Prediagnostic Plasma 25-Hydroxyvitamin D and Pancreatic Cancer Survival

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ABSTRACT

Purpose

Although vitamin D inhibits pancreatic cancer proliferation in laboratory models, the association of plasma 25-hydroxyvitamin D [25(OH)D] with patient survival is largely unexplored.

Patients and Methods

We analyzed survival among 493 patients from five prospective US cohorts who were diagnosed with pancreatic cancer from 1984 to 2008. We estimated hazard ratios (HRs) for death by plasma level of 25(OH)D (insufficient, < 20 ng/mL; relative insufficiency, 20 to < 30 ng/mL; sufficient ≥ 30 ng/mL) by using Cox proportional hazards regression models adjusted for age, cohort, race and ethnicity, smoking, diagnosis year, stage, and blood collection month. We also evaluated 30 tagging single-nucleotide polymorphisms in the vitamin D receptor gene, requiring $P < .002$ (0.05 divided by 30 genotyped variants) for statistical significance.

Results

Mean prediagnostic plasma level of 25(OH)D was 24.6 ng/mL, and 165 patients (33%) were vitamin D insufficient. Compared with patients with insufficient levels, multivariable-adjusted HRs for death were 0.79 (95% CI, 0.48 to 1.29) for patients with relative insufficiency and 0.66 (95% CI, 0.49 to 0.90) for patients with sufficient levels (P trend = .01). These results were unchanged after further adjustment for body mass index and history of diabetes (P trend = .02). The association was strongest among patients with blood collected within 5 years of diagnosis, with an HR of 0.58 (95% CI, 0.35 to 0.98) comparing patients with sufficient to patients with insufficient 25(OH)D levels. No single-nucleotide polymorphism at the vitamin D receptor gene met our corrected significance threshold of $P < .002$; rs7299460 was most strongly associated with survival (HR per minor allele, 0.80; 95% CI, 0.68 to 0.95; $P = .01$).

Conclusion

We observed longer overall survival in patients with pancreatic cancer who had sufficient prediagnostic plasma levels of 25(OH)D.

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INTRODUCTION

Pancreatic cancer is the third-leading cause of cancer-related deaths in the United States, and most patients die within 12 months of diagnosis.¹ Other than disease stage at presentation, few patient characteristics or circulating markers have been identified that predict survival time in patients with pancreatic ductal adenocarcinoma.²

Vitamin D is a fat-soluble vitamin that can be ingested in foods and is produced endogenously when sunlight triggers synthesis in the skin. Metabolites of vitamin D bind to the vitamin D

receptor (VDR) within cells, which regulate transcription of target genes by interacting with vitamin D response elements.³ In addition to the essential roles of vitamin D in calcium homeostasis and bone health, emerging evidence indicates that vitamin D and its analogs may inhibit cancer development and growth.³⁻⁵ Recent studies in laboratory models of pancreatic cancer have demonstrated the therapeutic potential of vitamin D analogs by engagement of VDR in tumor cells and supportive cells within the tumor stroma.⁶⁻⁸ Although studies that have evaluated circulating vitamin D and pancreatic cancer incidence have been conflicting,^{9,10} few studies have

been conducted to assess the association of vitamin D levels with survival in patients with pancreatic cancer.^{11,12}

To assess the potential prognostic effect of vitamin D status, we evaluated the association between prediagnostic plasma 25-hydroxyvitamin D [25(OH)D] and overall survival among patients with pancreatic cancer drawn from five large US prospective cohorts. In addition, we examined survival in relation to common germline genetic variants in *VDR*.

PATIENTS AND METHODS

Study Population

Our study population included patients with pancreatic cancer from five US prospective cohort studies. The Health Professionals Follow-up Study (HPFS) was established in 1986 when 51,529 male health professionals, age 40 to 75 years, responded to a mailed questionnaire. The Nurses' Health Study (NHS) was initiated in 1976 when 121,700 female registered nurses, age 30 to 55 years, responded to a mailed questionnaire. The Physicians' Health Study I (PHS I) is a randomized clinical trial of aspirin and β -carotene that enrolled 22,071 male physicians, age 40 to 84 years, in 1982. After completion of the randomly assigned components, study participants were observed. The Women's Health Initiative (WHI) Observational Study enrolled 93,676 postmenopausal women, age 50 to 79 years, between 1994 and 1998. The Women's Health Study (WHS) is a randomized, clinical trial of low-dose aspirin and vitamin E that enrolled 39,876 female professionals, age ≥ 45 years, between 1992 and 1995. The trial was completed in 2004, and participants were observed. Details of these cohorts have been described previously (HPFS,¹³ NHS,¹⁴ PHS,¹⁵ WHI,¹⁶ and WHS¹⁷). The study was approved by the Human Research Committee at the Brigham and Women's Hospital, and participants provided informed consent.

