Chapter 6

Vitamin D (25-OH D3) status and pathological response to neoadjuvant chemotherapy in stage II/III breast cancer

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Abstract

Background
Serum levels of 25-OH vitamin D3 (vitamin D) have been shown to be prognostic for disease-free survival in patients with breast cancer. We investigated the predictive value of these levels for pathological response in patients with breast cancer taking part in the NEOZOTAC phase-III trial. Additionally, the effect of chemotherapy on vitamin D levels was studied.

Materials and methods
Serum vitamin D was measured at baseline and before the last cycle of chemotherapy. The relationship between these measurements and clinical outcome, as defined by pathological complete response in breast and lymph nodes (pCR) was examined.

Results
Baseline and end of treatment vitamin D data were available in 169 and 91 patients, respectively. Median baseline vitamin D values were 58.0 nmol/L. In patients treated with chemotherapy only, serum vitamin D levels decreased during neoadjuvant chemotherapy (median decrease of 16 nmol/L, P=0.003). The prevalence of vitamin D levels <50 nmol/L increased from 38.3% at baseline to 55.9% after chemotherapy. In the total population, baseline and end of therapy vitamin D levels were not related to pathological response. No associations were found between pCR and vitamin D level changes.

Conclusion
The significant decrease in vitamin D post-neoadjuvant chemotherapy suggests that vitamin D levels should be monitored and in case of decrease of vitamin D levels, correction may be beneficial for skeletal health and possibly breast cancer outcome.

Introduction

Adequate vitamin D status is important for skeletal health. The prognostic and predictive value of vitamin D levels for patients with breast cancer remains to be established. In patients with breast cancer, vitamin D status has been suggested to be of prognostic value for survival as well as predictive value for a beneficial effect of the bisphosphonate zoledronic acid when added to adjuvant systemic treatment.(1) Data from a recent meta-analysis of 5.691 patients, mainly based on observational studies in breast cancer patients, suggested that low pretreatment vitamin D levels were associated with tumor recurrence.(2) Recently, it was postulated that low pretreatment vitamin D levels were associated with a more proliferative breast cancer phenotype. However, in this relative small study pre-neoadjuvant chemotherapy vitamin D levels were not prognostic for 3 year relapse-free survival. (3)
The proposed biological mechanism for an anti-tumor effect of vitamin D is based on the anti-proliferative and pro-apoptotic effects resulting from the binding of circulating 1,25(OH)2D3 vitamin D to its widely distributed vitamin D receptor. These receptor complexes may in turn promote transcription of genes involved in the process of cell growth and death.(4) Available data also suggest that neoadjuvant chemotherapy is associated with a higher incidence of vitamin D deficiency at the end of chemotherapy. (5)
To address the effect of chemotherapy on vitamin D levels and to investigate the relationship between vitamin D status and pathological response on neoadjuvant chemotherapy, we analyzed data of the NEOZOTAC trial.(6) In this trial patients with HER2-negative stage II/III breast cancer were randomized to 6 cycles of neoadjuvant chemotherapy (TAC) with or without zoledronic acid.

Methods

Patients and Material
Patients included in this study took part in the NEOZOTAC study. This was a Dutch multicenter study, conducted between March 2010 and April 2012 in 26 hospitals, which randomized 250 patients with early breast cancer to neoadjuvant chemotherapy (TAC) with or without zoledronic acid.(6) Patients allocated to the zoledronic acid arm of the study also received vitamin D and calcium supplements as per protocol at a fixed dose of 400 IU vitamin D and 500 mg calcium per day. Possible supplementation of patients allocated in the chemotherapy only arm was not protocol-driven and under the preference of the treating physician. Blood samples were drawn at baseline and before the last cycle of chemotherapy. The time of this last measurement was considered to be the end-of-treatment time-point. Vitamin D levels were measured in local laboratories of the participating centers using standard of care methods and results were documented in individual case report forms. All patients with available vitamin D measurements were included in this study.

Pathological response to chemotherapy
Pathological response was evaluated using the Miller and Payne classification system, which evaluates decreases in tumor cellularity (1=0% decrease, 2=0-30% decrease, 3=30-90%

decrease, 4=90% decrease, 5=100% decrease). Pathological complete response (pCR) was defined as the absence of invasive tumor cellularity in the resected breast tumor specimen and axillary lymph nodes after neoadjuvant chemotherapy. Good-response was defined as >90% decrease in tumor cellularity or total absence of invasive tumor cells in the breast only,

Study endpoints
The primary aim of this study was twofold: first to evaluate whether chemotherapy was associated with a change in serum 25-OH vitamin D3 (vitamin D) levels. Second, to investigate a possible association between vitamin D levels at baseline, levels at the end of neoadjuvant chemotherapy and changes in these levels during chemotherapy on pathological response.

