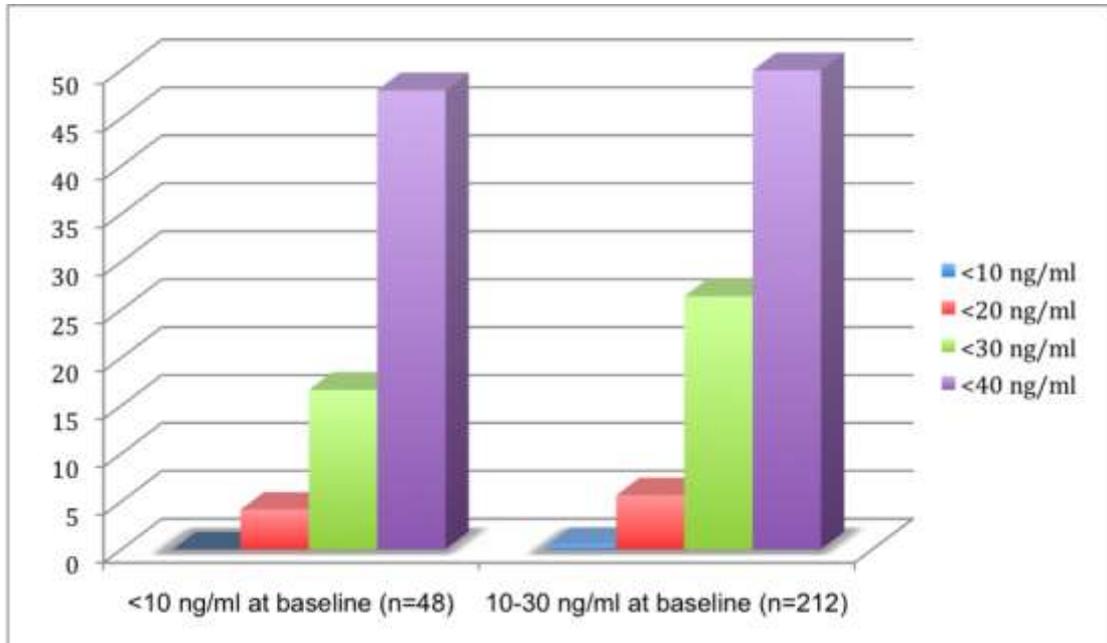
From these results, we conclude that most women requiring AI therapy have low concentrations of vitamin D at baseline and the appearance of AI-induced arthralgias in women with early breast cancer treated with these drugs is associated with their plasma concentrations of Vitamin D. The target concentration of 25OHD to achieve with supplementation in order to prevent incident arthralgias is  $\geq 40$  ng/ml. Similar concentrations might be necessary in order to prevent joint pain worsening in women treated with AI who are Vitamin D insufficient or deficient at baseline. The doses given in this study (approximately 170,000 IU over three months) was ineffective for almost half of the population to treat. Higher doses should be used in future trials to test the prevention of AI arthralgia with vitamin D supplementation.

## TABLES AND FIGURES

Figure 1. Population of study: Flow Diagram



**Figure 2.** Percentage of women with Vitamin D serum levels below different thresholds (10, 20, 30 and 40 ng/ml) after 3 months of supplementation with D3 daily 800 IU + 16,000 IU every two weeks, among patients who were Vitamin D insufficient and deficient at baseline.



**Table 1.** Baseline Characteristics

Patient Characteristic		Vitamin D at baseline <30ng/ml (n=251)	Vitamin D at baseline ≥30ng/ml (n=33)	p-value for a difference between groups
Patient Characteristic		Mean (SD) or n (%)	Mean (SD) or n (%)	
Age (years)		62.63 (8.77)	60.20 (9.64)	0.16
Age of menopause onset (years)		49.38 (4.19)	49.57 (4.54)	0.82
BMI (WHO Categories)	<25 kg/m <sup>2</sup>	57 (22.0%)	10 (33.3%)	0.004
	25 to <30	95 (36.5%)	14 (46.7%)	
	30 to <35	72 (27.7%)	5 (16.7%)	
	≥35 kg/m <sup>2</sup>	36 (13.8%)	1 (3.3%)	
Current Smokers		51 (19.6%)	6 (20.0%)	0.96
Diet Calcium intake (mg/day)		820.10 (327.47)	845.25 (304.28)	0.67
Dietary dairy Calcium (mg/day)		574.23 (297.82)	599.06 (265.63)	0.64
Aromatase Inhibitor	Exemestane	102 (39.2%)	19 (63.3%)	0.03
	Letrozole	147 (56.5%)	11 (36.7%)	
	Anastrozole	11 (4.3%)	0 (0.0%)	
Prior Tamoxifen therapy		146 (56.2%)	22 (73.3%)	0.07
Season sample was drawn	Winter	62 (23.8%)	8 (26.7%)	0.89
	Spring	63 (24.2%)	8 (26.7%)	
	Summer	47 (18.1%)	6 (19.9%)	
	Autumn	88 (33.8%)	8 (26.7%)	
Baseline joint pain (VAS score)	0	80 (30.8%)	10 (33.3%)	0.64
	>0 to 2	45 (17.3%)	4 (13.3%)	
	>2 to 4	59 (22.7%)	9 (30.0%)	
	>4 to 6	55 (21.2%)	5 (16.7%)	
	>6	21 (8.1%)	2 (6.7%)	
Baseline NTX levels (nmol / mmol creatinine)		46.32 (20.31)	39.40 (15.74)	0.03
Baseline Bone Mineral Density (T-Score)	Lumbar Spine	0.91 (0.14)	0.93 (0.12)	0.31
	Femoral Neck	0.71 (0.10)	0.75 (0.11)	0.03
	Total Hip	0.86 (0.12)	0.87 (0.10)	0.56
Baseline Vitamin D	<10 ng/ml	48 (18.5%)	40.20 (9.62)	<0.001
	10 to 30 ng/ml	212 (81.5%)		
Vitamin D at 3 months	<10 ng/ml	1 (0.4%)	35.79 (15.80)	0.005
	10 to 30	64 (24.6%)		
	≥30 ng/ml	195 (75.0%)		