We included 493 patients with pancreatic adenocarcinoma who were diagnosed through 2008 and who had available plasma and no prior history of cancer, except nonmelanoma skin cancer. Incident cases of pancreatic cancer were identified by self-report or during follow-up of participant deaths. Deaths were ascertained from next-of-kin or the US Postal Service and by searching the National Death Index; this method has been shown to capture $> 98\%$ of deaths.¹⁸ Diagnoses were confirmed by review of medical records, death certificates, and/or tumor registry data by study physicians who were blinded to exposure data. Patients with non-adenocarcinoma histology or unclear survival time were excluded.

Individual characteristics and habits were obtained from baseline questionnaires at study enrollment in PHS, WHI, and WHS, and from questionnaires that preceded the date of blood draw in HPFS and NHS. In all cohorts, data were available for age at blood draw, sex, race/ethnicity, weight, height, smoking status, and history of diabetes. Date of diagnosis and pancreatic cancer stage at diagnosis were obtained from medical record review. Cancer stage was classified as local disease amenable to surgical resection; locally advanced disease with extrapancreatic extension rendering it unresectable, but without distant metastases; distant metastatic disease; or unknown.

Plasma Samples

Blood samples were collected from 18,225 male patients in HPFS from 1993 to 1995; 32,826 female patients in NHS from 1989 to 1990; 14,916 male patients in PHS from 1982 to 1984; 93,676 female patients in WHI from 1994 to 1998; and 28,345 female patients in WHS from 1992 to 1995. All blood samples were continuously stored in well-monitored freezers. Details on blood draw procedures, transportation, and storage of plasma samples in these cohorts have been described elsewhere.¹⁹ As described previously, plasma levels of 25(OH)D were assayed in the laboratory of Nader Rifai, MD (Children's Hospital, Boston, MA), by using the 25-Hydroxyvitamin D Enzyme Immunoassay Kit from

Immunodiagnostic Systems (Tyne & Wear, United Kingdom) per manufacturer instructions.⁹ Mean intra-assay coefficients of variance were $\leq 9\%$ for blinded, replicate, quality control samples.⁹

Single Nucleotide Polymorphism Selection and Genotyping

We selected 36 tagging single nucleotide polymorphisms (SNPs) in the *VDR* gene ± 20 kb by using the tagger algorithm implemented in Haploview, with a cutoff of $r^2 = 0.8$ and a minor allele frequency of $\geq 5\%$ in Whites from the HapMap Project database, and we forced in one previously reported SNP related to prostate cancer risk (rs11574143).²⁰ Of 397 patients with pancreatic cancer, DNA was extracted from archived buffy coat samples with Qiagen QIAmp (Valencia, CA) and whole-genome amplified with GE Healthcare Genomiphi (Pittsburgh, PA). All genotyping was carried out at the Partners HealthCare Center for Personalized Genetic Medicine by using a custom-designed Illumina Golden Gate genotyping assay (San Diego, CA). Three tagging SNPs were not supported by the Golden Gate platform and so could not be genotyped. Three SNPs deviated from Hardy-Weinberg Equilibrium at $P < .01$ and were excluded. Replicate samples included for quality control ($n = 44$ sample groups) had mean genotype concordance of 97.8% across the 30 SNPs.

Statistical Analysis

Association of plasma 25(OH)D with overall survival was examined by using Cox proportional hazards regression models to calculate hazard ratios (HRs) and 95% CI. Overall survival time was calculated from the date of cancer diagnosis until the date of death or last follow-up if a participant was still alive. Although optimal levels of 25(OH)D have not been definitively determined, plasma 25(OH)D < 20 ng/mL has been defined as insufficiency, 20 to < 30 ng/mL as relative insufficiency, and ≥ 30 ng/mL as sufficient^{21,22}; therefore, we investigated patient survival by these categories of plasma 25(OH)D. To compare more extreme levels of 25(OH)D in secondary analyses, we assessed the association between quintiles of 25(OH)D and survival. We examined HRs for each gender-specific cohort individually and computed a summary HR by using the DerSimonian and Laird random effects model.²³ Heterogeneity was tested by using Cochran's Q statistic.²⁴ Two-sided tests for trend were calculated by entering log-transformed 25(OH)D as a continuous variable into Cox proportional hazards regression models, given that 25(OH)D was not normally distributed. In multivariable models, we adjusted for potential confounders, including age at diagnosis, race and ethnicity, smoking status, year of diagnosis, cancer stage, month of blood collection, and time between blood collection and cancer diagnosis. Subsequently, we adjusted for body mass index (BMI) and history of diabetes, which were prognostic factors identified in prior studies of these populations.^{25,26} The proportionality of hazards assumption was satisfied by evaluating a time-dependent variable, which was the cross-product of log-transformed 25(OH)D and time ($P = .58$).

We estimated median survival time and survival curves for participants in each category adjusted for covariates by using direct adjusted survival estimation.^{27,28} This method uses Cox proportional hazards regression to estimate probabilities of survival at each follow-up time point for each individual and averages them to obtain an overall survival estimate. We assessed statistical interaction by BMI, tobacco use, cancer stage, season of blood collection, and time between blood collection and cancer diagnosis by entering main effect terms and a cross-product term of log-transformed 25(OH)D and stratification variable into the model, and evaluated likelihood ratio tests.