Statistics
Differences in baseline parameters were analyzed using the Pearson's Chi-square test in case of categorical variables and the Mann-Whitney U-test in case of continuous variables (statistical significance defined as p value <0.05). Changes in levels between baseline and end of treatment were tested using the Wilcoxon signed rank test. Relations between baseline levels and changes in the levels of the tested markers after chemotherapy, and pathological response to neoadjuvant chemotherapy were also tested using multivariate logistic regression correcting for classical prognostic factors (cT-status, cN-status and ER status), age at randomization and Body Mass Index (BMI). In the logistical regression analysis regarding changes in vitamin D status, a dichotomous variable was calculated of decreased levels versus stabile and increased levels. The NEOZOTAC trial was approved by the Ethics Committee of Leiden University Medical Center and written informed consent was obtained from all patients.

Results

Baseline and paired 25-OH vitamin D3 levels were available in respectively 169 and 73 patients (Fig 1.) Demographic and tumor characteristics of patients in whom baseline vitamin D levels were available did not differ from the total study population except for age (Table 1). Patients in whom vitamin D measurements were available were significantly younger, than those in whom these values were not available (mean age 47.6 vs. 52.6, P<0.001). The median values of baseline and post-chemotherapy vitamin D levels were respectively 58.0 nmol/L (±27.5) and 51.0 nmol/L (±28.4). In patients that started treatment in the winter, lower average vitamin D levels (50.4 nmol/L) were measured than in patients that started treated in the summer (75.9 nmol/L), autumn (62.2 nmol/L), or spring (56.2 nmol/L; One-way ANOVA P value=0.001).

Effect of chemotherapy on vitamin D levels
A median decrease in vitamin D serum level at the end chemotherapy of 6 nmol/L was observed in the total study population (SD 23.8, P=0.032). In patients treated with chemotherapy only, there was a significant decrease in median vitamin D levels of 16 nmol/L (SD 25.2 nmol/L, P=0.003; Figure 1) at the end of neoadjuvant chemotherapy. In this chemotherapy only group, 38.8% of patients had baseline vitamin D levels of ≤50 nmol/L. At the end

Parameter		Total study population N(%)	Vitamin D baseline measured N(%)
N		246*	169
Age at randomisation	Median (range)	49.0 (29 – 70)	48.0 (29 – 68)
cT-stage	cT1	2 (0.8)	1 (0.6)
	cT2	142 (57.7)	97 (57.4)
	cT3/4	102 (41.5)	71 (42.0)
cN-stage	cN-	111 (45.1)	77 (45.6)
	cN+	135 (54.9)	92 (54.4)
Postmenopausal	Yes	98 (39.8)	110 (65.1)
	No	146 (59.3)	59 (34.9)
	Unknown	2 (0.8)	0 (-)
ER status	Positive	203 (82.5)	142 (84.0)
	Negative	43 (17.5)	27 (16.0)
PR status	Positive	154 (62.6)	109 (64.5)
	Negative	91 (37)	59 (34.9)
	Unknown	1 (0.4)	1 (0.6)
Zoledronic acid treatment	Yes	122 (49.6)	85 (50.3)
	No	124 (50.4)	84 (49.7)

Table 1. Patient and tumor characteristics of the total study populations of the NEOZOTAC trial and of the sub-group of patients in whom the vitamin D levels were measured. N=patient number

of neoadjuvant chemotherapy, the number of patients with vitamin D levels ≤50 nmol/L had increased to 55.9% (Fig 2, paired comparisons). In contrast, in the group of patients who also received zoledronic acid (and as per protocol also received vitamin D supplements at a standard fixed daily dose of 400 IU), no significant decrease was observed in vitamin D levels (median decrease 0.00, SD 20.61). There were differences in the decrease of vitamin D levels on basis of the season in which patients were treated mostly. In patients that were treated in the autumn or winter there was median decrease (chemotherapy and zoledronic acid: -12 nmol/L; chemotherapy only: -18 nmol/L), in contrary to patients that were treated in the summer or spring (chemotherapy and zoledronic acid +9.5 nmol/L; chemotherapy only; -13 nmol/L).

