

RESEARCH ARTICLE

Association Between Alterations in the Serum 25-Hydroxyvitamin D Status During Follow-Up and Breast Cancer Patient Prognosis

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Abstract

Background: Serum vitamin D status can affect the prognosis of breast cancer patients. Our aim was to determine the association between alterations in the 25-hydroxyvitamin D [25(OH)D] status during follow-up and the prognosis of breast cancer patients. Additionally, we evaluated the association between the 25(OH)D status at the time of diagnosis and the prognosis using a detailed age and stage categorization. **Materials and Methods:** Four hundred and sixty-nine Korean breast cancer patients were included. We collected patient clinicopathological data, including their serum 25(OH)D concentration at diagnosis and at the annual follow-up until 4 years after diagnosis. The patients were divided according to their 25(OH)D status at diagnosis into a deficient (<20 ng/ml) and a non-deficient (≥20 ng/ml) group. At follow-up, patients were categorized into the four following groups according to 25(OH)D status alterations: persistently deficient, improved, deteriorated and persistently non-deficient. **Results:** At diagnosis, 118 patients were classified into the deficient group and 351 into the non-deficient group. After a median follow-up period of 85.8±31.0 months, the patients with advanced-stage disease or an older age in the non-deficient group showed a significantly better survival compared with the deficient group. Furthermore, at the 1-year follow-up of 25(OH)D status, the persistently non-deficient group and the improved group had better survival compared with the other two groups. **Conclusions:** Our results suggest that maintaining an optimal 25(OH)D status at diagnosis and during the 1-year follow-up period is important for improving breast cancer patient survival.

Keywords: Breast cancer - 25-hydroxyvitamin D - follow-up - Survival

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Introduction

The anticarcinogenic properties of vitamin D, such as inhibiting cell proliferation and promoting apoptosis and cell differentiation in breast cancer, have been shown in several studies (Carlberg, 2003; Holick, 2006; Holick, 2009). Additionally, the importance of vitamin D in breast cancer epidemiology is being steadily revealed. In humans, most vitamin D is obtained in the form of vitamin D₃ (cholecalciferol) from the skin upon exposure to ultraviolet B solar radiation. Vitamin D₃ is transported to the liver and undergoes hydroxylation to form 25-hydroxyvitamin D [25(OH)D], the major circulating form of vitamin D. Subsequently, a second hydroxylation of 25(OH)D occurs in the kidneys and breast tissues to create 1,25-dihydroxyvitamin D, the active form of vitamin D (Bikle, 2009). The serum 1,25-dihydroxyvitamin D level does not provide a credible reflection of an individual's vitamin D status because of its short half-life and unmeasurable local production. Therefore, the serum 25(OH)D level is currently accepted as a better indicator of an individual's vitamin D status (Iqbal, 1994; Jacob et al., 2008; Crew et al., 2009). Several studies have reported that the serum 25(OH)D status is

associated with breast cancer risk, stage and patient age (Palmieri et al., 2006; Alipour et al., 2014; Shi et al. 2014).

To date, several studies have investigated the association between serum 25(OH)D status and breast cancer patient survival (Kermani et al., 2011). Although the results are conflicting, most of the studies reported a significant association between the serum 25(OH)D status and survival. For example, in 2009, Goodwin et al. reported that breast cancer patients with low 25(OH)D levels (<20 ng/ml) had poorer outcomes for long-term disease-free survival and overall survival compared with patients with an optimal 25(OH)D status (>28.8 ng/ml), as assessed with univariate analysis (Goodwin et al., 2009). Recently, in 2013, Villasenor et al. reported better overall survival in a 25(OH)D sufficient (>30 ng/ml) group compared with a 25(OH)D deficient (<20 ng/ml) group (Villasenor et al., 2013). Because of these reports, the interest in 25(OH)D status for breast cancer prognosis has notably increased.

