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Prediagnostic circulating concentrations of vitamin D binding protein and survival among colorectal cancer patients

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Abstract

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Background: Higher total 25-hydroxyvitamin D [25(OH)D] levels are associated with improved survival among colorectal cancer (CRC) patients, but the relationships between circulating vitamin D binding protein (VDBP), bioavailable or free 25(OH)D, and CRC survival remain unknown.

Methods: We examined the associations between prediagnostic plasma levels of vitamin D-related markers and survival among 603 white participants diagnosed with CRC from 2 prospective US cohorts. Plasma VDBP and total 25(OH)D were directly measured, while bioavailable and free 25(OH)D were calculated using a validated formula based on total 25(OH)D, VDBP, and albumin levels. Cox proportional hazards regression was used to estimate hazard ratios (HRs) for overall and CRC-specific mortality, adjusted for other prognostic markers and potential confounders.

Results: Higher VDBP levels were associated with improved overall (P_{trend} = 0.001) and CRC-specific survival (P_{trend} = 0.02). Compared to patients in the lowest quartile, those in the highest quartile of VDBP had a multivariable HR of 0.58 (95% confidence interval [CI], 0.41–0.80) for overall mortality and 0.58 (95% CI, 0.37–0.91) for CRC-specific mortality. The results remained similar after further adjustment for total 25(OH)D levels. In contrast, neither bioavailable nor free 25(OH)D levels were associated with overall or CRC-specific mortality (all P_{trend} > 0.15).

Conclusion: Prediagnostic circulating concentrations of VDBP were positively associated with survival among CRC patients.

Impact: The clinical utility of VDBP as a prognostic marker warrants further exploration, as well as research into underlying mechanisms of action.

Introduction

Vitamin D is hypothesized to play a role in the development and progression of colorectal cancer (CRC). Colon cancer cells express vitamin D receptor (VDR) (1, 2) and 1-α-hydroxylase (3), which converts the main circulating form of vitamin D, 25-hydroxyvitamin D [25(OH)D], into the active metabolite, calcitriol [1,25$(OH)_2$D]. Binding of 1,25$(OH)_2$D to VDR leads to several anti-cancer effects, including increased cell differentiation and apoptosis (4, 5) and decreased proliferation (6), angiogenesis (7, 8), and metastasis (9, 10).

Vitamin D binding protein (VDBP), also known as the group-specific component, is the major vitamin D carrier protein. Approximately 88% of circulating 25(OH)D is bound to VDBP, while 12% of 25(OH)D is loosely bound to albumin, leaving very little in the free form (11, 12). Experimental studies demonstrate that VDBP has important biological functions that may inhibit tumor growth, such as actin scavenging, macrophage activation, and chemotaxis (13). A meta-analysis of 28 studies examined VDBP levels in relation to the overall risk of multiple cancers including CRC and found borderline decreased risk in individuals with higher VDBP levels (odds ratio, 0.75; 95% confidence interval [CI], 0.56–1.00) (14). Previous studies did not find an association between VDBP levels and CRC risk (15–17). However, it is unknown whether prediagnostic VDBP levels influence survival outcomes among CRC patients.

The “free hormone hypothesis” postulates that the bound fraction of a hormone is not available to target cells for signaling and gene regulation (18), suggesting that free 25(OH)D

and albumin-bound 25(OH)D, which can dissociate during tissue perfusion, may be more biologically active than VDBP-bound 25(OH)D. However, more recent studies found that the 25(OH)D-VDBP complex can also be internalized into cells by transportation of megalin, an endocytic receptor that is expressed in epithelial cells of several organs including colon (19, 20). Although the link between higher total 25(OH)D levels and better CRC survival has been well documented (21–27), the association between bioavailable or free 25(OH)D levels and CRC survival is unknown.

Building upon our prior analyses of total 25(OH)D levels and CRC survival (21), we further investigated the associations of prediagnostic plasma levels of VDBP, bioavailable 25(OH)D, and free 25(OH)D with survival among participants diagnosed with CRC from 2 prospective US cohorts, the Nurses' Health Study (NHS) and the Health Professionals Follow-Up Study (HPFS).

