Plasma Vitamin D Concentration Influences Survival Outcome After a Diagnosis of Colorectal Cancer

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Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

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ABSTRACT

Purpose

We investigated whether the plasma level of 25-hydroxyvitamin D (25-OHD) after a diagnosis of colorectal cancer (CRC) influences survival outcome.

Patients and Methods

We prospectively studied 1,598 patients with stage I to III CRC. We sought association between plasma 25-OHD and stage-specific survival and tested for interaction between 25-OHD level and variation at the vitamin D receptor (VDR) gene locus. Blood was sampled postoperatively, and plasma was assayed for 25-OHD by liquid chromatography-tandem mass spectrometry. VDR polymorphisms (rs1544410, rs10735810, rs7975232, rs11568820) were genotyped, and haplotypes were inferred by using BEAGLE software. We tested for association between survival and 25-OHD, VDR genotype/haplotype, and after applying a VDR genotype–25-OHD interaction term. We conducted Kaplan-Meier survival analysis and used Cox proportional hazards models to estimate adjusted hazard ratios (HRs).

Results

We found strong associations between plasma 25-OHD concentration and CRC-specific ($P = .008$) and all-cause mortality ($P = .003$). Adjusted HRs were 0.68 (95% CI, 0.50 to 0.90) and 0.70 (95% CI, 0.55 to 0.89), respectively (highest v lowest 25-OHD tertile), particularly in stage II disease (HR, 0.44; $P = .004$ for CRC-specific mortality). We detected gene-environment interactions between 25-OHD concentration and rs11568820 genotype for CRC-specific ($P = .008$) and all-cause ($P = .022$) mortality, number of protective alleles ($P = .004$ and $P = .018$, respectively), and GAGC haplotype at the VDR locus for all-cause mortality ($P = .008$).

Conclusion

In patients with stage I to III CRC, postoperative plasma vitamin D is associated with clinically important differences in survival outcome, higher levels being associated with better outcome. We observed interactions between 25-OHD level and VDR genotype, suggesting a causal relationship between vitamin D and survival. The influence of vitamin D supplementation on CRC outcome will require further investigation.

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INTRODUCTION

Vitamin D deficiency is implicated in the etiology of several common diseases, including cancer.¹ Colorectal cancer (CRC) is the third most frequent cancer worldwide, with an estimated 1.2 million cases or more and 600,000 deaths annually, and yet it is potentially largely preventable.² Worldwide, there is substantial variation in CRC incidence (10-fold) and mortality (six-fold)² that may be attributable to different vitamin D levels between populations.³

Dietary vitamin D intake is generally low, even in those taking supplements.^{4,5} Hence, skin synthesis during exposure to sunlight (ultraviolet B) is the major source of vitamin D. Such synthesis is pro-

foundly influenced by geographical latitude and meteorologic factors. Modern lifestyle further compounds any deficit, because daylight hours are increasingly spent indoors. Thus, maintaining sufficient vitamin D levels is severely compromised, particularly in northern latitudes.

Establishing a causal relationship between CRC incidence and vitamin D deficiency is challenging because environmental risk factors associated with CRC may also be associated with vitamin D deficiency (co-causality; eg, level of physical activity), the presence of a tumor and its treatment may actually deplete vitamin D (reverse causation): decreased circulating 25-hydroxyvitamin D (25-OHD) concentration has been reported after

Table 1. Baseline Characteristics of Patients With CRC by May-Adjusted Serum 25-OHD Tertiles in SOCCS, 1999-2006