Despite these significant results, the previous studies presented several limitations. First, a single measurement of the 25(OH)D concentration was performed. The 25(OH)D status can change after a breast cancer diagnosis for many reasons, such as adjuvant chemotherapy, life-

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style modifications, or vitamin D supplementation (Kim et al., 2014; Vance et al., 2014; Heaney et al., 2003). It is plausible that alterations in the 25(OH)D status during the follow-up period might affect a breast cancer patient's prognosis, including the risks of recurrence, metastasis and death. However, none of the currently available reports overcame this limitation, most likely due to the lack of follow-up data. The second limitation is that all the subjects included in previous studies, except those from the study of Kim et al. (Kim et al., 2011), were weighted toward westerners. Ethnic differences must be considered in the analysis of serum 25(OH)D concentrations and breast cancer pathophysiologies (McCullough et al., 2010). For instance, the median age at diagnosis of Asian breast cancer patients is younger than the median age of their western counterparts (Mousavi-Jarrahi et al., 2013). Furthermore, because of the traditions of oriental cultures, the amount of sunlight exposure in Asian populations is lower compared with Western populations (Lee et al., 2009; Kim et al., 2010). However, the only previous report concerning an Asian population had limited power to evaluate the effect of the 25(OH)D status at the time of diagnosis on survival because of the relatively small sample size (n=310, including patients with ductal carcinoma in situ (DCIS) and the short-term nature of the follow-up (23 months).

Therefore, in this study, we collected data on the 25(OH)D status in Korean breast cancer patients at the time of diagnosis and at the annual follow-up for 4 years. Our primary aim was to determine the association between alterations in the 25(OH)D status during follow-up and the prognosis of breast cancer patients. Additionally, we evaluated the association between the 25(OH)D status at the time of diagnosis and the prognosis using detailed age and stage categorization.

Materials and Methods

Study participants

In this study, we retrospectively reviewed the medical records of 491 Korean women with breast cancer diagnosed at St. Vincent hospital between January 2000 and December 2008. The analysis included women aged 18-90 years old at the time of diagnosis who were histologically confirmed with primary invasive (stage I-IIIc) breast cancer after surgery. We excluded the patients who had metastatic disease at the time of breast cancer diagnosis, had a history of previous malignancy, or had undergone neoadjuvant chemotherapy. Patients who lacked data on the serum 25(OH)D concentration at diagnosis were also excluded. After exclusion, 469 patients were included in the final study. All patients who were at risk for relapse received adjuvant chemotherapy followed by local radiotherapy and/or hormonal therapy according to the recommended therapeutic regimen at the time of surgery as determined by international guidelines, such as the national comprehensive cancer network (NCCN). This study was approved by the institutional review board of St. Vincent hospital.

25(OH)D assessment

The serum 25(OH)D concentration at diagnosis was determined for all of the patients using a blood sample drawn after breast cancer surgery but before adjuvant treatment (on average, within 6.58 days after surgery). The serum samples were stored in aliquots at -80°C until analysis and the serum concentration of 25(OH)D was measured by chemiluminescent microparticle immunoassay using the ARCHITECT-i system (Abbott Diagnostics). The intra-assay coefficient of variation was 10%. The follow-up serum 25(OH)D concentration was determined annually in the same manner, until 4 years after the diagnosis of breast cancer. Although the follow-up concentration data were not complete, mostly due to patients declining to schedule follow-up visits, at least one follow-up blood sample from each patient was obtained during the follow-up period.

The patients were divided into two groups according to their 25(OH)D status at diagnosis as follows: a deficient group (<20 ng/ml) or a non-deficient group (≥20 ng/ml). During the follow-up period, patients were categorized into the four following groups according to alterations in their 25(OH)D status: persistently deficient (deficient state at diagnosis and at follow-up evaluations), improved (deficient state at diagnosis and non-deficient state at follow-up evaluations), deteriorated (non-deficient state at diagnosis and deficient state at follow-up evaluations) and persistently non-deficient (non-deficient state at diagnosis and at follow-up evaluations). Confounders

