Original Study

Serum Vitamin D Levels Affect Pathologic Complete Response in Patients Undergoing Neoadjuvant Systemic Therapy for Operable Breast Cancer

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Abstract

The impact of serum vitamin D levels on rate of pathologic complete response (pCR) after neoadjuvant chemotherapy in 144 patients with operable breast cancer was retrospectively investigated. Low serum vitamin D levels were associated with not attaining a pCR. Further study of vitamin D supplementation as a means to improve pCR rates is warranted.

Introduction: There has been increasing interest in the potential benefit of vitamin D in improving breast cancer outcome. Preclinical studies suggest that vitamin D enhances chemotherapy-induced cell death. We investigated the impact of serum vitamin D levels during neoadjuvant chemotherapy (NAC) on the rates of achieving pathologic complete response (pCR) after breast cancer NAC. **Patients and Methods:** Patients from 1 of 2 Iowa registries who had serum vitamin D level measured before or during NAC were included. French patients enrolled onto a previous study of the impact of NAC on vitamin D and bone metabolism were also eligible for this study. Vitamin D deficiency was defined as < 20 ng/mL. pCR was defined as no residual invasive disease in breast and lymph nodes. A Firth penalized logistic regression multivariable model was used. **Results:** The study included 144 women. There was no difference between the French and Iowan cohorts with regard to age at diagnosis ($P = .20$), clinical stage ($P = .22$), receptor status ($P = .32$), and pCR rate ($P = .34$). French women had lower body mass index (mean 24.8 vs. 28.8, $P < .01$) and lower vitamin D levels (mean 21.5 vs. 27.5, $P < .01$) compared to Iowan patients. In multivariable analysis, after adjusting for the effects of cohort, clinical stage, and receptor status, vitamin D deficiency increased the odds of not attaining pCR by 2.68 times (95% confidence interval, 1.12-6.41, $P = .03$). **Conclusion:** Low serum vitamin D levels were associated with not attaining a pCR. Prospective trials could elucidate if maintaining vitamin D levels during NAC, a highly modifiable variable, may be utilized to improve cancer outcomes.

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Introduction

The effects of vitamin D on cell cycle pathways as well as its role in breast cancer incidence, prevention, and progression have been extensively investigated. While the literature reports inconsistent results, it has been suggested that vitamin D may play a role in breast cancer progression,[1] and numerous studies have reported that adequate vitamin D levels are associated with improved overall survival and decreased risk of breast cancer.[2-9] Inverse associations

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between low serum vitamin D levels and breast cancer–specific outcomes, including recurrence and mortality, have been consistently reported in the recent literature.[10,11] Similarly, associations between higher vitamin D levels and superior breast cancer overall survival have been consistently reported.[11-15]

Preclinical studies have found that the vitamin D pathway in normal mammary tissue interacts with the cell cycle,[16,17] inflammatory pathways,[18] and estrogen pathways.[19] Additional laboratory data have demonstrated that the active form of vitamin D, 25-hydroxyvitamin D (calcitriol), inhibits cell proliferation while promoting apoptosis and cell differentiation in breast tumor tissue.[20] Furthermore, 25-hydroxyvitamin D has been demonstrated to enhance chemotherapy-induced cell death in vivo.[21-24]

Vitamin D deficiency is highly prevalent in women with operable breast cancer and is exacerbated further during neoadjuvant chemotherapy (NAC).[25,26] Thus, the period of NAC may serve as a potential ground for investigating the interactions between vitamin D levels and tumor responsiveness to chemotherapy.

We hypothesized that the pathologic outcomes after NAC for operable breast cancer may vary on the basis of serum vitamin D levels at diagnosis or during NAC. Pathologic complete response (pCR) is a measure of tumor responsiveness to chemotherapy and is increasingly recognized as a surrogate for improved long-term breast-cancer-specific survival.[27-30] Despite recent improvements in breast cancer survival, the Surveillance, Epidemiology, and End Results Program estimates 40,890 deaths from breast cancer in 2016.[31] Therefore, identifying modifiable factors that improve therapeutic efficacy remains an important goal. We investigated the impact of serum vitamin D level measured before or early in the course of NAC on achieving pCR after NAC in patients with operable breast cancer.