Characteristic	May-Adjusted Vitamin D Tertile												P ^a
	Tertile 1 (< 7.25 ng/mL)				Tertile 2 (7.25 to 13.25 ng/mL)				Tertile 3 (> 13.25 ng/mL)				
	No.	%	Mean	SD	No.	%	Mean	SD	No.	%	Mean	SD	
No. of patients	546				528				524				
25-OHD (ng/mL)			3.82	2.6			10.26	1.73			20.34	6.5	
Age (years)			62.56	10.48			62.73	10.71			59.65	10.6	$< .001^a$
Proportion of females													.69 ^b
In tertile		43				41				44			
All		34				32				34			
Vitamin D intake, μ g/day													
Diet			4.6	3.33			4.65	2.46			4.7	2.81	.64 ^a
Supplements													
< 5	485	88.8			451	85.4			419	80			
5-10	51	9.3			62	11.7			78	14.9			
> 10	10	1.8			15	2.8			27	5.2			$< .001^b$
Calcium intake (g per day)			1.18	0.46			1.22	0.7			1.21	0.51	.96 ^a
Energy intake (kCal)			2,671	1,032			2,627	940			2,672	1,128	.65 ^a
Alcohol (g per day)			13.22	17.39			12.52	15.9			13.38	15	.57 ^a
Red meat (portions per day)			1.42	0.89			1.36	0.77			1.35	0.74	.10 ^a
Deprivation score (% below median) ^c		52				57				58			.10 ^b
Family history risk of CRC													
Low	430	85.3			427	84.7			396	80			
Medium	67	13.3			68	13.5			92	18.6			
High	7	1.4			9	1.8			7	1.4			.12 ^b
Smoking status													
Ex-smoker		38				45				39			.0015 ^b
Current smoker		21				12				17			
NSAIDs ^d		33				31				28			.20 ^b
BMI ^e			27.27	4.86			26.65	4.11			26.03	4.08	$< .001^a$
AJCC stage													
I	119	21.8			122	23.1			128	24.4			
II	212	38.8			216	40.9			197	37.6			
III	215	39.4			190	36			199	38			.67 ^b
Physical activity category ^f													
1	317	67.2			262	58.2			248	55.1			
2	95	20.1			111	24.7			111	24.7			
3	41	8.7			47	10.4			59	13.1			
4	19	4			30	6.7			32	7.1			.0079 ^b
Time from diagnosis to sample (days)													
≤ 2 months	174	31.9			160	30.3			148	28.2			
2-6 months	220	40.3			214	40.5			235	44.8			
6-12 months	101	18.5			109	20.6			93	17.7			
1-2 years	51	9.3			45	8.5			48	9.2			.64 ^b
Season of treatment													
Fall	132	24.2			147	27.8			142	27.1			
Spring	145	26.6			115	21.8			149	28.4			
Summer	140	25.6			123	23.3			104	19.8			
Winter	129	23.6			143	27.1			129	24.6			.07 ^b
Season of blood collection													
Fall	158	29.2			124	23.6			130	25			
Spring	113	20.9			145	27.6			135	25.9			
Summer	154	28.5			125	23.8			140	26.9			
Winter	116	21.4			132	25.1			116	22.3			.05 ^b
Survival													
Alive	331	60.6			355	67.2			381	72.7			
Dead	215	39.4			173	32.8			143	27.3			$< .001^{b,g}$
Cause of death													
CRC	143	66.5			119	68.8			101	70.6			
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Table 1. Baseline Characteristics of Patients With CRC by May-Adjusted Serum 25-OHD Tertiles in SOCCS, 1999-2006 (continued)

May-Adjusted Vitamin D Tertile													P ^a
Characteristic	Tertile 1 (< 7.25 ng/mL)				Tertile 2 (7.25 to 13.25 ng/mL)				Tertile 3 (> 13.25 ng/mL)				
	No.	%	Mean	SD	No.	%	Mean	SD	No.	%	Mean	SD	
Other cause	45	20.9			32	18.5			25	17.5			
Other cancer	24	11.2			20	11.6			17	11.9			
Unknown	3	1.4			2	1.2			0	0			

NOTE. Values estimated from May-adjusted 25-hydroxyvitamin D (25-OHD) concentration.

Abbreviations: AJCC, American Joint Committee on Cancer; BMI, body mass index; CRC, colorectal cancer; NSAID, nonsteroidal anti-inflammatory drug; SD, standard deviation; SOCCS, Study of Colorectal Cancer in Scotland.

^aOne-way analysis of variance (continuous May-adjusted 25-OHD was used).

^b χ^2 test (May-adjusted 25-OHD tertiles were used).

^cSocioeconomic status (based on Carstairs deprivation index, 2001 Census).

^dRegular intake of NSAIDs (yes/no).

^eBMI calculated from self-reported height and weight a year before CRC diagnosis and subsequently validated.³²

^fLevel of physical activity (hours of running, cycling, and other sports activities; four categories).

^g χ^2 test on No. of patients dead or alive per tertile.

^hUnknown cause of death was omitted from χ^2 test.

elective knee surgery,⁶ and voluntary or involuntary changes in a patient's diet and lifestyle, such as being bed-bound or suffering fatigue, may decrease time spent outdoors and have further impact on 25-OHD levels. Evidence from studies investigating the relationship between CRC risk and exposure to solar radiation and vitamin D intake and/or circulating levels supports an inverse relationship between vitamin D and risk.^{7,8} Moreover, interaction between vitamin D level and functional sequence variants at the vitamin D receptor (VDR) gene locus also influence CRC risk.^{9,10}

The relationship between vitamin D and CRC mortality is even less well understood. An ecological study published more than 30 years ago was the first to report an inverse relationship between CRC mortality and exposure to solar radiation.¹¹ Similarly, better survival in patients diagnosed during summer and fall was shown more recently.¹² These provide circumstantial evidence linking vitamin D levels with cancer outcome. Higher intake of dietary vitamin D¹³ and higher circulating levels of 25-OHD¹³⁻¹⁷ have also been linked to better survival after CRC diagnosis. However, published work used proxies for plasma vitamin D level in patients with cancer (season of diagnosis, ultraviolet B intensity, dietary vitamin D). Prediagnostic plasma 25-OHD level that has been associated with cancer survival seems unlikely to reflect vitamin D level in the period following diagnosis, surgery, and chemotherapy. Predicted vitamin D level at diagnosis (from assays performed years before diagnosis) also suggests an association between low levels and poor CRC survival outcome.¹⁶

In this study, we investigated whether circulating vitamin D concentration influences survival after surgical intervention for CRC. We directly assayed total 25-OHD, which reflects both dietary intake and skin synthesis of vitamin D.^{18,19} To mitigate against potential confounding effects due to reverse causation, thereby implicating a causal relationship, we tested for interaction between plasma vitamin D level and genotype at the VDR locus.