Pancreatic cancer and stromal cells express *VDR*, which translocates to the nucleus and binds vitamin D response elements to regulate gene expression and mediate the activity of vitamin D. Thus, we examined the association of tagging SNPs in *VDR* with survival by including each three-level genotype as a continuous variable (additive model) in multivariable-adjusted Cox proportional hazards regression models. SNPs were considered statistically significant if $P < .002$ (0.05 divided by 30 genotyped variants). We subsequently examined the joint association of 25(OH)D

and VDR genotype in Cox regression models. All analyses were performed with SAS 9.3 statistical package. All *P* values were two sided.

RESULTS

Baseline characteristics of patients with pancreatic cancer by category of prediagnostic 25(OH)D and by cohort are listed in [Table 1](#) and [Appendix Table A1](#) (online only), respectively. Median time between blood draw and pancreatic cancer diagnosis was 6.7 years. Mean prediagnostic 25(OH)D was 24.6 ng/mL, with 33% of patients classified as vitamin D insufficient. Among those with known disease stage, 16.0% had localized disease, 29.6% had locally advanced disease, and 54.3% had metastatic disease. Median survival by cancer stage was 17 months for those with localized disease, 10 months for those with locally advanced disease, and 3 months for those with metastatic disease. At the end of follow-up, 464 patients (94%) were deceased.

Higher plasma 25(OH)D levels were associated with greater survival (*P* trend = .01; [Table 2](#) and [Fig 1](#)). Compared with patients with insufficient levels of vitamin D, the multivariable-adjusted HRs for death were 0.79 (95% CI, 0.48 to 1.29) for patients with relative insufficiency and 0.66 (95% CI, 0.49 to 0.90) for patients with sufficient 25(OH)D levels. After adjustment for time between

blood collection and diagnosis, BMI, and history of diabetes, our results remained largely unchanged ([Table 2](#)). In analyses to compare more extreme values of 25(OH)D, a similar inverse relationship was noted between plasma 25(OH)D and patient survival ([Table 3](#)). Compared with those in the bottom quintile of 25(OH)D, patients in the top quintile had a multivariable-adjusted HR for death of 0.68 (95% CI, 0.48 to 0.95). We considered the possible influence of subclinical malignancy on plasma 25(OH)D. After excluding 19 patients who were diagnosed with pancreatic cancer within one year of blood collection, our results were not materially altered ([Appendix Table A2](#), online only).

No statistically significant interactions were observed by BMI, tobacco use, cancer stage, season of blood collection, or time between blood collection and diagnosis ([Table 4](#)). A stronger association of 25(OH)D and patient survival was identified in patients with blood collected within 5 years of diagnosis. Among patients with blood collected ≤ 5 years before cancer diagnosis, HR for death was 0.58 (95% CI, 0.35 to 0.98), a comparison of those with sufficient levels and those with insufficient levels of 25(OH)D. We evaluated the association of 25(OH)D and patient survival across the cohort study populations ([Fig 2](#)), and noted no statistically significant heterogeneity (*P* heterogeneity = .78). In a comparison of patients with sufficient 25(OH)D levels and patients with insufficient levels of plasma vitamin D, multivariable-adjusted HRs for death in sex-specific meta-analyses were 0.61 (95% CI, 0.42 to 0.89) for females (NHS, WHI, and WHS) and 0.79 (95% CI, 0.45 to 1.39) for males (HPFS and PHS).

We next examined genotypic variation at VDR in relation to patient survival. Three tagging SNPs in VDR were associated with survival to *P* < .05 in an additive model of inheritance, but no SNP met the predefined significance threshold after multiple hypothesis testing correction ([Appendix Table A3](#), online only). The most significant SNP was rs7299460, which had a per-allele HR for death of 0.80 (95% CI, 0.68 to 0.95; *P* = .01). In an exploratory analysis, patients who were homozygous recessive at rs7299460 and with sufficient 25(OH)D had the best overall survival, with an HR for death of 0.45 (95% CI, 0.22 to 0.93; [Appendix Table A4](#), online only).

DISCUSSION

In this prospective study of patients with pancreatic cancer from five large US cohorts, patients with sufficient levels of prediagnostic 25(OH)D had a 35% lower hazard for death compared with those who were vitamin D deficient. Association of prediagnostic 25(OH)D with survival remained unchanged after consideration of multiple potential confounding factors and seemed to be strongest when blood was collected within five years before pancreatic cancer diagnosis. Although several polymorphisms at the VDR locus were nominally associated with survival, no SNP met the threshold for statistical significance after multiple hypothesis testing correction. In aggregate, these results indicate that prediagnostic plasma 25(OH)D is a prognostic factor in patients with pancreatic cancer.