We collected the data for potential confounders with known and suspected prognostic factors of breast cancer, including age at diagnosis, body-mass index (BMI), T stage, N stage, the stage of cancer based on the 7th American Joint Committee on Cancer (AJCC), lymphatic invasion (LI), vascular invasion (VI), Ki-67, histologic grade (HG) and the status of cancer treatment, such as radiotherapy, chemotherapy and hormonal therapy, as well as tumor characteristics including the expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor 2 (HER2) receptor. Fluorescence in situ hybridization (FISH) was unavailable during most of the study period. Therefore, we used immunohistochemical staining to classify the patients. A patient was considered ER- and PR-positive if 1% or more of the tumor was positively stained. An immunohistochemical staining score of 3+ for HER2 was considered positive and a HER2 score of 2+ was considered negative. The season when the blood sample at diagnosis was obtained was defined as follows: Spring, February-April; Summer, May-July; Autumn, August-October; and Winter, November-January.

Statistical analysis

We evaluated the association between the 25(OH)D status and the patients' disease-free survival (DFS), breast cancer-specific survival (BCSS) and overall survival (OS). DFS was defined as the period from surgery to the date of the first locoregional recurrence, distant metastasis, death from any cause or the date of the last follow-up. BCSS was defined as the period from the date of breast cancer diagnosis until the date of breast cancer-related death or the date of the last follow-up. OS was defined

as the period from the date of breast cancer diagnosis until the date of death (from any cause) or the date of the last follow-up. An independent t-test and chi-squared test were used to evaluate the significant differences between the clinicopathological features of the groups. The survival curves were obtained using the Kaplan-Meier method and log-rank tests were employed to compare the survival curves. Univariate and multivariate analyses were conducted using Cox's proportional hazard regression model to assess the independent prognostic significance of various confounders and hazard ratios (HRs) and 95% confidence intervals (95% CIs) were estimated for each variable. Age at diagnosis (years) was used as the time metric for all regression analysis and the multivariate models were adjusted for age; BMI; T stage; N stage; ER, PR and HER2 expression; chemotherapy; and lymphatic invasion. We tested and confirmed non-violation of the proportionality assumption based on Schoenfeld residuals. We conducted the assessments of effect modification by stratified analyses to examine whether the association between 25(OH)D status and survival varied by age and disease stage and heterogeneity between groups was tested by adding a multiplicative interaction term to the model and by using likelihood ratio tests for interactions. All statistical tests were two-sided and the statistical significance was set at $p < 0.05$. All statistical analyses were performed using SPSS version 12.0 (SPSS, Chicago, IL, USA).

Results

Association of the 25(OH)D status at diagnosis with tumor characteristics

The mean age at diagnosis was 49.61 ± 10.17 years and the mean serum 25(OH)D concentration at diagnosis was 33.53 ± 17.87 ng/ml (range: 2.6-100.8 ng/ml). A total of 118 (25.2%) patients were classified as 25(OH)D-deficient at diagnosis and the mean serum 25(OH)D concentration was 12.96 ± 4.16 ng/ml. Moreover, 351 (74.8%) patients were classified as 25(OH)D non-deficient at diagnosis with a mean serum 25(OH)D concentration of 40.44 ± 15.19 ng/ml. Table 1 presents the baseline characteristics of the 25(OH)D study population.

There was a significant difference in several characteristics between the two groups, which were as follows: PR positivity, chemotherapy, radiotherapy and follow-up duration. However, no significant differences were observed between the 25(OH)D status at diagnosis and BMI or cancer stage.

Associations between the 25(OH)D status at diagnosis and prognosis

Of the 469 breast cancer patients studied, 32 (6.8%) patients experienced locoregional recurrence or distant metastasis and 26 (5.5%) patients died during the follow-up period, including 22 (4.7%) patients who died of breast cancer. The median duration of follow-up for the entire cohort was 85.79 ± 31.01 months, which was significantly longer in the 25(OH)D non-deficient group (89.03 ± 30.01 months) compared with the 25(OH)D-deficient group (76.16 ± 32.05 months).

