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Association of Serum Level of Vitamin D at Diagnosis With Breast Cancer Survival
A Case-Cohort Analysis in the Pathways Study

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IMPORTANCE There are long-standing interests in the potential benefits of vitamin D for preventing breast cancer recurrence and mortality, yet data from prospective cohort studies are limited.

OBJECTIVE To investigate a serum biomarker of vitamin D status, 25-hydroxyvitamin D (25OHD) measured at the time of breast cancer diagnosis, to determine the association with prognosis.

DESIGN, SETTING, AND PARTICIPANTS The Pathways Study is a prospective cohort study of breast cancer survivors established in 2006. Enrollment was completed in 2013; follow-up is ongoing. The cohort was established in Kaiser Permanente Northern California, a large integrated health care delivery system in northern California. Women with a diagnosis of incident invasive breast cancer were typically consented and enrolled within 2 months of diagnosis. The overall enrollment rate was 46% (4505 of 9820). Participants are followed for health outcomes and comorbidities at 12, 24, 48, 72, and 96 months after baseline interview. A case-cohort design was used for efficiency assay of 25OHD, selecting 1666 cohort members with serum samples and ensuring representation in the subcohort of races and clinical subtypes. The data analysis was performed from January 5, 2014, to March 15, 2015.

MAIN OUTCOMES AND MEASURES Primary outcomes are breast cancer recurrence, second primary cancer, and death.

RESULTS Mean (SD) age was 58.7 (12.4) years. Serum 25OHD concentrations were lower in women with advanced-stage tumors, and the lowest in premenopausal women with triple-negative cancer. Levels were also inversely associated with hazards of disease progression and death. Compared with the lowest tertile, women with the highest tertile of 25OHD levels had superior overall survival (OS). This association remained after adjustment for clinical prognostic factors (hazard ratio [HR], 0.72; 95% CI, 0.54-0.98). Among premenopausal women, the association with OS was stronger, and there were also associations with breast cancer–specific survival and invasive disease–free survival (OS: HR, 0.45; 95% CI, 0.21-0.96; breast cancer–specific survival: HR, 0.37; 95% CI, 0.15-0.93; invasive disease–free survival: HR, 0.58; 95% CI, 0.34-1.01; all after full adjustment).

CONCLUSIONS AND RELEVANCE Serum 25OHD levels were independently associated with breast cancer prognostic characteristics and patient prognosis, most prominently among premenopausal women. Our findings from a large, well-characterized prospective cohort provide compelling observational evidence on associations of vitamin D with lower risk of breast cancer morbidity and mortality.

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Supplemental content

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Vitamin D deficiency has been implicated in a variety of cancers.[1,2] The level of 25-hydroxyvitamin D (25OHD), the major circulating metabolite, provides a direct assessment of vitamin D status in vivo.[3] Many epidemiological studies and meta-analyses have investigated the association of blood 25OHD levels with breast cancer risk, reporting mixed results.[4-9] This could be due, in part, to the etiological heterogeneity of breast cancer. We previously showed that, among premenopausal women, low 25OHD concentrations were associated with advanced tumor stage and triple-negative (TN) subtype.[10]

Compared with studies of breast cancer risk, only a few have examined the association of vitamin D status with prognosis. Goodwin et al[11] showed higher risk of distant recurrence and death among patients with vitamin D deficiency than those with sufficient levels. Although several later studies reported similar findings,[12-15] 2 studies reported null associations.[16,17] It is noted that previous studies invariably examined all-cause mortality, and only 1 examined breast cancer-specific mortality.[14] Because vitamin D status may be related to mortality due to all causes that are not necessarily specific to cancer,[18-20] it is important to consider breast cancer-specific survival (BCSS) and other outcomes.

In a large prospective cohort of breast cancer survivors, we investigated associations of serum 25OHD levels with breast cancer prognostic characteristics and outcomes, including recurrence, second primary cancers, and death.

Key Points

Question What is the association of serum vitamin D levels at the time of diagnosis with breast cancer survival?