Materials and Methods

Study population

In 1976, NHS was initiated when 121,700 US female registered nurses aged 30 to 55 years responded to a mailed questionnaire on risk factors for cancer and cardiovascular disease (28). Blood samples were collected from 32,826 NHS participants between 1989 and 1990. In 1986, HPFS was established when 51,529 US male dentists, optometrists, osteopaths, podiatrists, pharmacists, and veterinarians aged 40 to 75 years completed a mailed questionnaire on health-related behaviors and medical history (29). Blood samples were collected from 18,225 HPFS participants between 1993 and 1995. In both cohorts, participants received biennial questionnaires to update information on lifestyle factors and medical diagnoses. A high follow-up rate of more than 90% was achieved in both cohorts.

When an incident case of CRC was identified rom self-report or during follow-up of participant deaths, we asked permission to obtain hospital records and pathology reports. Physicians who were blinded to exposure data reviewed medical records, death certificates, or cancer registry data to ascertain the diagnosis of CRC and record information on important tumor characteristics. We have estimated that 96–97% of patients were captured by using these methods (30, 31).

Patients diagnosed with CRC after the date of blood collection through December 2011 were eligible for the current study. Patients were excluded if they were non-white (due to inability of the monoclonal assay to accurately measure VDBP in non-whites) or had reported any cancer (other than nonmelanoma skin cancer) prior to CRC diagnosis. Patients who were diagnosed with CRC within 2 years after blood collection were also excluded to minimize bias associated with presence of occult cancer. Among 627 eligible patients with total 25(OH)D levels, 603 had available VDBP levels, from which bioavailable and free 25(OH)D were calculated.

The study protocol was approved by the institutional review boards of the Brigham and Women's Hospital and the Harvard T.H. Chan School of Public Health, and those of participating registries as required. All participants provided written informed consent for

the researchers to access their medical records. The study was conducted in concordance with the International Ethical Guidelines for Biomedical Research Involving Human Subjects (CIOMS).

Measurement of plasma VDBP, total 25(OH)D, and albumin

Blood samples were shipped by overnight courier in chilled containers. On receipt, bloods were centrifuged, aliquoted, and stored in continuously-monitored liquid nitrogen freezers at -130°C or below. More than 95% of the blood samples arrived in our laboratory within 24 hours of phlebotomy.

Plasma VDBP was measured at Heartland Assays in 2013 by a monoclonal antibody-based, enzyme-linked immunosorbent assay (ELISA) (R&D Systems). Plasma total 25(OH)D was measured in the laboratory of Dr. Bruce Hollis (The Medical University of South Carolina, Charleston, SC) and Heartland Assays (Ames, IA) by radioimmunoassay (32). Plasma albumin was measured by a colorimetric assay (Roche Diagnostics) in the laboratory of Dr. Nader Rifai (Children's Hospital, Boston, MA). Although all samples were assayed at the same laboratory, cases identified from different questionnaires were assayed in different batches, which are detailed in Supplementary Table S1. The mean intra-assay coefficients of variation for VDBP, total 25(OH)D, and albumin were ≤14.8%, ≤11.8%, and ≤4.0%, respectively.

Calculation of plasma bioavailable and free 25(OH)D

Bioavailable and free 25(OH)D were calculated by the following equations:

- $$\text{Free 25(OH)D} = \text{Total 25(OH)D}/(1 + \text{Ka}_{\text{Albumin}} \times \text{Albumin} + \text{Ka}_{\text{VDBP}} \times \text{VDBP});$$
- $$\text{Bioavailable 25(OH)D} = \text{Free 25(OH)D} \times (1 + \text{Albumin} \times \text{Ka}_{\text{Albumin}}),$$

where $\text{Ka}_{\text{Albumin}}$ is the affinity of albumin for 25(OH)D (6×10^5), Ka_{VDBP} is the affinity of VDBP for 25(OH)D (7×10^8) (11), and all concentrations are in mol/L (18).