Figure 1 shows the association between the 25(OH)D status at diagnosis and survival. In the entire study population, only DFS showed a trend toward a poorer survival in the 25(OH)D-deficient group compared with the 25(OH)D non-deficient group ($p = 0.052$). However,

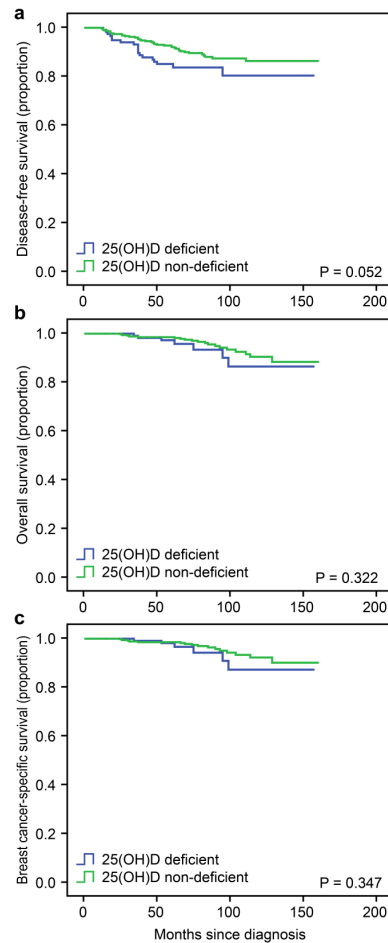


Figure 1. Association Between the 25(OH)D Status at Diagnosis and Survival. (a) disease-free survival (b) overall survival (c) breast cancer-specific survival

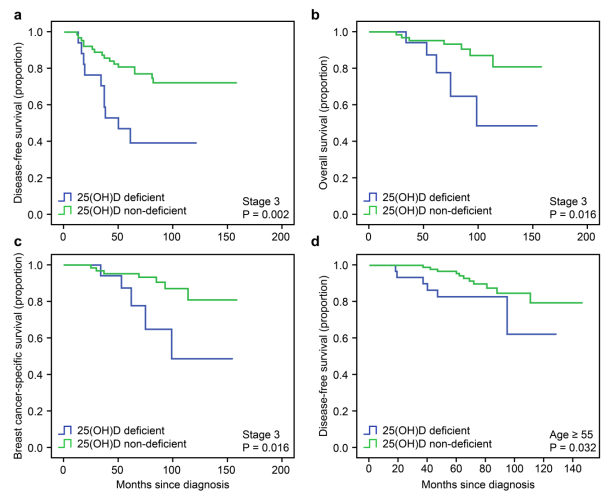


Figure 2. Association Between the 25(OH)D Status at Diagnosis and Survival After Stratification Based on the Stage and Patient Age. (a) disease-free survival in stage 3 patients (b) overall survival in stage 3 patients (c) breast cancer-specific survival in stage 3 patients (d) disease-free survival in patients with age ≥ 55

Table 1. Correlation Between the IER3 Expression and Clinicopathological Factors in 62 PHC P patients