Methods

Patient Selection

A total of 144 women who were diagnosed with clinical stage I to III breast cancer who had their vitamin D levels measured at diagnosis or during NAC and who underwent definitive surgery between October 2009 and December 2015 at the University of Iowa Holden Comprehensive Cancer Center and between March 2007 and August 2008 at the Institut Régional du Cancer in Montpellier, France, were included in the study. The University of Iowa cohort was identified using 2 data sources at the University of Iowa: the Breast Molecular Epidemiologic Resource and the Oncology Registry. Each registry database was queried to identify women at least 18 years of age with operable primary invasive mammary carcinoma treated with NAC followed by definitive surgical resection of the primary breast cancer. Patients were excluded if pathologic confirmation of pCR status after surgery was unavailable. The study was approved by the University of Iowa's institutional review board.

The French cohort has been previously reported and described.[25] It consisted of 77 patients with a history of histologically proven locally advanced breast cancer treated with a sequential anthracycline- and taxane-containing NAC regimen. Patients with human epidermal growth factor receptor 2 (*HER2*)-overexpressing tumors also received trastuzumab intravenously at the beginning of first taxane cycle. Definitive breast surgery with axillary lymphadenectomy was performed 4 to 6 weeks after the last treatment. Patients with known comorbid conditions that may affect vitamin D–calcium metabolism, such as osteoporosis and long-term corticosteroid use, were excluded from this study.

Serum Vitamin D Measurement

At Iowa, samples of plasma were tested for 25-hydroxyvitamin D using electrochemiluminescence immunoassay and multiplex flow immunoassay methodologies.

Montpellier Cancer Institute samples were tested using the DiaSorin 25-hydroxyvitamin D ^{125}I radioimmunoassay kit.

Clinical Staging and Pathology

Clinical breast cancer staging was performed in accordance with the 7th edition of the American Joint Committee on Cancer staging guidelines at both institutions. At Iowa, institutional practices are to confirm lymph node involvement by biopsy of any radiographically or clinically suspicious axillary lymph nodes. In the French cohort, axillary ultrasound was not routinely performed. All breast cancer was diagnosed by biopsy. Immunohistochemistry was used to determine estrogen receptor, progesterone receptor, and *HER2* status. For this analysis, hormone receptor (HR) positivity was defined as $\geq$ 1% expression of estrogen receptor or progesterone receptor on the tumor. For equivocal (2+) *HER2*/neu results on immunohistochemistry, in-situ hybridization was performed. Tumors that were HR negative and *HER2* negative were considered triple-negative breast cancer. pCR was defined as the absence of invasive disease in the breast and lymph nodes (ypT0/is ypN0), and was determined by reviewing pathology reports.