Using the TaqMan OpenArray SNP Genotyping Platform (Applied Biosystems), we successfully genotyped 2 common single-nucleotide polymorphisms (SNPs) in *VDBP*, rs4588 and rs7041, for 548 patients of our study population. The 2 SNPs give rise to 3 predominant haplotypes: GC1F, GC1S, and GC2. Regarding whether the affinity of VDBP for 25(OH)D is affected by these haplotypes, 1 study found the affinity of GC1F to be 4 times higher than that of GC2 and double that of GC1S (33), while 3 studies demonstrated no difference in the affinity (34–36). Therefore, we calculated bioavailable and free 25(OH)D using a constant affinity of VDBP for 25(OH)D, but our conclusions were essentially unchanged by using the genotype-specific affinities.

Mortality outcome

Patients were observed until date of death or last follow-up (June 2014 for NHS; January 2014 for HPFS), whichever came first. Ascertainment of deaths included reporting by family

or postal authorities, and interrogation of names of persistent nonrespondents in the National Death Index, which has been shown to capture approximately 98% of deaths (37). The primary outcome was overall mortality, and the secondary outcome was CRC-specific mortality. Because deaths from CRC mostly occur within the first 5 years after diagnosis, we evaluated 5-year overall mortality as an additional outcome by censoring patients who were alive at the end of the first 5 years.

Covariates

Cancer stage, grade of tumor differentiation, location of primary tumor, and year of diagnosis (as a surrogate for treatment) were extracted from medical records. Body mass index (BMI) and physical activity were obtained from the questionnaire returned before blood collection.

Statistical analyses

Plasma vitamin D-related markers were categorized into quartiles by batch (Supplementary Table S1) and analyzed (21). Follow-up time was calculated from CRC diagnosis to death or censoring. Cox proportional hazards regression was used to calculate hazard ratios (HRs) and 95% CIs for 3 outcomes: overall mortality, CRC-specific mortality, and 5-year overall mortality. We tested for a linear trend across quartiles using an ordinal variable. *A priori*, we included other prognostic markers and potential confounders in multivariable models, including age at diagnosis, sex, BMI, physical activity, cancer stage, grade of tumor differentiation, location of primary tumor, and year of diagnosis. We additionally adjusted for season of blood collection when the exposure was total, bioavailable, or free 25(OH)D. Interaction between VDBP and the potential effect modifier was assessed by entering in the model the cross product of the quartile of the biomarker and the stratification variable, evaluated by the likelihood ratio test. The Cox models were tested for and met the proportional hazards assumption. All analyses were performed with SAS software, version 9.4 (SAS Institute, Cary, NC). All P values are 2 sided.

Results

Baseline characteristics

Plasma samples were collected at a median of 9.3 years (interquartile range [IQR]: 6.1–13.3 years) before CRC diagnosis. The median VDBP level was 250 μg/mL (IQR: 175–311 μg/mL) and the median total 25(OH)D level was 27.3 ng/mL (IQR: 20.4–33.0 ng/mL), and both were modestly correlated ($r = 0.12$; $P < 0.01$; Supplementary Table S2). The median bioavailable 25(OH)D level was 3.36 ng/mL (IQR: 2.26–4.66 ng/mL). As expected, bioavailable 25(OH)D levels were positively correlated with total 25(OH)D ($r = 0.59$; $P < 0.0001$) and albumin levels ($r = 0.23$; $P < 0.0001$) and negatively correlated with VDBP levels ($r = -0.67$; $P < 0.0001$). The median free 25(OH)D level was 8.25 pg/mL (IQR, 5.66–11.19 pg/mL), and was nearly perfectly correlated with bioavailable 25(OH)D levels ($r = 0.99$; $P < 0.0001$); thus, we focused our analyses on bioavailable 25(OH)D that was much more abundant in the circulation.