		Total	Deficient (<20 ng/ml)	Non-deficient (≥20 ng/ml)	p value
		No. (%)	No. (%)	No. (%)	
Age	<55	343 (73.1%)	87 (73.7%)	256 (72.9%)	0.866
	≥55	126 (26.9%)	31 (26.3%)	95 (27.1%)	
BMI	≤25	268 (57.1%)	72 (61.0%)	196 (55.8%)	0.506
	25-30	176 (37.5%)	39 (33.0%)	137 (39.0%)	
	>30	25 (5.4%)	7 (6.0%)	18 (5.2%)	
T stage	1	204 (43.4%)	55 (46.6%)	149 (42.5%)	0.347
	2	237 (50.5%)	59 (50%)	178 (50.7%)	
	3	28 (6.1%)	4 (3.4%)	24 (6.8%)	
N stage	0	291 (62.0%)	75 (63.5%)	216 (61.5%)	0.389
	1	103 (21.9%)	27 (22.9%)	76 (21.7%)	
	2	43 (9.1%)	12 (10.2%)	31 (8.8%)	
AJCC stage	3	32 (6.8%)	4 (3.4%)	28 (8.0%)	0.6
	1	152 (32.4%)	41 (34.8%)	111 (31.6%)	
	2	236 (50.3%)	60 (50.8%)	176 (50.2%)	
Estrogen receptor	Positive	246 (52.4%)	59 (50%)	187 (53.3%)	0.538
	Negative	223 (47.5%)	59 (50%)	164 (46.7%)	
Progesteron receptor	Positive	244 (52.0%)	81 (68.6%)	163 (46.4%)	<0.001
	Negative	225 (48.0%)	37 (31.4%)	188 (53.6%)	
HER2 receptor	Positive	59 (12.6%)	10 (8.5%)	49 (14.0%)	0.12
	Negative	406 (86.8%)	107 (90.7%)	299 (85.2%)	
	Unknown	4 (0.8%)	1 (0.8%)	3 (0.8%)	
Ki-67	≤10%	188 (40.0%)	55 (46.6%)	133 (37.9%)	0.234
	>10%	153 (32.6%)	36 (30.5%)	117 (33.3%)	
	Unknown	128 (27.4%)	27 (22.9%)	101 (28.8%)	
Lymphatic invasion	Positive	122 (26.0%)	30 (25.4%)	92 (26.2%)	0.469
	Negative	286 (61.0%)	61 (51.7%)	225 (64.1%)	
	Unknown	61 (13.0%)	27 (22.9%)	34 (9.7%)	
Vascular invasion	Positive	8 (1.7%)	3 (2.6%)	5 (1.4%)	0.287
	Negative	400 (85.3%)	87 (73.7%)	313 (89.2%)	
	Unknown	61 (13.0%)	28 (23.7%)	33 (9.4%)	
Histologic grade	1	52 (11.1%)	12 (10.2%)	40 (11.4%)	0.914
	2	123 (26.2%)	30 (25.4%)	93 (26.5%)	
	3	84 (17.9%)	22 (18.6%)	62 (17.7%)	
	Unknown	210 (44.8%)	54 (45.8%)	156 (44.4%)	
Chemotherapy	Yes	301 (64.2%)	65 (55.1%)	236 (67.2%)	0.017
	No	168 (35.8%)	53 (44.9%)	115 (32.8%)	
Radiotherapy	Yes	273 (58.2%)	78 (66.1%)	195 (55.6%)	0.044
	No	196 (41.8%)	40 (33.9%)	156 (44.4%)	
Hormone therapy	Yes	353 (75.3%)	93 (78.8%)	260 (74.1%)	0.302
	No	116 (24.7%)	25 (21.2%)	91 (25.9%)	
Follow-up duration (months) Season of blood draw at diagnosis		85.79±31.01	76.16±32.05	89.03±30.01	<0.001
	Spring	116 (24.7%)	27 (22.9%)	89 (25.4%)	0.092
	Summer	117 (24.9%)	28 (23.7%)	89 (25.4%)	
	Autumn	149 (31.8%)	32 (27.1%)	117 (33.3%)	
	Winter	87 (18.6%)	31 (26.3%)	56 (15.9%)	

*SD standard deviation; BMI body mass index; AJCC American joint committee on cancer

after stratification based on tumor staging, the 25(OH)D-deficient group with advanced-stage disease showed significantly poorer survival for DFS ($p=0.002$), OS ($p=0.016$) and BCSS ($p=0.016$) compared with the non-deficient group (Figure 2). Similarly, the 25(OH)D-deficient group of older aged patients showed significantly poorer survival for DFS ($p=0.032$) compared with the 25(OH)D non-deficient group.