Findings In this cohort study of 1666 women with breast cancer, higher serum 25-hydroxyvitamin D levels were independently associated with better outcomes, including overall survival. Compared with women with the lowest third of 25-hydroxyvitamin D levels, those with the highest third had reduced hazards of all-cause death after full adjustment, and the associations were stronger in premenopausal women.

Meaning This study provides compelling observational evidence of vitamin D's benefits for breast cancer progression and mortality.

Methods

Study Population and Biospecimen Collection

The analyses were conducted within the Pathways Study, a prospective cohort of women with breast cancer. Established in January 2006 at Kaiser Permanente Northern California (KPNC), Pathways was designed specifically to examine factors associated with breast cancer recurrence and survival. As previously described,[21] women with newly diagnosed incident invasive breast cancer were identified through rapid case ascertainment and were typically consented in writing and enrolled within 2 months of diagnosis. The enrollment rate was 46% (4505 of 9820), and participants were representative of women who received a diagnosis of breast cancer at KPNC during the study period. Baseline interviews were conducted in person and included detailed questionnaires and anthropometric measures; blood samples were obtained shortly thereafter. Regular follow-ups were conducted via mailed or telephone questionnaires for lifestyle factors at 6, 24, and 72 months, and health outcomes and comorbidities at 12, 24, 48, 72, and 96 months. Blood samples were collected from 4034 (90%) of the women at a median of 69 days (range, 31-455 days) after diagnosis and shipped to Roswell Park Cancer Institute (RPCI) Data Bank and Biorepository laboratories for processing. The study was approved by the institutional review boards at KPNC and RPCI.

Clinical and Outcome Data Collection

Diagnostic and treatment data were obtained from the KPNC Cancer Registry and other electronic clinical and administrative databases. During follow-up interviews, women reported new breast or other cancers and conditions. On a monthly basis, KPNC electronic medical records were searched for reinitiation of chemotherapy and/or evidence of a potential recurrence using a computerized algorithm of *International Classification of Diseases, Ninth Revision (ICD-9),* or *International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10),* codes, based on research in similar integrated health systems.[22-25] Potential recurrences identified from both self-report and electronic medical records were confirmed by medical record review. Death information came from several sources, including family members, medical records, and linkage with the KPNC mortality file, which incorporates data from KPNC sources, the State of California, and the Social Security Administration. Underlying cause of death was determined from the death certificate, hospital discharge summary, autopsy or coroner's report, or physician notes.

Breast cancer clinical subtypes were classified using clinical data from KPNC, ascertained using immunohistochemistry (IHC) for estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status. For patients with equivocal HER2 status, fluorescence in situ hybridization was used. Subtypes were defined as follows: luminal A: ER positive or PR positive, and HER2 negative; luminal B: ER positive or PR positive, and HER2 positive; HER2 enriched: ER negative, PR negative, and HER2 positive; and triple negative: ER negative, PR negative, and HER2 negative.[26]

Case-Cohort Design and 25OHD Measurement

We used a case-cohort design to select a subcohort of patients from the total 3175 participants available at the time. All nonwhite cases and non-luminal A subtypes were included, along with a random sample of 400 white women with luminal A tumor. We also included women outside the subcohort who had an outcome during this time. In the final subcohort of 1666 women, serum samples were analyzed for 25OHD concentration by an immunochemiluminometric assay performed at Heartland Assays. The assay coefficient of variation was 8.8%.

Statistical Analysis

Analysis of variance (ANOVA) was used to assess associations of 25OHD concentrations with nonclinical factors that could potentially affect levels, including age at diagnosis, menopausal status, body mass index (BMI, calculated as weight in kilograms divided by height in meters squared), self-reported race/

ethnicity, socioeconomic status, physical activity, smoking, supplementary and dietary vitamin D intake, and season of blood collection. Because only weak seasonal variations in 25OHD concentrations were observed in this population (eFigure 1 in the Supplement), we used measured rather than season-adjusted concentrations of vitamin D levels, and accounted for residual confounding by including season in all models. We chose to divide vitamin D levels by tertiles rather than clinical cut points (sufficient, insufficient, and deficient) because of controversies over the deficiency definitions and the uncertainty of relevance of such definitions to breast cancer. In additional analyses of vitamin D levels classified by clinical cut points (deficient, <20.0 ng/mL; insufficient, 20.0-29.9 ng/mL; sufficient, ≥30.0 ng/mL; to convert to nanomoles per liter, multiply by 2.496),[27] the results were similar to those with tertiles.