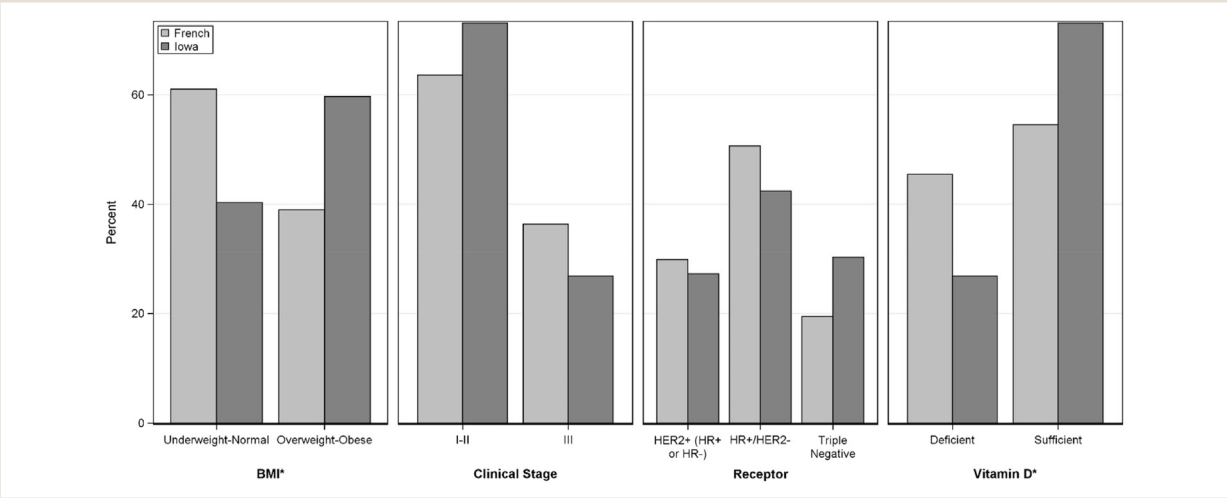
Statistical Analysis

Patient characteristics from both the Iowan cohort and the French cohort were compared by chi-square test (Fisher's exact test where appropriate) and *t* test. Firth penalized logistic regression models were used to determine whether vitamin D deficiency was associated with odds of not attaining pCR after adjusting for cohort, clinical stage, and receptor status. Estimated effects of predictors are reported as odds ratios (OR) and 95% confidence intervals (CIs). All statistical testing was 2 sided and was assessed for significance at the 5% level by SAS 9.4 (SAS Institute, Cary, NC).

Results

The final cohort included 144 women. Serum vitamin D levels were measured before starting NAC or during NAC in 77 patients from the French cohort and 67 from the Iowan cohort. Vitamin D levels were obtained before initiation of chemotherapy in 122 patients (84.7%). The median vitamin D level for the entire cohort was 23 ng/mL (range, 1.7-54 ng/mL). There was no difference between the French and Iowan cohorts with regard to age at diagnosis ($P = .20$), clinical stage ($P = .22$), disease type defined by receptor status (*HER2*$^+$, HR$^+$/*HER2*$^-$, triple-negative breast cancer, $P = .32$), and rate of pCR ($P = .34$) (Figure 1). French women had lower body mass index (mean 24.8 vs. 28.8 kg/m^2, $P < .01$), had lower vitamin D levels (mean, 21.5 vs. 27.5 ng/mL, $P < .01$), and underwent lumpectomy more frequently than mastectomy compared to Iowan women (75.3% vs. 47.8%, $P < .01$). Vitamin D was deficient (defined as < 20 ng/mL) in 53 women (36.8%).

Figure 1 Comparison Between French and Iowan Cohorts. *Statistically significant difference



There were no significant differences in clinicopathologic characteristics by vitamin D status (Table 1). All patients had received multiagent chemotherapy. In the Iowan cohort, all women had planned regimens with taxanes; 86% had planned regimens with anthracyclines. All women with *HER2*-positive tumors received anti-*HER2* therapy. Among the Iowan cohort, 47% of the patients required a reduction of at least one chemotherapy agent with a 17% and 13% median dose reduction for taxanes and anthracyclines, respectively. In the French cohort, all the patients received the planned treatment, as it was a selection criteria, as specified elsewhere.[25]

For the entire cohort, pCR was achieved in 48 women (33%) after NAC. On univariate analysis, there was a statistically significant association between vitamin D deficiency and the odds of not attaining a pCR (deficient vs. sufficient; OR = 2.98; 95% CI, 1.34-6.62). In addition, clinical stage (stage III vs. stage I-II; OR = 2.63; 95% CI, 1.15-5.88), HR status ($HR^+/HER2^-$ vs. $HR^-/HER2^-$; OR = 5.79; 95% CI, 2.27-14.78), nodal involvement (node positive vs. node negative; OR = 2.86; 95% CI, 1.41-5.88), and age (10-year increase in age, OR = 1.50; 95% CI, 1.06-2.11) were significantly associated with not attaining a pCR.