Association of alterations in the 25(OH)D status and prognosis

We determined the patient serum 25(OH)D concentrations annually until 4 years after diagnosis and

investigated the association between alterations in the 25(OH)D status during the follow-up period and survival. Regarding DFS, no statistically significant association was observed between an altered 25(OH)D status and survival until 4 years of follow-up after diagnosis. However, a trend in BCSS was observed, where the persistently non-deficient group and the improved group showed better survival compared with the other two groups at the 1-year follow-up ($p=0.051$) (Figure 3). Moreover, for OS, a significantly better survival was evident in the persistently non-deficient group and the improved group compared with the other two groups at the 1-year follow-up ($p=0.014$).

Table 2. Multivariate analysis evaluating the baseline risk factors that affect survival.

	Disease-free survival		Breast cancer-specific survival		Overall survival	
	Unadjusted HR (95% CI)	Adjusted HR (95% CI) ^a	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Unadjusted HR (95% CI)	Adjusted HR (95% CI) ^a
25(OH)D status at diagnosis						
Deficient (<20 ng/ml)	Referent	Referent	Referent	Referent	Referent	Referent
Non-deficient (≥20 ng/ml)	0.583 (0.336-1.012)	0.453 (0.251-0.817)*	0.638 (0.248-1.641)	0.464 (0.174-1.229)	0.646 (0.270-1.545)	0.455 (0.185-1.120)
25(OH)D status at 1 year follow-up						
Persistently deficient ^b	Referent	Referent	Referent	Referent	Referent	Referent
Improved ^c	0.725 (0.269-1.958)	0.411 (0.140-1.212)	0.201 (0.020-1.981)	0.128 (0.013-1.275)	0.151 (0.017-1.376)	0.040 (0.004-0.406)*
Deteriorated ^d	0.528 (0.209-1.333)	0.232 (0.080-0.674)*	0.746 (0.176-3.170)	0.288 (0.059-1.413)	0.561 (0.149-2.119)	0.129 (0.028-0.597)
Persistently non-deficient ^e	0.411 (0.190-0.888)*	0.176 (0.071-0.438)*	0.220 (0.054-0.892)*	0.125 (0.028-0.557)*	0.214 (0.063-0.728)*	0.032 (0.007-0.152)*
25(OH)D status at 2 years follow-up						
Persistently deficient	Referent	Referent	Referent	Referent	Referent	Referent
Improved	0.618 (0.086-4.412)	1.121 (0.188-6.664)	1.478 (0.152-14.34)	2.182 (0.207-23.01)	1.478 (0.152-14.34)	2.182 (0.207-23.01)
Deteriorated	0.599 (0.113-2.769)	0.329 (0.062-1.743)	0.801 (0.082-7.837)	0.356 (0.031-4.122)	0.801 (0.082-7.837)	0.356 (0.031-4.122)
Persistently non-deficient	0.542 (0.121-2.433)	0.345 (0.073-1.623)	0.385 (0.045-3.319)	0.271 (0.031-2.389)	0.385 (0.045-3.319)	0.271 (0.031-2.389)
25(OH)D status at 3 years follow-up						
Persistently deficient	Referent	Referent	Referent	Referent	Referent	Referent
Improved	1.133 (0.103-12.15)	0.286 (0.046-1.791)	0.282 (0.025-3.118)	0.383 (0.030-4.906)	0.316 (0.029-3.496)	0.370 (0.030-4.459)
Deteriorated	0.642 (0.067-6.185)	0.166 (0.039-0.709)	0.148 (0.013-1.668)	0.086 (0.06-1.179)	0.182 (0.016-2.070)	0.099 (0.070-1.322)
Persistently non-deficient	1.027 (0.133-7.966)	0.194 (0.0510-0.738)	0.221 (0.043-1.142)	0.209 (0.036-1.199)	0.294 (0.059-1.463)	0.249 (0.046-1.342)
25(OH)D status at 4 years follow-up						
Persistently deficient	Referent	Referent	Referent	Referent	Referent	Referent
Improved	0.512 (0.072-3.640)	0.586 (0.072-4.755)	0.233 (0.021-2.575)	0.360 (0.023-5.581)	0.228 (0.021-2.520)	0.277 (0.019-3.964)
Deteriorated	0.717 (0.139-3.700)	0.870 (0.154-4.922)	0.324 (0.045-2.318)	0.691 (0.061-7.806)	0.330 (0.046-2.365)	0.580 (0.059-5.737)
Persistently non-deficient	0.489 (0.109-2.186)	0.937 (0.104-8.489)	0.200 (0.039-1.033)	0.202 (0.033-1.237)	0.234 (0.047-1.160)	0.204 (0.035-1.188)