Serum 25OHD concentrations were compared by known prognostic characteristics at diagnosis, including tumor stage, grade, ER status, and clinical subtype, with adjustment for nonclinical covariates including age at diagnosis, BMI, race/ethnicity, and season of blood collection. Further adjustment for physical activity, smoking, and socioeconomic status variables in the models did not substantially change the results and were thus omitted. Multinomial logistic regression was used to assess odds ratios (ORs) for 25OHD tertiles with clinical subtypes by IHC, using the most common luminal A subtype as the referent group.

To account for the subcohort sampling scheme, sampling weights were incorporated in ANOVA and multivariable analyses, which generated similar estimates.

According to the standardized definitions in the STEEP system,[28] survival outcomes assessed included the following: recurrence-free survival (RFS), overall survival (OS), BCSS, and invasive disease-free survival (IDFS). The latter considers recurrence and secondary primary invasive cancers, as well as death due to any causes. We also examined second primary cancers (SPCs), including both invasive cancers and ductal carcinoma in situ. Follow-up began at the time of breast cancer diagnosis until the occurrence of a breast cancer event, and a patient with no event of interest during the follow-up was censored at the time of last outcome ascertainment (November 4, 2014). The median follow-up time was 7.0 years (range, 3.7-8.9 years), with 9% loss to active follow-up by telephone interview (passive follow-up by medical records continues).

The associations of serum 25OHD levels with time to each end point were examined using multivariable Cox proportional hazards models, modified for the case-cohort design by the method of Langholz and Jiao.[29] Minimally adjusted models included only significant nonclinical covariates (age at diagnosis, BMI, race/ethnicity, and season of blood sampling), followed by full adjustment for clinical prognostic factors. Time-covariate interactions were assessed, and no appreciable nonproportionality was found. Interactions were assessed using the Wald test. Nonlinearity was tested by including a squared term of ordered vitamin D levels in the models, which was not significant. Only a few covariates were missing with a small proportion, and observations with missing data were excluded from the multivariable models by default. All analyses were performed in SAS, version 9.4.

Results

Serum 25OHD concentrations by selected nonclinical factors are summarized in eTable 1 in the Supplement. As expected, concentrations were associated inversely with BMI and positively with physical activity, vitamin D supplement use, and dietary vitamin D intake; African Americans and Hispanics had lower 25OHD concentrations than whites; and current smokers had lower concentrations than never and former smokers. Older women tended to have higher 25OHD concentrations than younger women. Socioeconomic status variables, including education, household income, and marital status, were also associated. There were statistically significant yet small seasonal variations in 25OHD concentrations. At baseline, almost half (792 [48%]) of the patient population were vitamin D deficient, and another 584 (35%) insufficient.

As presented in **Table 1**, there were inverse associations of 25OHD concentrations with tumor stage and tumor grade. The results remained statistically significant for stage after adjustment for covariates, and did not vary by menopausal status (data not shown). No significant differences were found by ER or IHC subtype. Among premenopausal women, however, 25OHD concentrations were the lowest in TN cases (mean [SD], 20.0 [8.3], 19.8 [7.3], 19.3 [6.8], and 18.7 [9.6] ng/mL for luminal A, luminal B, HER2 enriched, and TN, respectively). Using the luminal A subtype as the referent group, premenopausal women with 25OHD levels in the higher two-thirds of the distribution had reduced odds of TN subtype compared with those in the lowest third (T2 vs T1: adjusted OR, 0.45; 95% CI, 0.25-0.83; T3 vs T1: OR, 0.53; 95% CI, 0.27-1.04; $P = .03$ for trend).