PATIENTS AND METHODS

Study Population and Follow-Up

The Study of Colorectal Cancer in Scotland (SOCCS) is a prospective, population-based case-control study designed to identify genetic and environ-

mental factors that have an impact on CRC risk and survival outcome.²⁰ All participants provided informed written consent, and research was approved by the MultiCentre Research Ethics Committee for Scotland, local research ethics committees, and National Health Service management. In this study, we measured 25-OHD plasma levels in 1,598 patients with stage I to III CRC who underwent surgical resection with curative intent and for whom we collected detailed clinical, pathologic, dietary, and lifestyle information.

Follow-up was through systematic search of the Scottish national records system. Deaths were ascertained through linkage to National Records of Scotland. Primary cause of death (CRC, other cancer, other cause) was assigned from death certificates separately by two researchers (L.Z., F.V.N.D.; > 99% concordance). Follow-up was calculated as time between blood sampling and death or between sampling and censor date (January 31, 2013) for patients who were not known to have died.

Plasma Vitamin D Assay

To minimize technical variability, all plasma samples were batched, and 25-OHD was assayed to a standardized protocol in the same laboratory. Total 25-OHD (25-OHD₂ and 25-OHD₃) was measured by liquid chromatography-tandem mass spectrometry.²¹ To account for seasonal differences, 25-OHD concentration was May-standardized by adjusting for sampling month using differences in age- and sex-adjusted monthly averages generated from SOCCS control participants.⁴ May-adjusted measurements were used in analyses throughout, unless otherwise specified.

Vitamin D deficiency is defined in terms of circulating 25-OHD as vitamin D deficient (< 10 ng/mL), at high (10 to 16 ng/mL) or low (16 to 20 ng/mL) risk of deficiency, or as vitamin D sufficient (> 20 ng/mL), although these thresholds are subject to debate.^{4,18} Because few individuals in Scotland are vitamin D sufficient,⁴ we grouped patients into 25-OHD tertiles to maximize statistical power. Vitamin D intake was calculated from a food frequency questionnaire.⁴

Genotype at the VDR Locus

Blood leukocyte DNA was genotyped for polymorphisms at the VDR gene locus (rs1544410, rs10735810, rs7975232, rs11568820) by using either an Illumina Infinium array or DNA sequencing. Protective allele score was calculated by summing the following: rs10735810 G allele,²² a variant that determines the translational start site and downstream 1,25-dihydroxycholecalciferol (1,25[OH]₂D) effects²³; rs1544410 T allele, a variant that has been associated with increased VDR mRNA expression and increased serum levels of 1,25(OH)₂D²⁴; and rs11568820 A allele, a variant that is located in the VDR promoter region and directly influences transcriptional activity.²⁵ rs7975232 has been excluded, given high correlation with rs1544410 ($r^2 = 0.63$ in the United Kingdom; $\chi^2 P < .001$ in this cohort). We estimated VDR phased haplotypes for the four genotyped VDR

Table 2. Unadjusted and Multivariable Adjusted HRs of Death According to May-Adjusted 25-OHD Tertile

Model	Tertile 1 (< 7.25 ng/mL)	Vitamin D Tertiles (May-adjusted 25-OHD)						<i>P</i> _{trend}	No. of Patients
		Tertile 2 (7.25-13.25 ng/mL)			Tertile 3 (> 13.25 ng/mL)				
		HR	95% CI	<i>P</i>	HR	95% CI	<i>P</i>		
CRC mortality*									
Unadjusted	Ref	0.8	0.63 to 1.02	.08	0.66	0.51 to 0.85	.001	.0012	1,594
Adjusted†	Ref	0.81	0.63 to 1.03	.09	0.67	0.52 to 0.87	.002	.002	1,573
Fully adjusted‡	Ref	0.86	0.66 to 1.13	.27	0.68	0.50 to 0.90	.008	.009	1,336
All-cause mortality*									
Unadjusted	Ref	0.78	0.64 to 0.95	.01	0.62	0.5 to 0.77	< .001	< .001	1,598
Adjusted†	Ref	0.77	0.63 to 0.94	.01	0.66	0.53 to 0.81	< .001	< .001	1,577
Fully adjusted‡	Ref	0.81	0.65 to 1.01	.056	0.70	0.55 to 0.89	.0034	.0029	1,338

NOTE. Final model is shown in **bold**.

Abbreviations: 25-OHD, 25-hydroxyvitamin D; CRC, colorectal cancer; HR, hazard ratio; Ref, reference.

*Follow-up from time of sampling until death or censor date.

†Multivariate model additionally adjusted for tumor site (colon/rectum), surgery (yes/no), time between definitive treatment and sampling, and season of blood collection. Data were incomplete for 21 participants, so they were excluded from adjusted model.

‡Multivariate model additionally adjusted for body mass index and level of physical activity.

polymorphisms for all samples by using BEAGLE software (version 3.3.2) with standard settings.²⁶ BEAGLE builds a model of haplotypes from which it identifies likely haplotypes that underlie the genotype data. The resulting haplotypes were tabulated so that each sample was assigned two haplotypes. Accordingly, variables corresponding to the dose of each haplotype were created. For each haplotype, we set the dose to give values of 0 (haplotype not present), 1 (heterozygous), or 2 (homozygous), with the sum of haplotype doses equaling 2 for any individual.