*HR hazard ratio; CI confidence interval. ^aAdjusted for age (<55, ≥55), BMI (<25, 25-30, >30), T stage (I,2,3), N stage (0,1,2,3), ER, PR, HER2, chemotherapy, and lymphatic invasion. ^bPersistently deficient; deficient state (<20 ng/ml) at diagnosis and at follow-up measurement. ^cImproved; deficient state (<20 ng/ml) at diagnosis and non-deficient state (≥20 ng/ml) at follow-up measurement. ^dDeteriorated; non-deficient state (≥20 ng/ml) at diagnosis and deficient state (<20 ng/ml) at follow-up measurement. ^ePersistently non-deficient; non-deficient (≥20 ng/ml) state at diagnosis and at follow-up measurement. *Statistical significance with P≤0.05

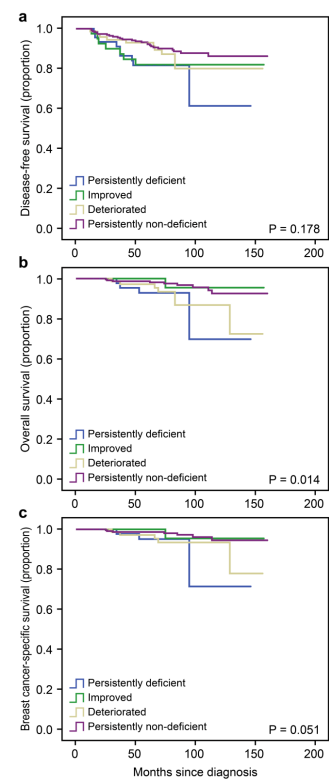


Figure 3. Association Between Alterations in the 25(OH)D Status at the 1-Year Follow-Up and Survival. (a) disease-free survival (b) overall survival (c) breast cancer-specific survival

Univariate and multivariate analyses for survival

We conducted univariate and multivariate analyses to determine the risk factors that can affect survival. The univariate analysis showed that the T stage, N stage, AJCC stage, LI, chemotherapy and alterations in the 25(OH)D status at the 1-year follow-up were significantly associated with DFS, OS and BCSS. Subsequently, the multivariate analysis showed that the 25(OH)D status at diagnosis was an independent prognostic indicator for DFS and that an alteration in the 25(OH)D status at the 1-year follow-up was similarly an independent prognostic indicator for DFS, OS and BCSS (Table 2).

Discussion

In this study, we observed a significant association between alterations in the 25(OH)D status during a 4-years follow-up after

diagnosis and breast cancer patient survival. To our knowledge, this study is the first to investigate alterations in the 25(OH)D status during the follow-up period as a prognostic predictor for breast cancer patients. Furthermore, we confirmed the association between the 25(OH)D status at diagnosis and breast cancer patient survival in detail by using stage and age-based stratifications.

Although many studies have been performed to date, a standard optimal 25(OH)D concentration is still under debate. Several reports from Western countries suggested that the optimal serum 25(OH)D concentration is >30 ng/ml (Holick., 2007; Peppone et al., 2011; Bilinski et al., 2013), but geographical and sociocultural differences can and do exist between Western and Asian populations. McCullough et al. reported a relatively low serum 25(OH)D concentration in Asian people compared with Western people, most likely due to the low incidence of supplement use, the lack of food fortification and a low amount of sunlight exposure (McCullough et al., 2010). Moreover, Manson et al. mentioned that a serum 25(OH)D concentration of >20 ng/ml was adequate to maintain bone health in 97.5% of North Americans (Manson et al., 2011). Thus, in our study, we established a cut-off value for the serum 25(OH)D concentration of 20 ng/ml. However, additional research is needed to determine the optimal value of the serum 25(OH)D concentration that prudently considers the various possible confounding factors.