Kaplan-Meir survival curves by tertile of serum 25OHD are shown in the **Figure.** In multivariable analyses of survival outcomes with adjustment for nonclinical factors, higher 25OHD levels were associated with superior OS (HR, 0.54; 95% CI, 0.40-0.72; $P < .001$ for trend), BCSS (HR, 0.58; 95% CI, 0.38-0.90; $P = .01$ for trend), and IDFS (HR, 0.61; 95% CI, 0.44-0.85; $P = .004$ for trend), but not with RFS or SPC (**Table 2**). The associations with BCSS and IDFS were attenuated and became nonsignificant after further adjustment for clinical factors including tumor stage, grade, and IHC subtype; while the association with OS remained significant (T3 vs T1: HR, 0.72; 95% CI, 0.54-0.98; $P = .03$ for trend) (Table 2).

When stratified by menopausal status, higher 25OHD levels were associated with superior RFS, OS, BCSS, and IDFS among premenopausal women (survival curves in eFigure 2 in the Supplement and adjusted HRs in eTable 2 in the Supplement). These associations remained significant after adjustment for nonclinical factors and further for clinical factors. Among postmenopausal women, there was a significant association of 25OHD levels with OS when adjusted for nonclinical factors, which, however, became nonsignificant after further adjustment for clinical factors. Serum 25OHD level was not associated with SPC in either premenopausal or postmenopausal women. Interactions of 25OHD levels with menopausal status on associations with outcomes were nonsignificant. Additional adjustment for treatment regimens (surgery

Table 1. Multivariable-Adjusted Serum 25-Hydroxyvitamin D (25OHD) Levels by Breast Cancer Prognostic Characteristics in the Pathways Study Cohort

Characteristic	No.	LS Mean 25OHD Level (95% CI), ng/mL[a]	*P* Value
American Joint Committee on Cancer stage			
I	824	21.5 (20.9-22.1)	<.001
II	606	19.3 (18.6-20.0)	
III	202	19.0 (17.8-20.2)	
IV	34	18.4 (15.7-21.2)	
Tumor grade			
Well differentiated	329	20.8 (19.9-21.8)	.15
Moderately differentiated	675	20.5 (19.9-21.2)	
Poorly differentiated	559	19.8 (19.0-20.6)	
ER status			
Positive	1226	20.4 (19.8-20.9)	.95
Negative	440	20.3 (19.5-21.2)	
Clinical subtype by immunohistochemical analysis[b]			
Luminal A	1000	20.4 (19.9-21.0)	.56
Luminal B	213	19.8 (18.7-21.0)	
HER2 enriched	113	21.0 (19.4-22.6)	
Triple-negative	323	20.0 (19.1-21.0)	

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; LS, least squares; PR, progesterone receptor.

SI conversion factor: To convert 25OHD level to nanomoles per liter, multiply by 2.496.

[a] After adjustment for age at diagnosis, body mass index at baseline, race/ethnicity, and season of blood sample collection.

[b] Definitions: luminal A: ER positive or PR positive, and HER2 negative; luminal B: ER positive or PR positive, and HER2 positive; HER2 enriched: ER negative, PR negative, and HER2 positive; and triple negative: ER negative, PR negative, and HER2 negative.

and adjuvant chemotherapy, radiation therapy, and hormonal therapy) in the multivariable models already containing clinical prognostic factors did not change the results.

Discussion

In this case-cohort analysis of serum 25OHD levels with outcomes in a prospective study of women with breast cancer, we found that higher serum levels of 25OHD were associated with superior prognosis. Women with higher levels of 25OHD had better overall survival, and in premenopausal women, also better BCSS, RFS, and IDFS. No impact of 25OHD levels was observed for risk of SPCs.

Several previous studies examined blood 25OHD levels with breast cancer survival outcomes: 5 reporting superior OS in patients with high 25OHD levels[11-15]; 4 remained significant and 1 became nonsignificant after adjustment.[14] Our findings are thus consistent with the majority of the literature demonstrating better OS among patients with higher 25OHD levels, following a dose-response pattern. This largely consistent trend was confirmed in 2 recent meta-analyses.[4,30] A Cochrane systematic review commissioned by the Institute of Medicine also concluded that mortality is probably inversely related to blood 25OHD concentrations among cancer patients.[31]