Relationship between vitamin D levels and pathological response to chemotherapy

There was no association between baseline vitamin D levels and pCR (P=0.76, OR 1.00, 95% C.I. 0.98-1.02 for pCR) or pathological good-response (P=0.08, OR 0.99, 95% C.I. 0.97-1.00) in the total analysed population studied after correction for the predefined variables. There was also no significant association between end of treatment vitamin D levels and pCR (56.0

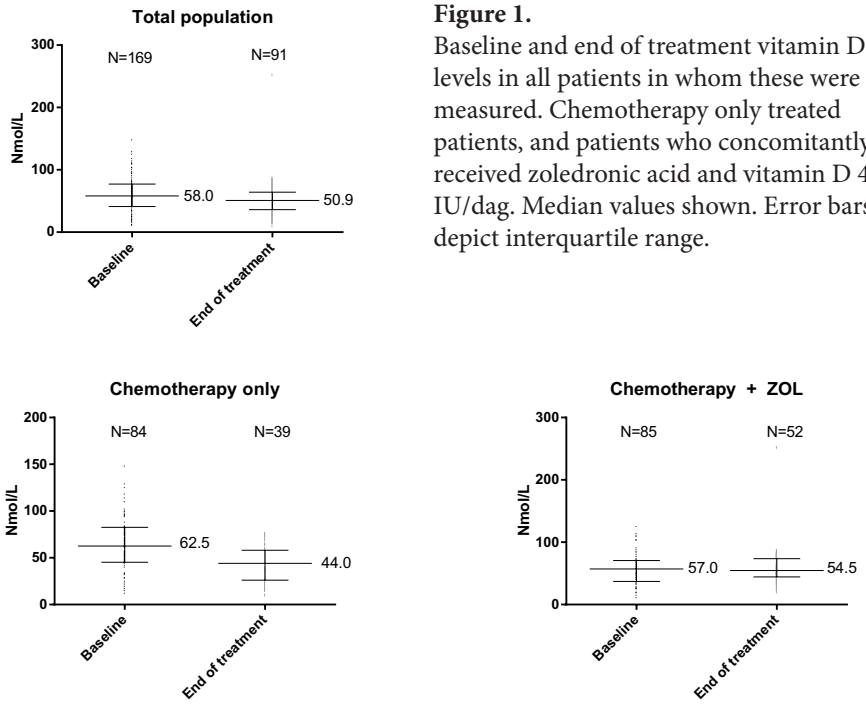

Figure 1.
Baseline and end of treatment vitamin D levels in all patients in whom these were measured. Chemotherapy only treated patients, and patients who concomitantly received zoledronic acid and vitamin D 400 IU/dag. Median values shown. Error bars depict interquartile range.

for pCR vs. 50.8, P=0.80) or pathological good-response (56.2 nmol/L for good-response vs. 49.5, P=0.66). Although, in women with end of treatment vitamin D levels above the median of measured values (50.99 nmol/L), pCR (17.8% vs. 11.6%, P=0.57) and good-response (46.7% vs. 29.5%, P=0.10) was numerically observed more often than in those with vitamin D levels below the median value. Additionally, positive changes in vitamin D levels were significantly associated with pathological good-response, with better response observed in patients with increase or stable vitamin D levels (change >= 0 nmol/L) rather than decrease in vitamin D levels (change <0 nmol/L; median change in levels 3.00 nmol/L in good-responders vs. -13.4 nmol/L in poor-responders, P=0.01; Figure 3). This association was still observed after multivariate analysis (P=0.003, OR 6.15, 95% CI. 1.8-20.42). When changes in vitamin D were analyzed as a continuous variable the difference was also significant (P=0.02). In the patients that were studied for changes 68 patients (93.2%) experienced at least one gastrointestinal toxicity during neoadjuvant chemotherapy (grade I-IV). In an exploratory analysis, there was no difference in decrease of vitamin D serum levels between patients that experienced gastrointestinal toxicities (-11.5 nmol/L) and those wo did not (-4.5 nmol/L;P=0.45).

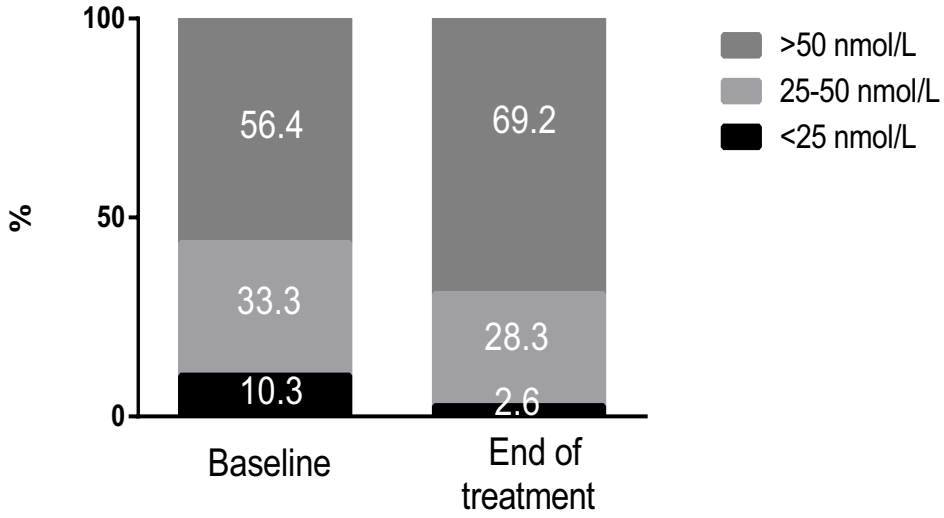

Figure 2. Distribution of vitamin D serum levels in patients who were treated with chemotherapy only (2A) and chemotherapy with zoledronic acid and vitamine D 400 IU/day(2B) and in whom paired baseline and end of treatment values were available.