Given sample size constraints, we considered only haplotypes with frequencies of more than 10% and included them as dose variables in the adjusted Cox proportional hazards regression models (rarer haplotypes were ignored). We first ran models that investigated main effects of VDR haplotypes and then extended the models to include interaction terms between VDR haplotypes and May-adjusted vitamin D tertiles.

Patient- and Tumor-Related Variables

We considered factors with an established contribution to CRC mortality or etiology, including sex; smoking; physical activity; family history; non-

steroidal anti-inflammatory drugs; total daily energy; red meat, calcium, and alcohol intake; and socioeconomic status.²⁰ We collected detailed information on tumor site and multiplicity and clinicopathologic staging from clinical records and tumor boards (Scottish Managed Clinical Networks). Preoperative imaging was collected from participating centers. By using collated pathology, imaging, and clinical data, tumor stage was assigned according to the Union for International Cancer Control TNM staging system and was mapped onto the American Joint Committee on Cancer (AJCC) staging system (AJCC stage I to IV). AJCC stage IV was excluded from the analysis of vitamin D effect on survival.

Surgery and Chemotherapy

Date of definitive treatment is the date of surgical treatment. Extent of surgery was recorded (as polypectomy and/or local excision or resection). All patients were treated with curative intent, so any chemotherapy was administered in the adjuvant setting. We assessed whether chemotherapy modified associations between 25-OHD concentration and mortality in stage II and III

Table 3. Unadjusted and Multivariable Adjusted HRs of CRC-Specific Death According to May-Adjusted 25-OHD Tertile and AJCC Cancer Stage

Vitamin D Deficiency Category (estimated from May-adjusted 25-OHD)										
AJCC Stage	Model	Tertile 1 (7.25 ng/mL)	Tertile 2 (7.25-13.25 ng/mL)			Tertile 3 (13.25 ng/mL)			<i>P</i> _{trend}	No. of Patients
			HR	95% CI	<i>P</i>	HR	95% CI	<i>P</i>		
CRC mortality*										
I	Unadjusted	Ref	0.63	0.26 to 1.54	.31	0.57	0.23 to 1.41	.23	.22	369
	Adjusted†	Ref	0.59	0.24 to 1.46	.25	0.65	0.26 to 1.61	.36	.33	365
	Fully adjusted‡	Ref	0.61	0.22 to 1.71	.35	1.08	0.40 to 2.91	.88	.96	313
II	Unadjusted	Ref	0.68	0.45 to 1.05	.08	0.46	0.28 to 0.75	.0018	.0014	622
	Adjusted†	Ref	0.64	0.41 to 1	.05	0.46	0.28 to 0.75	.0019	.0014	613
	Fully adjusted‡	Ref	0.68	0.42 to 1.09	.11	0.44	0.25 to 0.76	.004	.0028	517
III	Unadjusted	Ref	0.97	0.71 to 1.32	.83	0.82	0.6 to 1.13	.23	.24	603
	Adjusted†	Ref	0.96	0.7 to 1.31	.78	0.82	0.59 to 1.13	.22	.23	595
	Fully adjusted‡	Ref	1.01	0.72 to 1.43	.94	0.80	0.55 to 1.16	.23	.25	506

NOTE. Final model is shown in **bold**.

Abbreviations: 25-OHD, 25-hydroxyvitamin D; AJCC, American Joint Committee on Cancer; CRC, colorectal cancer; HR, hazard ratio.

*Follow-up from time of sampling until death or censor date.

†Multivariate model additionally adjusted for tumor site (colon/rectum), time between definitive treatment and sampling, and season of blood collection. Also adjusted for surgery (yes/no) for AJCC stage I subgroup (all patients with stage II and III disease had surgery with curative intent).

‡Multivariate model additionally adjusted for body mass index and level of physical activity.

disease in a subset of patients for whom these data were available ($n = 758$; chemotherapy administered to 365 patients).

Time to Sampling

Median time to sampling was 105 days after the definitive treatment (interquartile range [IQR], 53 to 200 days). A variable describing time from definitive treatment to blood sampling was created, because acute illness, surgery, and postoperative recovery may influence vitamin D levels.⁶

Statistical Analysis and Survival

Data were analyzed by using R program and survival package for R. The associations between circulating vitamin D and patient characteristics were investigated by one-way analysis of variance or by χ^2 test for categorical variables. We first examined the association between vitamin D and CRC-specific and all-cause mortality by using Kaplan-Meier survival analysis. Cox proportional hazards models were used to calculate hazard ratios (HRs), adjusting the analysis for other relevant factors. Test of the proportional hazards assumptions was performed. HRs were calculated for vitamin D tertiles, with the lowest category as the reference. To explore effect modification, we assessed the risk in an analysis stratified for cancer stage. Trend tests were calculated based on 25-OHD as a continuous variable. $P < .05$ was considered statistically significant unless otherwise stated. Main effects of *VDR* polymorphisms and haplotypes and their multiplicative interaction with vitamin D on survival were assessed by using a Cox proportional hazards model.

Because four single nucleotide polymorphisms (SNPs) in the *VDR* gene were tested, the corrected P value threshold was set to .013 for this analysis.