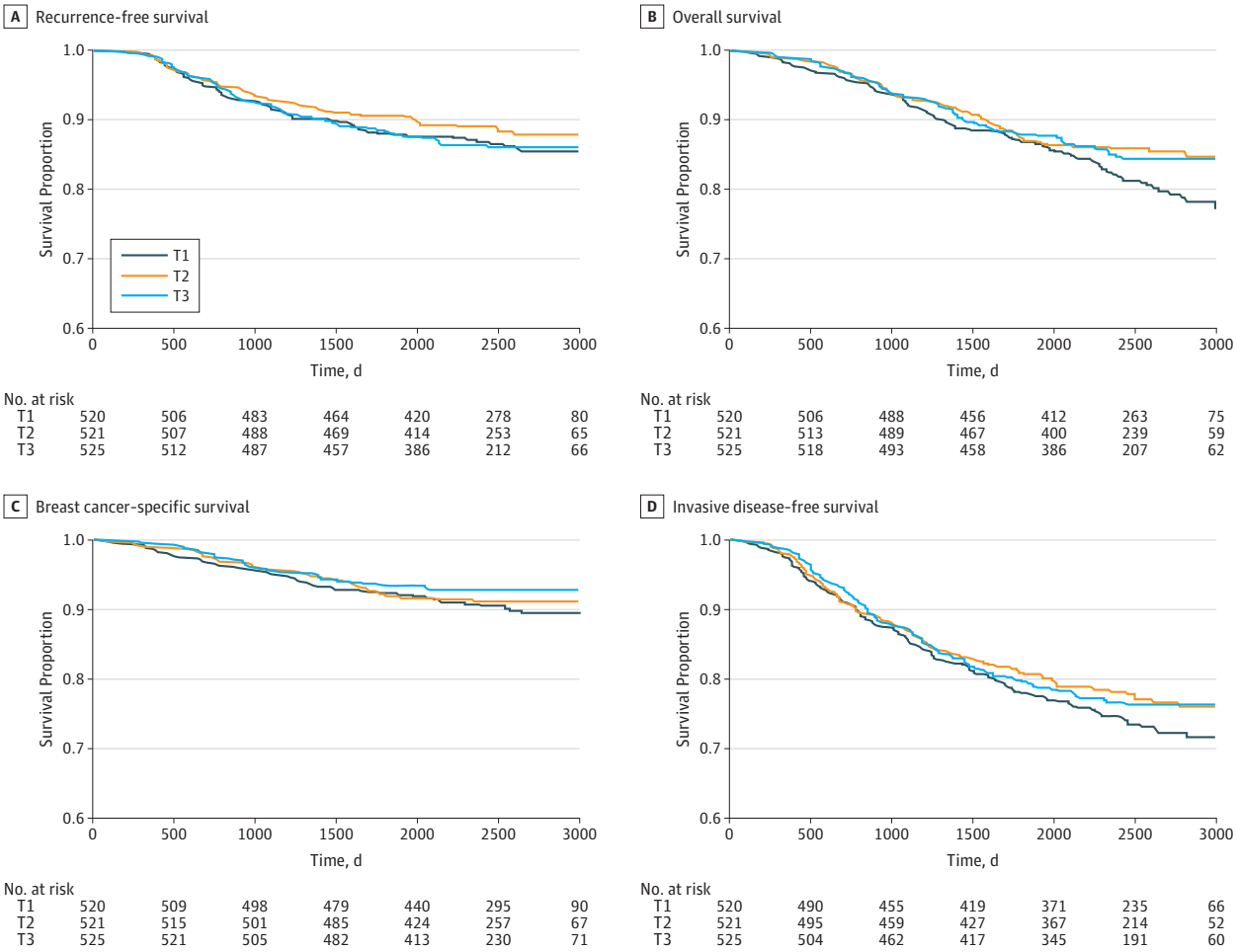
In addition to OS, the relationship of recurrence to plasma 25OHD levels has also been studied in the literature, yet the results have been mixed. Three studies reported an inverse association[11,12,15] and another 2[16,17] reported null associations. In our study, 25OHD levels were not related to RFS in the overall patient population or among postmenopausal women, but an inverse association was found among premenopausal women. Other survival outcomes were only occasionally evaluated in previous studies: BCSS was reported in 1 study with no association,[14] and SPC was reported in the MA14 trial with no association.[16] While our results of SPC were similar to the MA14 trial, we did observe a significant association of serum 25OHD levels with BCSS in premenopausal women.