Discussion

The classical function of vitamin D in bone and mineral homeostasis is well-established. It has also been shown that high serum vitamin D levels are associated with better survival in breast cancer and that vitamin D plays an important pleotropic role in cancer.(4;7-13)
Data on the association between vitamin D levels and pathological response to neoadjuvant chemotherapy are scarce in patients with breast cancer and have been presented once before. (3) Our data are in respect with this side-study of the I-SPY trial, which showed no association between baseline vitamin D levels and pathological tumor response.(3) In our breast cancer population, a relatively high number of patients had vitamin D levels <50 nmol/L (38.3%) before start of treatment. A significant decrease in vitamin D levels to < 50 nmol/L was observed in patients who received chemotherapy only. In addition, at the end of chemotherapy more patients were vitamin D deficient (<50 nmol/L).
A number of different mechanisms may be responsible for the observed chemotherapy-induced decreases in vitamin D levels in breast cancer. First, a biological explanation may be that active forms of vitamin D might be converted to inactive metabolites by upregulation of the CYP3A4 enzyme, a member of the cytochrome P450 superfamily of drug-metabolizers, which may be up-regulated as a defense mechanism against chemotherapy.(14)Another possible explanation might be the advice which is often given to breast cancer patients to

avoid sunlight during treatment, for the prevention of chemotherapy-induced photosensitivity.(15) Patients with breast cancer also often experience gastrointestinal side-effects such as mucositis, nausea and diarrhea, which may influence nutritional vitamin D intake and absorption. Our study shows no association between vitamin D levels and pCR. However patients with good pathological response (>90% decrease of tumor cells) had an increase rather than a decrease of vitamin D levels at the end of neoadjuvant therapy. Of note, this finding is mere hypothesis-generating as to date pCR is the only clinically relevant endpoint linked to breast cancer outcome.(16)
The hypothesis-generating association between a better pathological response to chemotherapy and positive changes in vitamin D levels may be a result of the pleiotropic effect of vitamin D on tumor cells.(17) The binding of 1,25 OH-vitamin D to its receptor would potentially lead to enhanced expression of genes with anti-proliferative and pro-apoptoptic effects, potentially explaining why low levels of vitamin D may be associated with the less favorable pathological responses.(18-20) However it remains unclear why absolute vitamin D baseline levels seem to have no predictive value for response to chemotherapy.
Our study has strengths as well as limitations. Its main strength lies in the availability of baseline vitamin D data in a relatively large group of patients (n=169), although paired data on changes in vitamin D levels were only available in 73 patients, as it is not yet standard practice to routinely measure vitamin D levels in breast cancer patients receiving chemotherapy. Another limitation was that we did not have data on the use of supplements or dietary vitamin D intake of patients.

In conclusion, our findings from this post hoc analysis of data of a subgroup of patients from the NEOZOTAC trial do confirm that vitamin D levels significantly decrease as a result of neoadjuvant chemotherapy. As previously data has shown that low vitamin D levels are associated with worse survival and because of the known beneficial effects of vitamin D on the skeleton, our data highlight and reinforce the premise that clinicians should monitor vitamin D levels in patients with breast cancer receiving chemotherapy. Further prospective studies are required to evaluate whether or not vitamin D levels are correlated with pathological response in patients with breast cancer.

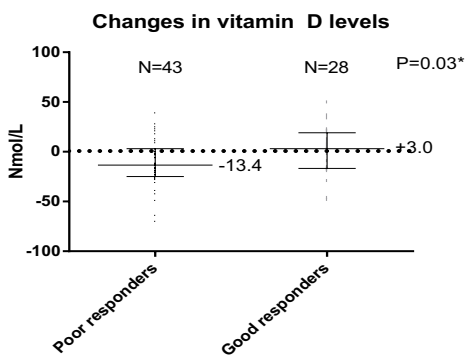

Figure 3. Changes in vitamin D levels during neoadjuvant chemotherapy in relation to pathological response (good reponse: >90% decrease of tumor cellularity). Error bars depict interquartile range. P is signifant at p<0.05.

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