Meta-Analysis

We searched PubMed and Cochrane Collaboration databases for studies that investigated the relationship between circulating 25-OHD and survival in patients with colorectal cancer by using search terms “25-hydroxyvitamin D”, “vitamin D”, “colon cancer”, “colorectal cancer”, “rectal cancer”, “bowel cancer”, “survival”, and “mortality”, and we hand-searched resultant bibliographic references. We identified only six relevant studies^{13,16,27-30} and one recent systematic review.³¹ Exclusions were as follows: two studies^{13,16} reported analyses of the same patient cohort; one study¹⁶ used predicted rather than measured 25-OHD; and another study²⁹ included patients with metastatic (stage IV) CRC. We performed a fixed effects meta-analysis of our data with that from the four remaining studies.^{13,27,28,30}

RESULTS

At the time of sampling, 49.7% of patients fulfilled criteria for vitamin D deficiency (25-OHD below 10 ng/mL), and a further 26.8% were at high risk of deficiency (10 to 16 ng/mL). Baseline characteristics of study participants according to vitamin D deficiency status are shown

Fig 1. Colorectal cancer (CRC) –specific survival (after sampling) according to tertile of postoperative 25-hydroxyvitamin D (VtD) levels in patients with CRC. (A) American Joint Committee on Cancer (AJCC) CRC stage I, (B) stage II, (C) stage III, and (D) stages I to III (see Data Supplement for relationship with all-cause mortality). t1 (< 7.25 ng/mL) median: 4.80 ng/mL; t2 (7.25 to 13.25 ng/mL) median: 10.11 ng/mL; t3 (> 13.25 ng/mL) median: 18.26 ng/mL. No. at risk at 24 months: t1 (for AJCC stage I, AJCC stage II, and AJCC stage III [all]): 114, 188, and 178 (480); t2: 118, 204, and 163 (485); and t3: 126, 191, and 175 (492). No. at risk at 60 months: t1: 108, 158, and 138 (404); t2: 110, 178, and 126 (414); and t3: 120, 177, and 141 (438).

in the Data Supplement and according to tertiles in Table 1. Among the 1,598 patients, there were 12,323 person-years of follow-up, 363 deaths as a result of CRC, and 168 deaths as a result of other causes (Table 1). Median follow-up was 8.9 years (IQR, 6.0 to 9.9 years) overall and 9.6 years (IQR, 8.8 to 10.2 years) for those alive at censor date. Median survival time by stage for patients who died as a result of CRC was 5.5 years (IQR, 3.6 to 7.4 years; $n = 28$) for stage I, 3.4 years (IQR, 2.1 to 5.6 years; $n = 110$) for stage II, and 2.7 years (IQR, 1.6 to 4.5 years; $n = 227$) for stage III patients (Data Supplement).

Mortality

Higher postoperative 25-OHD levels were strongly associated with lower CRC-specific ($P = .008$) and all-cause ($P = .003$) mortality (Table 2). Comparing patients with May-adjusted 25-OHD levels in the highest versus the lowest tertile, the adjusted HR was 0.68 (95% CI, 0.50 to 0.90) for CRC-specific mortality and 0.70 (95% CI, 0.55 to 0.89) for all-cause mortality. Results were consistent using unadjusted 25-OHD (Data Supplement). The effect was particularly apparent for patients with stage II disease. Comparing the highest to the lowest tertile in patients with stage II CRC, the adjusted HR was 0.44 (95% CI, 0.25 to 0.76) for CRC-specific mortality (Table 3). CRC-specific mortality reached 10% after 2.5 years for patients in the lowest tertile (AJCC II) and after 6.6 years for patients in the highest tertile of 25-OHD (Fig 1). Trends were similar for all-cause mortality (Data Supplement).

Analysis of deficiency categories (< 10 , 10 to 16, 16 to 20, and > 20 ng/mL) gave results consistent with those using tertiles (data not shown) and when we calculated follow-up from incidence instead of sampling date adjusting for survival before sampling.

Adjuvant Chemotherapy

The protective effect of higher 25-OHD levels was stronger for patients not receiving chemotherapy (disease stage II and III) than for

those receiving chemotherapy (lowest v highest tertiles; HR, 0.42; 95% CI, 0.22 to 0.80; $P = .008$; $P_{\text{trend}} = .006$). Again, the effect size was most prominent in stage II disease (HR, 0.34; 95% CI, 0.14 to 0.81; Data Supplement).

Meta-Analysis

In multivariable analysis, each study showed either an association between lower mortality and higher 25-OHD levels or a nonsignificant trend with the same direction of effect (Data Supplement). In a meta-analysis of four published studies and our data, adjusted HR for CRC-specific mortality was 0.67 (95% CI, 0.54 to 0.81), and it was 0.65 (95% CI, 0.55 to 0.77; Data Supplement) for all-cause mortality.