The lack of consistency in results of outcomes other than OS may be, in part, due to differences across studies in validity and completeness of data on recurrence, SPCs, or cause of death. Compared with all-cause mortality, these outcomes might be challenging to track, collect, and ascertain, especially when the data are scattered across the health care system. Our study minimizes this limitation by being conducted within a single large integrated health care delivery system. There is also a possibility that the consistently observed associations of high blood 25OHD concentrations with lower risk of all-cause mortality reflect more of a general relationship that is not specific to patients with breast cancer.[18-20] We cannot completely refute this possibility, yet the significant associations with RFS and BCSS in our study suggest otherwise.

We advise caution when interpreting our findings of vitamin D association with outcomes due to potential residual confounding, given that serum 25OHD concentrations are subject to many environmental and physiological changes. To assess causality and place our findings in the context of the literature, we adapted the Bradford-Hill criteria as recently discussed by Robsahm et al,[32,33] regarding the association of vitamin D level and cancer risk. These criteria include temporality, strength, exposure-response, biological plausibility, and consistency. Because there is strong biological plausibility of vitamin D's anticancer properties from experimental studies,[1,2] good consistency across studies of OS, and a clear dose-response relationship, we focused our discussion on temporality and confounding effects.

In our study, blood samples were collected typically within 2 months after diagnosis, a timing likely prior to the development of symptomatic disease progression events. Given that treatment may affect 25OHD levels,[34-37] we assessed the impact of blood sample collection time relative to cancer diagnosis and treatment (surgery and initiation of radiation therapy, chemotherapy, or endocrine therapy). Only the time intervals from diagnosis to chemotherapy or endocrine therapy had a moderate influence on measured 25OHD levels. Even so, consideration of timing of blood sampling relative to these clinical events did not change associations of 25OHD with outcomes, when the other covariates were already in the models (data not shown). It is also possible that disease severity might adversely affect 25OHD concentrations. Thus, in addition to obesity and other nonclinical factors affecting 25OHD levels, we also adjusted for tumor stage, grade, and clinical subtype. Some of the associations were attenuated, yet the associations of 25OHD

Figure. Kaplan-Meier Survival Curves by Tertiles of Serum 25-Hydroxyvitamin D Levels



T1, T2, and T3 indicate first, second, and third tertiles.

with OS and among premenopausal women remained. There is also a concern of confounding by the systemic inflammatory response.[38] However, the relationship between inflammation and serum 25OHD concentrations is complex and also subject to reverse causation. In fact, vitamin D is known to be anti-inflammatory,[39,40] and suppression of tumor-caused inflammation may not be confounding, but along the causal pathway of vitamin D's association with breast cancer prognosis.

Based on this assessment, we agree with the conclusion drawn by Robsahm et al[33] that the relationship between serum 25OHD level and cancer survival may be causal. To definitively prove this, randomized clinical trials (RCTs) of vitamin D supplementation vs placebo would be necessary. However, in a feasibility study, 84.4% of patients with newly diagnosed breast cancer reported use of vitamin D–containing supplements, and only 12.7% of patients met the eligibility criteria,[41] possibly due to increasing public and medical recognition of the issue of vitamin D deficiency. The low levels of deficiency or insufficiency among those cancer patients led the authors to conclude that such an RCT would have limited feasibility. This issue may be further complicated by individuals' changing behaviors of sun exposure and dietary pattern, which may also "contaminate" an RCT schema. Indeed, except for an RCT in the United Kingdom to test the feasibility of a trial on vitamin D and longevity (VIDAL) (http://vidal.lshtm.ac.uk/), we are unaware of any other RCTs on vitamin D with cancer survival as the primary end point. The ongoing Vitamin D and Omega-3 Trial (VITAL) may eventually provide some data on cancer survival outcomes with continued follow-up[42]; however, it may take many years to accumulate enough events. In this regard, observational studies like ours from large prospective breast cancer cohorts are valuable to advance our understanding of the relationship between vitamin D level and breast cancer survival. In particular, studies of serum levels of vitamin D per se—as contrasted with studies of vitamin D supplementation—are not amenable to primary investigation through RCT study designs.