In laboratory models, vitamin D and its analogs have been shown to induce differentiation, promote apoptosis, and inhibit proliferation of pancreatic cancers.^{3,5,29-31} Furthermore, recent studies have indicated direct effects of ligand binding to VDR in pancreatic cancer cells, but also indirect effects by VDR

Table 1. Baseline Characteristics of Patients With Pancreatic Cancer by Prediagnostic Plasma 25(OH)D Level

Characteristic	Plasma 25(OH)D		
	Insufficient	Relative Insufficiency	Sufficient
25(OH)D level			
ng/mL	< 20	20 to < 30	≥ 30
nM	< 50	50 to < 75	≥ 75
No. of patients	165	212	116
Age at blood draw, years	63.9 (7.7)	64.0 (8.8)	61.6 (8.8)
Age at diagnosis, years	71.1 (8.1)	71.7 (8.1)	70.2 (8.7)
Female sex	126 (76.4)	152 (71.7)	67 (57.8)
Race/ethnicity			
White	142 (86.1)	188 (88.7)	106 (91.4)
Black	14 (8.5)	4 (1.9)	0 (0)
Other	5 (3.0)	8 (3.8)	2 (1.7)
Missing	4 (2.4)	12 (5.7)	8 (6.9)
Body mass index, kg/m ²	27.2 (5.0)	26.7 (5.5)	24.9 (3.5)
Physical activity, MET h/wk	11.5 (14.7)	16.8 (20.6)	26.9 (29.4)
Diabetes at blood draw	10 (6.1)	12 (5.7)	7 (6.0)
Tobacco use			
Never	65 (39.4)	96 (45.3)	43 (37.1)
Past	69 (41.8)	90 (42.5)	62 (53.4)
Current	29 (17.6)	25 (11.8)	11 (9.5)
Missing	2 (1.2)	1 (0.5)	0 (0)
Multivitamin use	60 (36.4)	104 (49.1)	53 (45.7)
Alcohol, ≥ 1 drink/d	46 (27.9)	45 (21.2)	32 (27.6)
Diagnosis period			
1984-2000	94 (57.0)	111 (52.4)	65 (56.0)
2001-2008	71 (43.0)	101 (47.6)	51 (44.0)
Cancer stage			
Localized	21 (12.7)	25 (11.8)	19 (16.4)
Locally advanced	48 (29.1)	49 (23.1)	23 (19.8)
Metastatic	68 (41.2)	97 (45.8)	55 (47.4)
Unknown	28 (17.0)	41 (19.3)	19 (16.4)

NOTE. Continuous variables reported as mean (standard deviation) and categorical variables reported as No. (%), unless otherwise noted.

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; MET, metabolic equivalent.

Table 2. HRs for Death Among Patients With Pancreatic Cancer by Plasma 25(OH)D Level

Model	Plasma 25(OH)D			<i>P</i> _{trend} *
	Insufficient	Relative Insufficiency	Sufficient	
25(OH)D level				
ng/mL	< 20	20 to < 30	≥ 30	
nM	< 50	50 to < 75	≥ 75	
Median survival, months	5	7	8	
Person-months	1,712	2,843	1,348	
Patients/deaths	165/154	212/199	116/111	
HR (95% CI)†	1.0	0.79 (0.48 to 1.29)	0.66 (0.49 to 0.90)	.01
HR (95% CI)‡	1.0	0.83 (0.52 to 1.32)	0.67 (0.49 to 0.92)	.02
HR (95% CI)§	1.0	0.77 (0.46 to 1.29)	0.62 (0.44 to 0.86)	.02

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; HR, hazard ratio.

*Test for trend calculated by entering log-transformed 25(OH)D as a continuous variable in Cox proportional hazards regression models.

†Meta-analysis of cohort-specific HRs (95% CI) from Cox proportional hazards regression models adjusted for age at diagnosis, race and ethnicity (white, black, other, unknown), smoking status (never, past, current, or missing), month of blood draw (2-month intervals), stage at diagnosis (localized, locally advanced, metastatic, or unknown), and year of diagnosis (1984-2000 or 2001-2008).

‡Model further adjusted for time between blood collection and cancer diagnosis (0 to < 5 years, 5 to < 10 years, or ≥ 10 years).

§Model further adjusted for body mass index (continuous) and history of diabetes (yes or no).

engagement in stromal cells.⁶⁻⁸ In a study by Sherman and colleagues,⁸ VDR acted as a master transcriptional regulator of pancreatic stellate cells, which reside within the tumor microenvironment. Treatment of pancreatic cancer in genetically engineered mice with a vitamin D analog led to remodeling of the stroma, increased concentration of intratumoral chemotherapy, and prolonged survival.

Few studies have investigated whether plasma levels of 25(OH)D predict patient survival. Circulating 25(OH)D is a preferred measure of vitamin D status as a result of its 2-week half-life and its reflection of both vitamin D ingested in the diet and synthesized in the skin.³² In a retrospective study of 178 patients who were treated at a tertiary cancer center and who underwent vitamin D measurement as part of clinical care, serum 25(OH)D < 20 ng/mL at the initial clinical visit was associated with poor prognosis (HR, 1.99; 95% CI, 1.16 to 3.43) in patients with advanced disease.¹² In contrast, baseline serum 25(OH)D levels were not

associated with overall survival in 256 patients with advanced pancreatic cancer who were enrolled in a randomized clinical trial of gemcitabine with or without bevacizumab.¹¹ However, these studies had relatively small sample sizes, different patient populations, and plasma 25(OH)D measurements made after cancer diagnosis. Because inadequate nutrition and limited outdoor activity as a result of morbidity of recently diagnosed pancreatic cancer can reduce 25(OH)D levels, measured levels likely do not reflect the long-term exposure to vitamin D before acute illness. Although little data are available related to pancreatic cancer, studies of several other malignancies have suggested that single nucleotide variants at VDR are associated with patient survival, including in smoking-associated cancers.³³⁻³⁵