Genotype and Haplotypes at the VDR Locus

There was no association between survival and VDR SNPs individually or with protective allele score (Table 4). However, there was evidence for gene-environment interaction effects on CRC-specific mortality between May-adjusted 25-OHD level and rs11568820 genotype ($P = .008$) and also protective allele score ($P = .004$; Table 4 and Data Supplement for all-cause mortality). A trend toward a reduction in cancer mortality was observed for a majority of genotype subgroups (Table 5). Interestingly, if the number of protective alleles was low (two or fewer), adjusted HR for the highest tertile of May-adjusted 25-OHD was highly significant (HR, 0.46; 95% CI, 0.33 to 0.70; $P < .001$), although if there were three or more protective alleles, May-adjusted 25-OHD was not significantly associated with survival (HR, 1.01; 95% CI, 0.65 to 1.58). Notwithstanding that dietary history is an imprecise measure of vitamin D intake, we observed a suggestive interaction between intake and rs11568820 genotype (adjusted $P = .09$ for CRC-specific mortality; unadjusted $P = .11$).

Analysis of haplotypes with frequencies of more than 10% is shown in the Data Supplement. In the adjusted Cox proportional hazards model, there was no association between haplotype dose

Table 4. Multivariable-Adjusted Analysis of HR of Death as a Result of CRC According to Polymorphisms in VDR Gene and Their Interaction With Plasma 25-OHD Concentration (unadjusted and May-adjusted)

SNP*	Model	Unadjusted 25-OHD			May-Adjusted 25-OHD		
		SNP	25-OHD	$P_{\text{interaction}}$	SNP	25-OHD	$P_{\text{interaction}}$
rs7975232	Simple	0.93	0.13		0.93	0.18	
	With interaction	0.94	0.47	.88	0.97	0.47	.99
rs1544410	Simple	0.74	0.11		0.73	0.15	
	With interaction	0.91	0.27	.71	0.74	0.55	.87
rs10735810	Simple	0.67	0.11		0.65	0.14	
	With interaction	0.76	0.38	.93	0.44	0.21	.53
rs11568820 (AA + AG v GG)†	Simple	0.80	0.10		0.80	0.13	
	With interaction	0.06	0.05	.01	0.05	0.04	.008
Protective alleles ≤ 2 v 2+)	Simple	0.63	0.10		0.60	0.12	
	With interaction	0.04	0.01	.03	0.008	0.002	.004

NOTE. Multivariate model adjusted for age at diagnosis, sex, American Joint Committee on Cancer stage, tumor site (colon/rectum), surgery (yes/no), time between definitive treatment and sampling, season of blood collection, body mass index, and level of physical activity. P values that are significant at multiple testing adjusted level ($P < .013$) are shown in **bold**.

Abbreviations: 25-OHD, 25-hydroxyvitamin D; CRC, colorectal cancer; HR, hazard ratio; HWE, Hardy-Weinberg equilibrium; LD, linkage disequilibrium; SNP, single nucleotide polymorphism; VDR, vitamin D receptor.

*Several functional VDR gene polymorphisms were considered. rs11568820 is located in the VDR promoter region and directly influences transcriptional activity.²⁵ rs10735810 determines the translational start site and downstream 1,25-dihydroxycholecalciferol effects.²³ rs1544410 has been associated with increased VDR messenger RNA expression and increased serum levels of 1,25-dihydroxycholecalciferol.²⁴ rs1544410 is in high LD with rs7975232, and both are located in an intron and so may tag a causal variant rather than affect VDR function directly. There were no deviations from HWE for examined SNPs.

†AA and AG genotypes were analyzed jointly because of the low frequency of AA genotype (5.4%). In the analysis in which the three genotypes were kept separate, $P = .03$ for the interaction with unadjusted 25-OHD, and $P = .016$ for the interaction with May-adjusted 25-OHD.

Table 5. Unadjusted and Multivariable Adjusted HRs of CRC-Specific Death According to May-Adjusted 25-OHD Tertile and VDR Polymorphisms