Limitations

Some limitations of our study should be noted. Although we show stronger associations of 25OHD levels with survival in premenopausal women, interaction testing with menopausal sta-

Table 2. Multivariable-Adjusted Associations of Tertiles of Serum 25-Hydroxyvitamin D (25OHD) Levels With Survival Outcomes in the Pathways Study Cohort

25OHD Level[a]	No. of Events/ Total	Nonclinical Factors[b]		Clinical Factors[c]	
		HR (95% CI)	*P* Value for Trend[d]	HR (95% CI)	*P* Value for Trend[d]
Recurrence-free survival					
T1	71/520	1 [Reference]		1 [Reference]	
T2	59/521	0.76 (0.55-1.06)	.88	0.87 (0.62-1.21)	.47
T3	70/525	0.98 (0.71-1.36)		1.13 (0.82-1.58)	
Overall survival					
T1	100/520	1 [Reference]		1 [Reference]	
T2	74/521	0.61 (0.46-0.80)	<.001	0.78 (0.59-1.04)	.03
T3	76/525	0.54 (0.40-0.72)		0.72 (0.54-0.98)	
Breast cancer-specific survival					
T1	51/520	1 [Reference]		1 [Reference]	
T2	45/521	0.81 (0.55-1.19)	.01	1.12 (0.76-1.67)	.53
T3	37/525	0.58 (0.38-0.90)		0.85 (0.55-1.33)	
Invasive disease-free survival					
T1	137/520	1 [Reference]		1 [Reference]	
T2	116/521	0.63 (0.45-0.88)	.004	0.81 (0.57-1.14)	.36
T3	119/525	0.61 (0.44-0.85)		0.85 (0.60-1.20)	
Second primary cancer-free survival					
T1	30/520	1 [Reference]		1 [Reference]	
T2	34/521	0.98 (0.61-1.59)	.64	0.98 (0.61-1.60)	.49
T3	32/525	0.89 (0.54-1.46)		0.84 (0.51-1.39)	

Abbreviations: HR, hazard ratio; T1, first tertile; T2, second tertile; T3, third tertile.

SI conversion factor: To convert 25OHD level to nanomoles per liter, multiply by 2.496.

[a] Cutoff points for serum 25OHD levels: T1, less than 16.75 ng/mL; T2, 16.75 to 25.09 ng/mL; T3, 25.10 ng/mL or greater.

[b] Adjusted for age at diagnosis, race/ethnicity, body mass index, and season of blood sample collection.

[c] Adjusted additionally for tumor stage, grade, and subtype by immunohistochemical analysis. Further adjustment for cancer treatment (surgery, radiation therapy, chemotherapy, and endocrine therapy) on the basis of clinical factors models did not substantially change the results.

[d] The tertiles were treated as an ordered value in the models.

tus was not significant. Based on ad hoc power calculation, this is likely due to inadequate sample size, especially when the interaction is quantitative, that is, in the same direction but with different magnitudes of the associations. We did not explicitly control for multiple comparison testing considering the 5 survival end points examined, as our analyses were conducted with a priori hypothesis based on the literature and our previous study. Last, our classification of IHC subtypes was based on 3 markers available from the clinical record, ER, PR, and HER2. The lack of Ki-67 data may have misclassified some luminal B cases featuring ER/PR positivity and high Ki-67 as luminal A. However, the proportion of these cases is expected to be small and our main findings are not focused on luminal tumors.

Conclusions

We found that low serum 25OHD levels were associated with poorer survival in this prospective cohort study of women with breast cancer. Furthermore, low serum 25OHD levels were also associated with prognostic characteristics, including TN subtype. The associations with prognostic characteristics and outcomes were independent of each other and were most prominent among premenopausal women. Our findings provide compelling observational evidence for inverse associations between vitamin D levels and risk of breast cancer progression and death.

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