Patient characteristics were well balanced by quartile of VDBP, except that patients with higher VDBP levels had a lower BMI (Table 1). Patients with higher total 25(OH)D levels had a lower BMI and higher physical activity, compared to those with lower levels (Supplementary Table S3). In addition, patients with higher total or bioavailable 25(OH)D levels were more likely to have their blood collected in the summer or fall.

Causes of death

The median time of follow-up among patients who were alive at the end of follow-up was 12.4 years (IQR: 8.0–15.3 years). During the follow-up, we documented 328 deaths, 187 (57.0%) of which were due to CRC. Non-CRC causes of death included other malignancies (n = 26), cardiovascular disease (n = 33), neurological disorders (n = 21), cerebrovascular disease (n = 13), respiratory disease (n = 12), and other or unknown reasons (n = 36). A summary of causes of death by follow-up period after diagnosis is presented in Supplementary Table S4. Of the 187 deaths due to CRC, 163 (87.2%) occurred within the first 5 years after diagnosis.

Association between prediagnostic VDBP levels and patient survival

Higher VDBP levels were significantly associated with improved overall (P_{trend} = 0.001) and CRC-specific survival (P_{trend} = 0.02) (Table 2). Compared to patients in the lowest quartile, those in the highest quartile of VDBP had a multivariable HR of 0.58 (95% CI, 0.41–0.80) for overall mortality and 0.58 (95% CI, 0.37–0.91) for CRC-specific mortality. The HRs were not materially changed after further adjustment for total 25(OH)D levels. In addition, higher VDBP levels were associated with improved 5-year overall survival (P_{trend} = 0.001), with a multivariable HR of 0.50 (95% CI, 0.32–0.76) comparing extreme quartiles.

To further address concerns about the possible influence of occult cancer on VDBP levels, we performed sensitivity analyses by excluding patients who developed CRC within 3, 4, and 5 years after blood collection, respectively. Although statistical power was diminished, the association between VDBP levels and patient survival remained largely unchanged (Table 3).

We next evaluated the associations between 2 *VDBP* polymorphisms and patient survival. Neither rs7041 nor rs4588 was significantly associated with overall or CRC-specific survival ($P \geq 0.08$; Supplementary Table S5). In models additionally adjusted for these polymorphisms, the significant association between VDBP levels and patient survival remained unchanged (P_{trend} = 0.001 and 0.008 for overall and CRC-specific mortality, respectively).

The association of VDBP levels with overall and CRC-specific survival was examined across strata of potential effect modifiers, including age at diagnosis, time from blood collection to diagnosis, sex, BMI, physical activity, cancer stage, grade of tumor differentiation, location of primary tumor, year of diagnosis, and total 25(OH)D levels, and remained largely unchanged in most subgroups ($P_{interaction} \geq 0.18$; Fig. 1).

Associations between prediagnostic levels of total, bioavailable, and free 25(OH)D and patient survival

Total 25(OH)D levels were not significantly associated with overall (P_{trend} = 0.09) or CRC-specific survival (P_{trend} = 0.08) (Table 4). However, higher total 25(OH)D levels were associated with improved 5-year overall survival (P_{trend} = 0.01), with a multivariable HR of 0.48 (95% CI, 0.30–0.78) comparing the highest to the lowest quartile. The association remained significant after further adjustment for VDBP levels (P_{trend} = 0.02).

Bioavailable 25(OH)D levels were not associated with either overall (P_{trend} = 0.39) or CRC-specific survival (P_{trend} = 0.43), even in the analyses with 5-year overall survival as the outcome (P_{trend} = 0.66; Table 4). Free 25(OH)D levels were also not associated with any of these outcomes (P_{trend} = 0.15, 0.32, and 0.31, respectively; Table 4).

Discussion

We found that CRC patients with the highest prediagnostic plasma VDBP levels had a significant improvement in overall and CRC-specific survival, independent of total 25(OH)D levels. Bioavailable and free 25(OH)D levels were not associated with overall or CRC-specific mortality.