Vitamin D Deficiency (estimated from May-adjusted 25-OHD)										
SNP	Genotype	Model	Tertile 1	Tertile 2		Tertile 3		<i>P</i> _{trend}	No. of Patients	
			(< 7.25 ng/mL) HR	(7.25-13.25 ng/mL) HR	95% CI	(> 13.25 ng/mL) HR	95% CI			
rs7975232	CC	Unadjusted	Ref	0.68	0.39 to 1.18	0.43	0.24 to 0.79	.006	325	
		Full	Ref	0.85	0.46 to 1.55	0.28	0.13 to 0.6	.001	263	
	AC	Unadjusted	Ref	0.89	0.63 to 1.25	0.86	0.61 to 1.22	.41	807	
		Full	Ref	1.05	0.71 to 1.54	1.15	0.77 to 1.72	.5	689	
	AA	Unadjusted	Ref	0.74	0.47 to 1.18	0.54	0.32 to 0.9	.02	420	
		Full	Ref	0.58	0.34 to 0.99	0.43	0.24 to 0.79	.005	354	
rs11568820*	AA †	Unadjusted	Ref	2.27	0.88 to 5.85	0.45	0.14 to 1.5	.26	82	
		Full	Ref	4.72	1.22 to 18.27	0.81	0.17 to 3.92	.81	64	
	AG	Unadjusted	Ref	1.06	0.67 to 1.68	1.06	0.67 to 1.67	.81	501	
		Full	Ref	1.16	0.7 to 1.91	1.04	0.61 to 1.75	.89	423	
	GG	Unadjusted	Ref	0.65	0.47 to 0.89	0.54	0.38 to 0.76	< .001	944	
		Full	Ref	0.69	0.49 to 0.98	0.54	0.36 to 0.79	.001	799	
rs1544410*	AA †	Unadjusted	Ref	0.74	0.41 to 1.34	0.64	0.33 to 1.23	.16	258	
		Full	Ref	0.55	0.28 to 1.09	0.46	0.21 to 1.01	.04	218	
	AG	Unadjusted	Ref	0.88	0.63 to 1.24	0.73	0.51 to 1.03	.08	766	
		Full	Ref	0.99	0.67 to 1.45	0.83	0.56 to 1.24	.37	649	
	GG	Unadjusted	Ref	0.72	0.47 to 1.1	0.58	0.37 to 0.91	.02	561	
		Full	Ref	0.83	0.53 to 1.32	0.54	0.31 to 0.92	.03	460	
rs10735810*	AA	Unadjusted	Ref	0.81	0.44 to 1.47	0.52	0.27 to 1	.05	233	
		Full	Ref	0.83	0.41 to 1.68	0.51	0.23 to 1.1	.09	191	
	AG	Unadjusted	Ref	0.8	0.56 to 1.15	0.74	0.51 to 1.06	.1	757	
		Full	Ref	0.86	0.58 to 1.27	0.67	0.44 to 1.01	.05	636	
	GG †	Unadjusted	Ref	0.83	0.56 to 1.24	0.61	0.39 to 0.95	.03	585	
		Full	Ref	0.9	0.58 to 1.39	0.75	0.45 to 1.24	.26	500	
VDR protective alleles	0, 1	Unadjusted	Ref	0.6	0.34 to 1.06	0.4	0.21 to 0.74	.003	302	
		Full	Ref	0.67	0.35 to 1.3	0.37	0.17 to 0.79	.009	250	
	2	Unadjusted	Ref	0.68	0.45 to 1.03	0.55	0.35 to 0.86	.008	500	
		Full	Ref	0.65	0.41 to 1.03	0.52	0.31 to 0.86	.009	431	
	3	Unadjusted	Ref	1.1	0.66 to 1.82	1.21	0.73 to 2.01	.45	426	
		Full	Ref	1.61	0.92 to 2.83	1.46	0.82 to 2.62	.17	357	
	4+	Unadjusted	Ref	0.99	0.57 to 1.7	0.6	0.32 to 1.13	.12	290	
		Full	Ref	0.93	0.49 to 1.76	0.65	0.31 to 1.36	.26	248	
	≤ 2	Unadjusted	Ref	0.65	0.46 to 0.91	0.49	0.34 to 0.7	< .001	802	
		Full	Ref	0.65	0.45 to 0.95	0.46	0.3 to 0.7	< .001	681	
	> 2	Unadjusted	Ref	1.05	0.72 to 1.52	0.92	0.62 to 1.35	.68	716	
		Full	Ref	1.16	0.77 to 1.75	1.01	0.65 to 1.58	.92	605	

NOTE. Full model is adjusted for age at diagnosis, sex, American Joint Committee on Cancer stage, tumor site (colon/rectum), surgery (yes/no), time between definitive treatment and sampling, season of blood collection, body mass index, and level of physical activity. Results that are significant at multiple testing adjusted level ($P < .013$) are shown in **bold**.

*Included in protective allele score calculation.

†Protective allele.

(alone) and survival (Data Supplement). However, after including an interaction term in the model, there was an interaction between dose of GAGC haplotype and May-adjusted vitamin D. This interaction was highly significantly associated with overall survival, even after accounting for multiple testing ($P = .008$).

DISCUSSION

In this prospective cohort study of 1,598 patients sampled after treatment for stage I to III CRC, we show that higher plasma 25-OHD level is associated with lower cancer-specific and all-cause mortality. We assayed plasma total 25-OHD, the best biomarker of both dietary intake and sunlight exposure. The interaction between

25-OHD and genetic variation at the VDR locus in relation to survival provides additional evidence implicating the vitamin D pathway and provides support for a causal relationship (see the Data Supplement for a discussion of causality). The magnitude of effect was substantial and clinically important. Mortality was about one third lower in the patient group with the highest compared with the lowest plasma 25-OHD tertiles. The direction of protective effect was consistent across different strata of patients. CRC-specific mortality was 20% at 5 years in patients with the lowest plasma 25-OHD tertile, and 10 years elapsed before mortality reached 20% in those with the highest tertile. There was a similar differential in all-cause mortality between plasma tertile groups (20% mortality after 3.5 and 6.0 years).

Because of Scotland's northern latitude, vitamin D deficiency is highly prevalent in the Scottish population⁴ and, unsurprisingly, this is reflected in the patients with CRC studied here. However, even within this narrow, deficient range, we have shown that vitamin D has a protective effect from death after a diagnosis of CRC. This suggests that plasma 25-OHD assayed postoperatively may have utility as a biomarker for survival in patients without evidence of systemic metastases. Furthermore, the gene-environment interaction between genetic variation at the VDR locus and vitamin D level suggests that vitamin D may actively protect from death, rather than merely reflecting tumor aggression and an inevitable outcome. Although the evidence presented here linking plasma 25-OHD level with outcome is persuasive, there is now a pressing need for prospective randomized trials to establish definitively whether dietary vitamin D supplementation reduces cancer-specific and all-cause mortality following a diagnosis of CRC.