On multivariate analysis, after adjusting for the effects of cohort, clinical stage, and disease type by receptor status, vitamin D deficiency put a woman at 2.68 times increased odds of not attaining a pCR (95% CI, 1.12-6.41, $P = .03$) (Table 2, Figure 2). This variable remained significant with vitamin D deficiency defined as < 30 ng/mL and considering this variable continuously. While age was significantly associated with odds of pCR at the univariate level, no independent effect of age on pCR was seen in the multivariate analysis.

Discussion

In this retrospective cohort study, we found an independent and statistically significant association between vitamin D deficiency at the time of diagnosis or early during NAC and an increased odds of not attaining a pCR after adjusting for known powerful predictors of pCR: clinical stage and receptor expression profile. Women with serum vitamin D levels < 20 ng/mL were considered vitamin D deficient and had 2.68 times increased odds of not attaining a pCR after NAC. Our result is consistent with laboratory studies demonstrating that vitamin D sensitizes breast cancer cells to chemotherapy agents. Vitamin D has been shown to potentiate apoptosis induced by Adriamycin, paclitaxel, tamoxifen, and radiotherapy.[21,32,33]

Few studies to date have reported the association between serum vitamin D level and its effect on pCR rates in patients receiving NAC. The data from the NEOZOTAC trial reported a significant decrease in the vitamin D level in patients receiving NAC (median decrease of 16 nmol/L, $P = .003$). There was a higher rate of pCR seen in patients who were found to have an adequate vitamin D level of > 20 ng/mL at the completion of NAC (pCR 17.8% vs. 11.6%, $P = .57$); however, this was not statistically significant, and such a presurgical landmark analysis is unable to guide early vitamin D supplementation.[26] Clark et al[34] evaluated the correlation between pretreatment vitamin D levels and response to NAC. They also demonstrated that vitamin D insufficiency was a frequent occurrence at the time of breast cancer diagnosis among women who were candidates for NAC and was associated with a more proliferative breast cancer phenotype based on higher Ki-67. However, vitamin D levels did not demonstrate an increased response to NAC and was not prognostic for 3-year relapse-free survival. The results seen in these 2 studies did not reach statistical significance. This may be explained by the fact that both of these studies excluded patients with *HER2*-positive disease, and the majority of the patients had HR-positive disease, thereby both excluding the group of patients most likely to achieve a pCR and including largely patients who are less likely to attain pCR. This may account for an overall low rate of pCR, a gross imbalance between the pCR and non-pCR groups, and an inability to detect statistically significant differences attributed to vitamin D levels.

A study evaluating the use of vitamin D supplementation during adjuvant chemotherapy demonstrated improved disease-free

Table 1 Analysis of Covariates for Vitamin D Deficiency (<20 Ng/mL)

Covariate	Level	Deficient (N = 53)	Sufficient (N = 91)	*P*
Mean age at diagnosis (years)		48.19	50.82	.15
BMI	Underweight—normal	27 (50.9)	47 (51.6)	.93
	Overweight—obese	26 (49.1)	44 (48.4)	
Collection time point	Before chemotherapy	45 (84.9)	77 (84.6)	.96
	During chemotherapy	8 (15.1)	14 (15.4)	
Grade[a]	G1-2	24 (47.1)	41 (46.1)	.91
	G3	27 (52.9)	48 (53.9)	
Clinical T[a]	T0	0 (0)	1 (1.1)	.54
	T1	7 (13.2)	7 (7.7)	
	T2	31 (58.5)	51 (56)	
	T3	8 (15.1)	22 (24.2)	
	T4	7 (13.2)	10 (11)	
Clinical N	N0	19 (35.8)	43 (47.3)	.14
	N1	25 (47.2)	41 (45.1)	
	N2	9 (17)	6 (6.6)	
	N3	0 (0)	1 (1.1)	
Clinical stage	I-II	37 (69.8)	61 (67)	.73
	III	16 (30.2)	30 (33)	
ER	Negative	18 (34)	44 (48.4)	.09
	Positive	35 (66)	47 (51.6)	
PR	Negative	23 (43.4)	51 (56)	.14
	Positive	30 (56.6)	40 (44)	
HER2[a]	Negative	41 (77.4)	61 (67.8)	.22
	Positive	12 (22.6)	29 (32.2)	
Receptor[a]	*HER2*$^+$ (HR$^+$ or HR$^-$)	12 (22.6)	29 (32.2)	.20
	HR$^+$/*HER2*$^-$	30 (56.6)	37 (41.1)	
	Triple negative	11 (20.8)	24 (26.7)	
Type of surgery	Lumpectomy	32 (60.4)	58 (63.7)	.69
	Mastectomy	21 (39.6)	33 (36.3)	
PCR	No	43 (81.1)	53 (58.2)	<.01*
	Yes	10 (18.9)	38 (41.8)	