To date, several studies have shown worse DFS in breast cancer patients with a low 25(OH)D concentration at diagnosis. In 2011, Kim et al. reported that a worse DFS occurred in the low 25(OH)D concentration (<20 ng/ml) group compared with the high 25(OH)D concentration (≥ 30 ng/ml) (HR=3.97; 95% CI 1.77-8.91) (Kim et al., 2011). Likewise, in 2012, Hatse et al. reported a worse DFS for postmenopausal breast cancer patients with a low 25(OH)D concentration (≤ 30 ng/ml) compared with patient with a high 25(OH)D concentration (>30 ng/ml) (relapse rate of 7.8% versus 5.6%, respectively, after 4.7 years of follow-up) (Hatse et al., 2012). These reports did not apply stage stratification to the analysis of DFS, however; the low 25(OH)D concentration groups of both studies included patients with significantly more advanced T- or N-stage disease which might have skewed the survival analysis results. In our study, after stage- and age-based stratifications, the 25(OH)D deficient (<20 ng/ml) group showed a significantly worse DFS, OS or BCSS compared with the non-deficient (≥ 20 ng/ml) group with advanced-stage (stage 3) disease and the older (≥ 55 years) patients. The explanation for these results is unclear; however, it is plausible that the low-estrogen environment of older aged women or the relatively increased risk of post-therapeutic micro-residual disease in advanced-stage breast-cancer patients may lead to an enhanced prognostic impact of the 25(OH)D status.

Our study showed a significant association between alteration in the 25(OH)D status at the 1-year follow-up and OS and BCSS. Clinically, the serum 25(OH)D status can be greatly affected within 1-year after surgery by adjuvant chemotherapy and by drastic lifestyle changes. Therefore, based on our findings, maintaining

an optimal serum 25(OH)D status within 1 year after diagnosis is important for improving patient's survival. Interestingly, the improved group and the persistently non-deficient group showed better survival compared with the persistently deficient group. This result suggested that even if a patient's 25(OH)D status at diagnosis was not optimal, an opportunity to improve the patient's survival arises by correcting their 25(OH)D status through vitamin D supplementation or appropriate life-style modifications. Although the institute of medicine and the endocrine society recommend 600-800 IU/day of vitamin D supplementation for healthy women to maintain bone health and optimal calcium homeostasis (Institute of Medicine., 2010; Holick., 2011), no clear consensus about a reasonable supplementation dose for breast cancer patients has emerged. For this reason, we emphasize the necessity of further studies to investigate this subject.

Similar to previous studies, our study had several limitations. First, our results had limited power because of the incomplete follow-up data for 25(OH)D measurement, including the season of blood sampling for the follow-up measurements. This limitation most likely resulted from the retrospective study design. Second, the sample size and incidence of survival events in our study were relatively small. Finally, we were not able to collect data about the patients' vitamin D supplementation, amount of physical activity and amount of sunlight exposure, all of which can influence an individual's 25(OH)D status. However, we expect that these limitations can be overcome by a larger, prospective study and thorough collection of clinical data.

In conclusion, our results demonstrate that the 25(OH)D status at diagnosis and at the 1-year follow-up is significantly associated with the survival of breast cancer patients. These findings may provide the grounds to justify clinical efforts to maintain and improve a patient's 25(OH)D status through vitamin D supplementation or lifestyle modifications, not only before diagnosis but also after surgery, to enhance breast cancer patients' survival particularly in advanced-stage cancer and older patients. To confirm our results, a more properly designed, larger scale study is urgently warranted.

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