To date, only 1 study has examined the association between circulating VDBP levels and CRC survival. This analysis of 206 Chinese CRC patients measured VDBP at surgery and detected no association with overall survival (38). Additionally, a Canadian study found that the C allele at rs2282679 (a perfect proxy for rs4588) in *VDBP* was significantly associated with worse disease-free survival among 488 CRC patients (39). Our observation that higher VDBP levels were associated with increased survival is biologically plausible. As the major carrier protein of circulating 25(OH)D, VDBP may boost the anti-cancer effects of 25(OH)D by prolonging its half-life. In addition, VDBP has independent biological functions that may inhibit tumor growth. First, VDBP functions as an actin scavenger, binding to circulating actin released from tissue injury, and thereby preventing vascular occlusion and organ dysfunction (40). Second, VDBP plays a role in immune response through the inflammation-primed conversion to VDBP-macrophage activating factor, which has direct anti-angiogenic and anti-proliferative activities in addition to its ability to activate tumoricidal macrophages (41–44). Third, VDBP has an anti-inflammatory effect by directing neutrophils to sites of inflammation (neutrophil chemotaxis) (45, 46). In the current study, the association between VDBP levels and patient survival remained significant after controlling for total 25(OH)D levels. To minimize bias in the plasma VDBP levels by the presence of occult cancer, we excluded patients diagnosed within 2 years of blood collection in the main analyses, and continued to note an association even when extending this restriction to 5 years.

Multiple prospective cohort studies (21–24), as well as a phase II randomized clinical trial (26), have suggested a benefit of higher vitamin D levels on survival among CRC patients. In our previous analysis of 304 CRC patients from the same cohort studies, higher total 25(OH)D levels were associated with improved overall survival (21). In the current study with a larger sample size and extended follow-up, higher total 25(OH)D levels were

associated with improved 5-year overall survival, but not with overall survival during the entire follow-up. One possible explanation is that vitamin D may not reduce excess mortality from causes other than CRC. In a recent meta-analysis of 52 randomized controlled trials, vitamin D supplementation was found to reduce the risk of cancer death by 16% without being associated with all-cause mortality (47). Another potential explanation is that some patients provided blood samples many years before their diagnosis. Whereas the prospective design of our study is advantageous in reducing bias that results from reverse causation, total 25(OH)D levels measured remotely from diagnosis may not accurately reflect the relevant vitamin D status that influences long-term CRC survival.

In this study, we found no association between bioavailable or free 25(OH)D levels and CRC survival even in analyses with 5-year overall survival as the outcome. As higher VDBP levels were associated with improved survival, and resulted in lower concentrations of bioavailable and free 25(OH)D, we would not expect that higher levels of bioavailable or free 25(OH)D would also be associated with improved survival. In a previous case-control study including participants from NHS, total 25(OH)D levels, but not bioavailable or free 25(OH)D levels, were inversely associated with CRC risk (15). Taken together, these data do not support the “free hormone hypothesis” within the context of circulating 25(OH)D and colorectal carcinogenesis, indicating that total 25(OH)D remains the best measure of clinically relevant vitamin D status.

Our study has several strengths, including the prospective design, long follow-up, high follow-up rate, and detailed data on potential confounders. The prospective design reduces reverse causation, as blood samples were collected years before inadequate nutrition and limited performance status that commonly develop at the time of CRC diagnosis. The comprehensive assessment of VDBP, total 25(OH)D, and bioavailable and free 25(OH)D, along with availability of albumin levels and *VDBP* genotype, allowed for a better understanding of the roles of vitamin D-related biomarkers in CRC survival.