Data are presented as n (%) unless otherwise indicated.
Abbreviations: BMI = body mass index; ER = estrogen receptor; *HER2* = human epidermal growth factor receptor 2; HR = hormone receptor; PR = progesterone receptor.
[a]Analysis included for those with information available.
*Statistically significant (0.05 level).

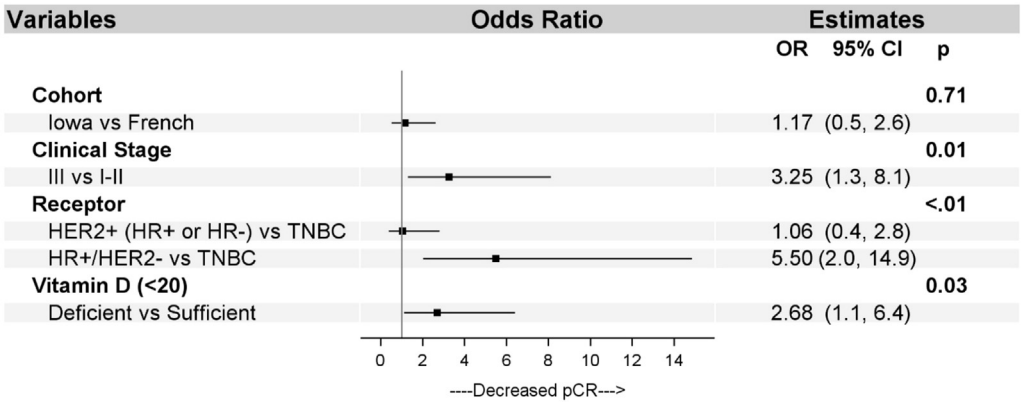
survival in *HER2*-positive breast cancer.[35] Yao et al[6] reported among premenopausal women with low vitamin D levels an association with advanced tumor stage and triple-negative subtype. These data suggest that the association between vitamin D levels during NAC and pCR may vary by tumor receptor expression profile.

Table 2 Multivariable Analysis of Odds of Not Attaining Pathologic Complete Response

Covariate	Level	N	Odds Ratio	95% CI		*P*
Cohort	Iowan	66	1.17	0.52	2.63	.71
	French	77	Ref	—	—	—
Vitamin D (<20 ng/mL)	Deficient	53	2.68	1.12	6.41	.03*
	Sufficient	90	Ref	—	—	—
Clinical stage	III	46	3.25	1.30	8.11	.01*
	I-II	97	Ref	—	—	—
Receptor	*HER2*$^+$ (HR$^+$ or HR$^-$)	41	1.06	0.40	2.81	.91
	HR$^+$/*HER2*$^-$	67	5.50	2.04	14.85	<.01*
	Triple negative	35	Ref	—	—	—

Abbreviations: CI = confidence interval; *HER2* = human epidermal growth factor receptor 2; HR = hormone receptor.
*Statistically significant (0.05 level) using Firth penalized logistic regression models.