**Table 2.** Association between change in VAS score (3 months – baseline) and Vitamin D thresholds at 3 months in 260 women with baseline 25(OH)D <30ng/ml who were commenced on AI for early breast cancer.

	All seasons			Season Stratification			
				Winter/ Spring		Summer / Autumn	
3 month threshold:	N (%) above the threshold	Crude Beta Coeff [95%CI]	Adjusted Beta † [95%CI]	N (%) above threshold	Crude Beta Coeff [95%CI]	N (%) above threshold	Crude Beta Coeff [95%CI]
≥ 20 ng/ml	246 (94.6)	-0.81 [-2.24 to 0.62]	-0.52 [-1.97 to 0.92]	152 (95.0)	-0.05 [-1.92 to 1.83]	94 (93.9)	-1.84 [-4.07 to 0.40]
≥ 30 ng/ml	196 (75.4)	-0.65 [-1.40 to 0.10]	-0.76* [-1.51 to -0.01]	119 (74.4)	-0.25 [-1.19 to 0.68]	77 (76.8)	-1.31* [-2.57 to -0.06]
≥ 40 ng/ml	131 (50.4)	-0.68* [-1.33 to -0.04]	-0.75* [-1.39 to -0.10]	81 (50.6)	-0.33 [-1.15 to 0.48]	50 (50.5)	-1.28* [-2.33 to -0.24]
≥ 50 ng/ml	73 (28.1)	-0.23 [-0.95 to 0.49]	-0.32 [-1.05 to 0.40]	47 (31.1)	-0.18 [-1.08 to 0.71]	26 (26.3)	-0.33 [-1.56 to 0.89]

\* p-value <0.05; \*\* p-value <0.01; \*\*\* p-value <0.001

† Adjusted for: age, BMI (WHO categories), season when the 3-months sample was drawn, aromatase inhibitor (exemestane vs letrozole/anastrozole), bisphosphonate therapy, prior tamoxifen therapy, and previous fracture/s.

**Table 3.** Association between Vitamin D thresholds at 3 months and incident pain in 79 women with vitamin D deficiency (25OHD <30ng/ml) and no pain (VAS score=0) at baseline, who were treated with oral daily 800 IU and fortnightly 16,000 IU D3.

	All seasons			Season stratification			
				Winter / Spring		Summer / Autumn	
3 month threshold:	N (%) above threshold	Crude OR [95%CI]	Adjusted OR <sup>†</sup> [95%CI]	N (%) above threshold	Crude OR [95%CI]	N (%) above threshold	Crude OR [95%CI]
≥20 ng/ml	75 (93.8)	0.42 [0.05 to 2.68]	0.33 [0.08 to 1.13]	49 (92.3)	0.58 [0.08 to 4.48]	26 (96.3)	0.00 [0 to >999]
≥30 ng/ml	55 (68.8)	0.62 [0.24 to 1.62]	0.55 [0.17 to 1.66]	34 (65.4)	0.75 [0.24 to 2.37]	21 (77.8)	0.31 [0.05 to 2.08]
≥40 ng/ml	33 (41.3)	0.24 ** [0.08 to 0.63]	0.12 ** [0.03 to 0.40]	20 (38.5)	0.27* [0.07 to 0.97]	13 (48.1)	0.17* [0.03 to 0.90]
≥50 ng/ml	23 (28.8)	0.31 * [0.09 to 0.89]	0.17 * [0.04 to 0.62]	17 (32.7)	0.38 [0.10 to 1.41]	6 (22.2)	0.18 [0.02 to 1.83]

\* p-value <0.05; \*\* p-value <0.01; \*\*\* p-value <0.001

† Adjusted for: age, BMI (WHO categories), season when the 3-months sample was drawn, aromatase inhibitor (exemestane vs letrozole/anastrozole), bisphosphonate therapy, prior tamoxifen therapy, and previous fracture/s.

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