All published studies to date have a fundamental methodologic limitation: 25-OHD concentration was not determined in cancer survivors but was instead measured years before cancer diagnosis,^{13,27,30} perioperatively,²⁸ or was predicted from the determinants of 25-OHD concentration.¹⁶ We designed this study to investigate whether vitamin D deficiency contributes to survival outcome in a well-characterized cohort in which plasma 25-OHD was measured after CRC diagnosis and surgical treatment. Thus, to the best of our knowledge, this is the first study to examine the effect of postoperative vitamin D levels on survival in patients with CRC and the first to investigate gene-environment interaction between vitamin D level and genetic variation at the VDR gene locus.

This study has some limitations. One single measurement of 25-OHD was taken. Although this is likely to reduce statistical power, it is unlikely to bias findings. In this population, 25-OHD concentrations are generally low, preventing us from systematically examining the effect of higher concentrations. The time period between cancer treatment and 25-OHD sampling levels was not constant (although there was no detectable association between time interval to sampling and 25-OHD levels or outcome). The limited number of SNPs in the analysis may reduce the accuracy of inferred haplotypes. We cannot exclude the possibility that low 25-OHD is associated with other predictors of poor prognosis because this was an observational study.

Evidence from epidemiologic, preclinical, and clinical studies indicates that vitamin D (and analogs) might have application as therapeutic agents in patients with cancer following a diagnosis because their administration can activate apoptotic pathways,³³ inhibit angiogenesis,^{34,35} and can have prodifferentiative³⁶ and antiproliferative³⁷ effects (Data Supplement).³⁸ Recent randomized clinical trials of the effects of supplemental vitamin D report a reduction in tumor-promoting inflammation biomarkers with vitamin D₃ supplementation,³⁹ decreased oxidative DNA damage,⁴⁰ and modification of the APC/β-catenin pathway⁴¹ in the normal mucosa of patients with colorectal adenoma, further highlighting the need to explore vitamin D as a preventative and therapeutic anticancer agent.³⁸

In this large study, we report for the first time an association between mortality following a diagnosis of CRC and an interaction between GAGC haplotype dose and vitamin D. Plasma 25-OHD, along with genotype at the VDR locus, has potential utility as a prognostic biomarker. In conclusion, our findings suggest a causal relationship between vitamin D and survival outcome in patients with cancer. Hence, there is now a compelling case for appropriately designed and adequately powered randomized clinical trials aimed at testing whether actively supplementing the diet of patients recently diagnosed with CRC with vitamin D can have a favorable impact on survival.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

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GLOSSARY TERMS

1,25-dihydroxycholecalciferol [1,25(OH)₂D]: a steroid hormone (also known as calcitriol) that functions as the physiologically most active form of vitamin D. It acts in an autocrine and paracrine manner and binds to the vitamin D receptor to regulate calcium homeostasis and bone growth and mineralization, among other cellular processes.

APC: a tumor suppressor gene. Mutations in the APC gene are responsible for familial adenomatous polyposis (germline mutations) or sporadic (somatic mutations) colorectal tumors. The gene product is known to interact with adherens junction proteins, a- and b-catenins, suggesting a role in cell adhesion.

American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC)

TNM staging: a cancer staging system that describes the extent of cancer in a patient's body. "T" describes the size of the tumor and whether it has invaded nearby tissue; "N" describes regional lymph nodes that are involved; "M" describes distant metastasis (spread of cancer from one body part to another). The TNM Classification of Malignant Tumours was developed and maintained by the UICC to achieve consensus on one globally recognized standard for classifying the extent of spread of cancer. The TNM classification was also used by the AJCC. In 1987, the UICC and AJCC staging systems were unified into a single staging system. Prognosis of a patient is defined by TNM classification.

angiogenesis: the process involved in the generation of new blood vessels. Although this is a normal process that naturally occurs and is controlled by so-called on and off switches, blocking tumor angiogenesis (antiangiogenesis) disrupts the blood supply to tumors, thereby preventing tumor growth.

apoptosis: also called programmed cell death. Apoptosis is a signaling pathway that leads to cellular suicide in an organized manner. Several factors and receptors are specific to the apoptotic pathway. The net result is that cells shrink and develop blebs on their surface, and their DNA undergoes fragmentation.

β-catenin: originally identified as a component of cell-cell adhesion complexes composed of cadherins and actin. β-catenin has now been shown to be a downstream signaling molecule in the Wnt signaling pathway.

Cox proportional hazards regression model: a statistical model for regression analysis of censored survival data, examining the relationship of censored survival distribution to one or more covariates. This model produces a baseline survival curve, covariate coefficient estimates with their standard errors, risk ratios, 95% CIs, and significance levels.

genetic polymorphisms: a genetic variant seen in at least 1% of the population. Because proteins are gene products, their polymorphisms reflect allelic differences in the gene. The advent of restriction enzymes, which digest DNA to fragments based on sequence specificity, has ushered in an era of restriction fragment length polymorphisms in which changes in DNA sequences manifest as restriction fragments of different sizes when cleaved with a specific restriction enzyme. Polymorphisms are used in tissue typing, in determining disease, in pharmacogenetics, and in assessing genetic diversity.

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