Median survival times were longer by 3 to 4 months in patients with sufficient circulating 25(OH)D compared with patients with deficient levels. Two multiagent chemotherapy programs have recently been adopted for treatment of patients with metastatic pancreatic cancer, and have improved median overall survival by 1.8 months (gemcitabine plus nab-paclitaxel)³⁶ and 4.3 months (FOLFIRINOX)³⁷ compared with single-agent gemcitabine. Whether addition of vitamin D or its analogs to systemic chemotherapy can further improve patient outcomes is currently being investigated (eg, [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02030860) NCT02030860).

Several strengths of this study are notable, including large sample size, inclusion of patients with all stages of disease and from all geographic regions of the United States, and a prospective cohort study design. An important aspect of the prospective cohort design is its ability to fully capture the spectrum of patients with pancreatic cancer in terms of disease aggressiveness and stage of disease, as individuals are enrolled before their diagnosis and are not identified at selected tertiary care centers. Of note, survival times and stage distribution for patients in the five cohorts were similar to 121,713 patients who were included in the National Cancer Database, which is thought to capture 76% of patient cases of pancreatic cancer diagnosed in the United States each year.³⁸ The prospective cohort design reduces bias that results from reverse causation, as blood samples were collected before nutritional deficiencies and limited performance status that commonly develop at the time of pancreatic cancer diagnosis. Furthermore,

Fig 1. Overall survival curves by prediagnostic plasma 25-hydroxyvitamin D [25(OH)D] among patients with pancreatic cancer.

Table 3. HRs for Death Among Patients With Pancreatic Cancer by Quintile of Plasma 25(OH)D

Model	Quintile of Plasma 25(OH)D*				
	1	2	3	4	5
Median 25(OH)D, nM	35.7	48.2	58.3	68.8	88.1
Person-months	1,024	1,172	1,371	1,165	1,171
Patients/deaths	97/92	99/91	101/94	99/95	97/92
HR (95% CI)†	1.0	0.91 (0.64 to 1.30)	0.72 (0.51 to 1.01)	0.74 (0.50 to 1.09)	0.68 (0.48 to 0.95)
HR (95% CI)‡	1.0	0.91 (0.64 to 1.31)	0.77 (0.54 to 1.09)	0.76 (0.53 to 1.09)	0.66 (0.47 to 0.94)
HR (95% CI)§	1.0	0.96 (0.67 to 1.39)	0.74 (0.52 to 1.06)	0.76 (0.51 to 1.13)	0.66 (0.46 to 0.95)

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; HR, hazard ratio.

*Cohort-specific quintile ranges of plasma 25(OH)D: Health Professionals Follow-up Study (< 46.9, 46.9 to < 62.0, 62.0 to < 72.3, 72.3 to < 83.9, ≥ 83.9), Nurses' Health Study (< 44.3, 44.3 to < 54.1, 54.1 to < 64.7, 64.7 to < 82.9, ≥ 82.9), Physicians' Health Study (< 45.0, 45.0 to < 58.3, 58.3 to < 68.6, 68.6 to < 86.9, ≥ 86.9), Women's Health Initiative (< 38.2, 38.2 to < 49.4, 49.4 to < 59.7, 59.7 to < 68.9, ≥ 68.9), and Women's Health Study (< 44.9, 44.9 to < 56.4, 56.4 to < 61.8, 61.8 to < 79.7, ≥ 79.7).

†Meta-analysis of cohort-specific HRs (95% CI) from Cox proportional hazards regression models adjusted for age at diagnosis, race and ethnicity (white, black, other, unknown), smoking status (never, past, current, or missing), month of blood draw (2-month intervals), stage at diagnosis (localized, locally advanced, metastatic, or unknown), and year of diagnosis (1984-2000 or 2001-2008).

‡Model further adjusted for time between blood collection and cancer diagnosis (0 to < 5 years, 5 to < 10 years, or ≥ 10 years).

§Model further adjusted for body mass index (continuous) and history of diabetes (yes or no).

exclusion of patients who were diagnosed with pancreatic cancer within 1 year of blood collection did not materially alter our results. Circulating 25(OH)D was measured in a single laboratory as a single batch, with low coefficients of variance for blinded, replicate quality control samples, and extensive covariate data from all five cohorts allowed for rigorous control of potential confounding factors and evaluation of effect modification.