Figure 2 Multivariate Analysis of Odds of Pathologic Complete Response (pCR)



Associations between higher levels of vitamin D levels and improved breast cancer overall survival have been consistently reported in the previous literature. Multiple studies have reported improved overall survival in patients with higher serum vitamin D levels.[2,11-15] Similar results were also observed in 2 meta-analyses.[2,36] Furthermore, vitamin D deficiency was reported to be a high-risk factor for breast cancer recurrence, especially in those with HR-positive breast cancer.[10] Identifying modifiable factors to improve overall survival and therapeutic efficacy would benefit breast cancer patients. Notably, the relationship of vitamin D supplementation and breast cancer incidence may be different than that seen between vitamin D levels and cancer outcomes in women who have breast cancer. In 2 large randomized clinical trials, vitamin D supplementation was not found to confer a decrease in the risk of developing breast cancer.[37,38]

Several limitations of this study should be noted. First, our sample size was small: 144 patients with operable breast cancer. However, despite the small sample size, this study was adequately powered to detect statistically significant relationships between vitamin D levels and responses to NAC. In addition, we can assume generalizability of the data as consistent results were seen between the Iowan and French cohorts. Second, because this study is a retrospective cohort study, causality cannot be concluded, and caution should be used when interpreting the result. Therefore, this result should be repeated in a larger, prospective study that will further investigate the association between vitamin D levels and the rate of pCR. Another limitation of our work is that we are unable to account for self-administered supplements; a limitation seen in most studies, as an exhaustive retrospective identification of self-administered supplements taken while receiving chemotherapy appears challenging. More specifically, vitamin D and bisphosphonates are not recommended as a standard part of cancer care in upfront therapy for operable breast cancer. None of our patients specifically received vitamin D or bisphosphonate therapy as part of their treatment during their NAC. Finally, a relationship between low vitamin D levels and physical activity has been reported outside of the breast cancer setting;[36,37] however, it is difficult to know the causes of this association. Whether it is a direct relationship due to sun exposure (linked to outdoor activities) or an indirect cause remains unclear. We did not perform a prospective evaluation of the physical activity in this cohort, and retrospective evaluation of this parameter would be challenging.

Our observation that vitamin D–deficient women with operable breast cancer have a lower likelihood of attaining pCR suggests that supplementing patients with vitamin D could potentially increase the rate of pCR and in turn could lead to improved breast cancer outcomes, including better disease-free and overall survival. Importantly, this is a modifiable clinical variable that is highly actionable and could be addressed at a relatively low cost.[39] Further validations in a larger data set will be important to confirm our findings and to better understand our results. Additionally, further study could help to understand if this effect is also seen with other neoadjuvant systemic therapies, including antiestrogen therapy and novel biologic therapies. Finally, prospective trials could elucidate if maintaining adequate vitamin D levels during NAC can be utilized to improve cancer outcomes in addition to benefiting other established health outcomes.

Conclusion

In this retrospective cohort analysis of women with operable breast cancer, vitamin D deficiency at initiation or early in the course of NAC was associated with not attaining a pCR. Vitamin D supplementation during NAC may benefit patients receiving NAC by increasing its therapeutic efficacy. Further investigation, including prospective clinical trials and meta-analyses, would be valuable to better understand the role of vitamin D supplementation in improving breast cancer outcomes.

Clinical Practice Points

- Inverse associations between low serum vitamin D levels and breast cancer–specific outcomes including recurrence and mortality have been reported in the literature.
- Preclinical studies demonstrate that vitamin D may enhance chemotherapy-induced cell death.
- Vitamin D deficiency (levels < 20 mg/mL) is highly prevalent during breast cancer NAC.

- We analyzed the association between pCR and serum vitamin D levels in 144 patients receiving NAC while accounting for the powerful known predictors of pCR, such as stage and tumor receptor expression profile.
- On multivariable analysis, after adjusting for the effects of cohort, clinical stage, and receptor status, vitamin D deficiency before or early in the course of NAC increased the likelihood of not attaining a pCR by 2.68-fold.
- Follow-up of this cohort for longer-term disease-free survival and overall survival outcomes will be important to better understanding the role of vitamin D in NAC and other neoadjuvant systemic therapies.
- Further research is necessary to understand if vitamin D supplementation during NAC might benefit breast cancer patients.

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Disclosure

The authors have stated that they have no conflict of interest.

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