Limitations of the current study must also be considered. Among patients with pancreatic cancer, treatment programs likely varied, and we could not control for differences in treatment because this information was not collected in our cohorts. Nevertheless, chemotherapy and radiation have had only modest impact on patient survival,² and treatment programs were unlikely to have varied meaningfully by baseline 25(OH)D measured years

before diagnosis. We used overall mortality data in our analyses, as opposed to pancreatic cancer-specific mortality; however, pancreatic cancer is a highly lethal malignancy, with overall cure rates of < 5%, such that overall mortality is a good surrogate for cancer-specific mortality. Although circulating 25(OH)D was measured at a single time point, we have previously shown a high correlation of 0.70 for repeated measures of plasma 25(OH)D within individuals over time,³⁹ which suggests that a single measurement is a reasonable proxy for long-term levels of 25(OH)D. We cannot rule out that our findings may be influenced, in part, by residual confounding or that prediagnostic plasma 25(OH)D levels may mark overall health status impacting patient survival. Nonetheless, we included multiple possible confounding covariates in multivariable models without observing meaningful changes in risk estimates.

Table 4. HRs for Death Among Patients With Pancreatic Cancer by Plasma 25(OH)D Level Stratified by Covariates

Stratification Covariate	No. of Patients	Plasma 25(OH)D, HR (95% CI)*			P Interaction
		Insufficient	Relative Insufficiency	Sufficient	
BMI, kg/m ²					.44
< 25.0	216	1.0	0.92 (0.63 to 1.35)	0.86 (0.57 to 1.30)	
25.0-29.9	182	1.0	0.67 (0.46 to 0.97)	0.70 (0.44 to 1.12)	
≥ 30.0	95	1.0	0.64 (0.35 to 1.17)	0.37 (0.15 to 0.93)	
Tobacco use					.60
Never	204	1.0	0.87 (0.60 to 1.25)	0.64 (0.41 to 1.01)	
Past	221	1.0	0.68 (0.48 to 0.96)	0.68 (0.46 to 1.00)	
Current	65	1.0	1.12 (0.47 to 2.72)	1.17 (0.41 to 3.32)	
Cancer stage					.13
Localized	65	1.0	0.51 (0.21 to 1.24)	1.07 (0.39 to 2.96)	
Locally advanced	120	1.0	0.91 (0.53 to 1.56)	0.67 (0.35 to 1.27)	
Metastatic	220	1.0	0.91 (0.65 to 1.28)	0.63 (0.41 to 0.96)	
Season of blood draw					.66
Summer/autumn	207	1.0	0.91 (0.65 to 1.26)	0.76 (0.48 to 1.23)	
Winter/spring	286	1.0	0.71 (0.52 to 0.98)	0.64 (0.45 to 0.90)	
Time of blood draw to diagnosis, years					.20
≤ 5	165	1.0	0.64 (0.43 to 0.95)	0.58 (0.35 to 0.98)	
5-10	183	1.0	1.03 (0.70 to 1.50)	0.75 (0.47 to 1.19)	
> 10	145	1.0	0.88 (0.54 to 1.44)	1.02 (0.60 to 1.75)	

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; HR, hazard ratio.

*Adjusted for age at diagnosis, cohort (also adjusts for sex), race and ethnicity (white, black, other, or unknown), smoking status (never, past, current, or missing), month of blood draw (2-month intervals), stage at diagnosis (localized, locally advanced, metastatic, or unknown), and year of diagnosis (1984-2000 or 2001-2008).

Fig 2. Forest plot and meta-analysis of hazard ratios (HRs) for death among patients with pancreatic cancer, comparing those with sufficient levels of 25-hydroxyvitamin D with those with insufficient levels in the Health Professionals Follow-up Study (HPFS), Nurses' Health Study (NHS), Physicians' Health Study (PHS), Women's Health Initiative (WHI), and Women's Health Study (WHS). Solid squares and horizontal lines correspond to the cohort-specific multivariable-adjusted hazard ratios and 95% CIs. Area of the solid square reflects the cohort-specific weight (inverse of the variance). Diamond represents the meta-analysis multivariable-adjusted HR and 95% CI. Vertical line indicates an odds ratio of 1.0. HRs adjusted for age at diagnosis, race and ethnicity (white, black, other, unknown), smoking status (never, past, current, or missing), month of blood draw (2-month intervals), stage at diagnosis (localized, locally advanced, metastatic, or unknown), and year of diagnosis (1984-2000 or 2001-2008).

Finally, our study participants were predominantly individuals of European descent. African Americans have higher rates of vitamin D deficiency⁴⁰ and pancreatic cancer mortality.^{41,42} Additional studies of vitamin D levels and pancreatic cancer survival are warranted in racially diverse patient populations.⁴³

Higher prediagnostic plasma levels of 25(OH)D were associated with a statistically significant improvement in survival among patients with pancreatic cancer who were enrolled in five large, prospective cohorts. When considering these findings together with previously reported preclinical data in pancreatic cancer models, agonists of the vitamin D receptor are a potentially attractive therapeutic approach for investigation in this highly lethal malignancy.

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Disclosures provided by the authors are available with this article at www.jco.org.

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Appendix**Table A1.** Baseline Characteristics of Patients With Pancreatic Cancer by Cohort

Characteristic	HPFS	NHS	PHS	WHI	WHS	Total
No. of patients	76	101	72	208	36	493
Plasma 25(OH)D, nM	68.5 (24.3)	65.4 (25.7)	65.8 (22.1)	55.2 (19.6)	60.4 (19.5)	61.3 (22.7)
Age at blood draw, years	65.6 (7.8)	60.5 (6.7)	56.9 (8.6)	67.0 (7.3)	58.9 (8.9)	63.4 (8.5)
Age at diagnosis, years	72.7 (8.4)	70.7 (7.0)	72.0 (9.4)	71.9 (7.5)	63.1 (9.1)	71.2 (8.3)
Female sex	0 (0)	101 (100)	0 (0)	208 (100)	36 (100)	345 (70.0)
Race/ethnicity						
White	71 (93.4)	100 (99.0)	53 (73.6)	178 (85.6)	34 (94.4)	436 (88.4)
Black	1 (1.3)	1 (1.0)	1 (1.4)	14 (6.7)	1 (2.8)	18 (3.7)
Other	1 (1.3)	0 (0)	0 (0)	14 (6.7)	0 (0)	15 (3.0)
Missing	3 (3.9)	0 (0)	18 (25.0)	2 (1.0)	1 (2.8)	24 (4.9)
Body mass index, kg/m ²	25.7 (3.3)	25.6 (5.0)	25.7 (2.9)	27.4 (5.9)	26.4 (5.3)	26.4 (5.0)
Physical activity, MET h/wk	36.9 (38.5)	15.4 (17.8)	12.0 (11.2)	13.0 (13.2)	17.8 (21.8)	17.4 (22.1)
History of diabetes	3 (3.9)	5 (5.0)	3 (4.2)	15 (7.2)	3 (8.3)	29 (5.9)
Tobacco use						
Never	26 (34.2)	40 (39.6)	29 (40.3)	96 (46.2)	13 (36.1)	204 (41.4)
Past	42 (55.3)	41 (40.6)	30 (41.7)	92 (44.2)	16 (44.4)	221 (44.8)
Current	8 (10.5)	20 (19.8)	13 (18.1)	17 (8.2)	7 (19.4)	65 (13.2)
Missing	0 (0)	0 (0)	0 (0)	3 (1.4)	0 (0)	3 (0.6)
Multivitamin use	41 (53.9)	46 (45.5)	21 (29.2)	99 (47.6)	10 (27.8)	217 (44.0)
Alcohol ($\geq$ 1 drink/d)	32 (42.1)	24 (23.8)	24 (33.3)	36 (17.3)	7 (19.4)	123 (24.9)
Median time blood draw to diagnosis, years	6.5	10.6	16.6	5.2	4.1	6.7
Diagnosis period						
1984-2000	39 (51.3)	52 (51.5)	45 (62.5)	103 (49.5)	31 (86.1)	270 (54.8)
2001-2008	37 (48.7)	49 (48.5)	27 (37.5)	105 (50.5)	5 (13.9)	223 (45.2)
Cancer stage						
Localized	11 (14.5)	19 (18.8)	14 (19.4)	13 (6.3)	8 (22.2)	65 (13.2)
Locally advanced	11 (14.5)	12 (11.9)	14 (19.4)	77 (37.0)	6 (16.7)	120 (24.3)
Metastatic	33 (43.4)	44 (43.6)	32 (44.4)	91 (43.8)	20 (55.6)	220 (44.6)
Unknown	21 (27.6)	26 (25.7)	12 (16.7)	27 (13.0)	2 (5.6)	88 (17.8)
Median survival time, months						
All patients	5	5	6.5	8	5	6
By stage						
Localized	14	21	14.5	25.0	11.5	17
Locally advanced	10	9	8.5	12.0	10.5	10
Metastatic	4	3	3.5	4.0	4.5	3
Unknown	4	6	7.0	5.0	3.0	5

NOTE. Continuous variables reported as mean (standard deviation) and categorical variables reported as No. (%), unless otherwise noted.

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; HPFS, Health Professionals Follow-up Study; MET, metabolic equivalent; NHS, Nurses' Health Study; PHS, Physicians' Health Study; WHI, Women's Health Initiative; WHS, Women's Health Study.

Table A2. HRs for Death Among Patients With Pancreatic Cancer by Plasma 25(OH)D Level, Excluding Patients Diagnosed Within 1 Year of Blood Draw

Model	Plasma 25(OH)D			<i>P</i> _{trend} *
	Insufficient	Relative Insufficiency	Sufficient	
Range				
ng/mL	< 20	20 to < 30	≥ 30	
nM	< 50	50 to < 75	≥ 75	
Median survival, months	5	7	8	
Person-months	1,662	2,541	1,325	
Patients/deaths	159/148	201/189	113/108	
HR (95% CI)†	1.0	0.84 (0.53 to 1.33)	0.62 (0.45 to 0.85)	.02
HR (95% CI)‡	1.0	0.87 (0.55 to 1.36)	0.63 (0.45 to 0.87)	.03
HR (95% CI)§	1.0	0.75 (0.41 to 1.36)	0.59 (0.41 to 0.84)	.04

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; HR, hazard ratio.

*Test for trend calculated by entering log-transformed 25(OH)D as a continuous variable in Cox proportional hazards regression models.

†Meta-analysis of cohort-specific HRs (95% CI) from Cox proportional hazards regression models adjusted for age at diagnosis, race and ethnicity (white, black, other, or unknown), smoking status (never, past, current, or missing), month of blood draw (2-month intervals), stage at diagnosis (localized, locally advanced, metastatic, or unknown), and year of diagnosis (1984-2000 or 2001-2008).

‡Model further adjusted for time between blood collection and cancer diagnosis (0 to < 5 years, 5 to < 10 years, or ≥ 10 years).

§Model further adjusted for body mass index (continuous) and history of diabetes (yes or no).

Table A3. SNPs in *VDR* Ranked by Association *P* Value With Overall Survival Among Patients With Pancreatic Cancer

SNP*	No. of Patients	Minor Allele Frequency, %	Additive Model	<i>P</i>
			HR (95% CI)	
rs7299460	391	0.30	0.80 (0.68 to 0.95)	.01
rs11568820	388	0.22	0.79 (0.65 to 0.96)	.02
rs4334089	388	0.23	0.81 (0.68 to 0.98)	.03
rs886441	392	0.18	0.82 (0.67 to 0.99)	.04
rs2853564	396	0.38	1.16 (0.99 to 1.35)	.07
rs2239179	392	0.44	1.14 (0.99 to 1.33)	.08
rs2239182	392	0.47	0.89 (0.77 to 1.03)	.12
rs7295021	395	0.20	0.86 (0.70 to 1.05)	.13
rs11168293	392	0.34	1.13 (0.96 to 1.32)	.15
rs2239186	395	0.20	0.89 (0.74 to 1.06)	.20
rs7132324	391	0.35	1.10 (0.94 to 1.29)	.24
rs7963776	388	0.46	0.91 (0.79 to 1.06)	.24
rs2254210	396	0.39	1.09 (0.94 to 1.26)	.28
rs2189480	390	0.34	0.92 (0.79 to 1.08)	.30
rs2283342	392	0.15	0.90 (0.72 to 1.11)	.31
rs4760648	391	0.43	0.92 (0.78 to 1.08)	.32
rs10875693	389	0.33	1.08 (0.92 to 1.27)	.35
rs2238136	393	0.27	0.92 (0.78 to 1.09)	.35
rs11574077	391	0.05	1.18 (0.83 to 1.66)	.36
rs1859281	395	0.07	1.13 (0.83 to 1.53)	.44
rs1544410	359	0.37	1.05 (0.90 to 1.23)	.53
rs7310552	393	0.40	1.04 (0.88 to 1.22)	.64
rs10747524	329	0.49	0.97 (0.81 to 1.16)	.71
rs2107301	394	0.28	0.97 (0.83 to 1.14)	.71
rs11168275	392	0.24	0.97 (0.81 to 1.16)	.73
rs2544037	394	0.42	1.02 (0.87 to 1.18)	.84
rs11574032	297	0.09	1.02 (0.75 to 1.40)	.89
rs12721364	391	0.14	1.01 (0.81 to 1.26)	.93
rs11574143	391	0.11	1.01 (0.80 to 1.27)	.96
rs3819545	393	0.39	1.00 (0.86 to 1.17)	.96

Abbreviations: HR, hazard ratio; SNP, single nucleotide polymorphism; VDR, vitamin D receptor.

*Effect of each minor allele of the SNP on survival in Cox proportional hazards regression models adjusted for age at diagnosis (years, continuous), cohort (also adjusts for sex), race and ethnicity (white, black, other, or unknown), smoking status (never, past, current, or missing), stage at diagnosis (localized, locally advanced, metastatic, or unknown), and year of diagnosis (1984-2000 or 2001-2008).

Vitamin D and Pancreatic Cancer Survival

Table A4. HRs (95% CI) for Death Among Patients With Pancreatic Cancer by Plasma 25(OH)D and Stratified by rs7299460 Genotypes in *VDR*

SNP	Genotype	No.	Plasma 25(OH)D, HR (95% CI)		
			Insufficient	Relative Insufficiency	Sufficient
rs7299460	GG	194	1.0	0.97 (0.69 to 1.38)	0.78 (0.51 to 1.18)
	AG	162	0.84 (0.57 to 1.25)	0.67 (0.46 to 0.98)	0.85 (0.56 to 1.30)
	AA	35	0.71 (0.31 to 1.59)	0.62 (0.33 to 1.16)	0.45 (0.22 to 0.93)

NOTE. Adjusted for age at diagnosis (years, continuous), cohort (also adjusts for sex), race and ethnicity (white, black, other, or unknown), smoking status (never, past, current, or missing), month of blood draw (2-month intervals), stage at diagnosis (localized, locally advanced, metastatic, or unknown), and year of diagnosis (1984-2000 or 2001-2008).

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; SNP, single nucleotide polymorphism; VDR, vitamin D receptor.