Several limitations of our study deserve comment. We used a single measurement of VDBP and total 25(OH)D from plasma samples collected years before diagnosis, so we were unable to assess the influence of the dynamic changes of these markers. We did not directly measure bioavailable and free 25(OH)D; however, calculated and directly measured concentrations of these markers have been found to be well correlated (48). We used a monoclonal antibody-based ELISA to measure VDBP, which is inferior to a polyclonal assay and incapable of measuring VDBP levels in blacks (48, 49). To address this concern, we excluded non-white patients in the main analyses, and the results were similar after further adjustment for *VDBP* polymorphisms. Finally, information on treatment was not systematically collected in NHS and HPFS. However, treatment programs were unlikely to have varied by VDBP levels years before diagnosis.

In conclusion, higher prediagnostic plasma VDBP levels were associated with improved overall and CRC-specific survival among CRC patients. Bioavailable or free 25(OH)D levels were not associated with CRC survival. Additional efforts to understand the mechanisms through which the vitamin D pathway influences colorectal carcinogenesis and cancer progression are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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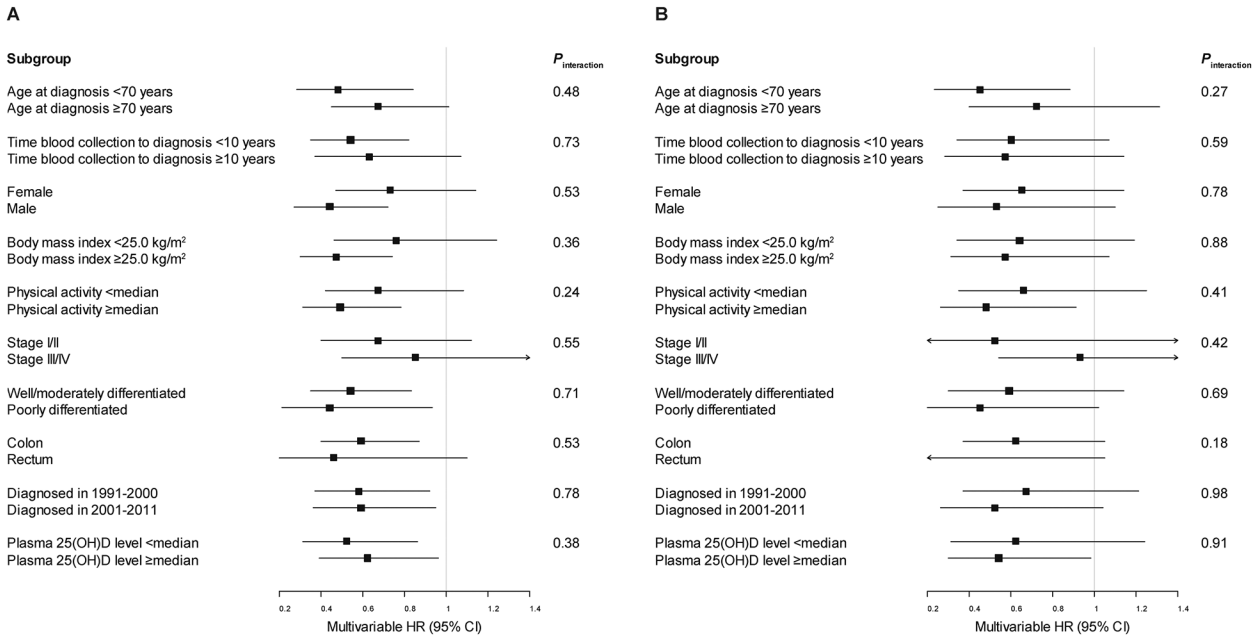

Figure 1.

Multivariable hazard ratios (HRs) and 95% confidence intervals (CIs) for (A) overall and (B) colorectal cancer-specific mortality comparing the highest to the lowest quartile of plasma vitamin D binding protein among colorectal cancer patients, stratified by covariates. Adjusted for age at diagnosis (continuous), sex, body mass index (continuous), physical activity (continuous), cancer stage (I to IV or unknown), grade of tumor differentiation (well differentiated, moderately differentiated, poorly differentiated, unknown), location of primary tumor (proximal colon, distal colon, rectum, unknown), and year of diagnosis (continuous), excluding the stratification covariate. 25(OH)D, 25-hydroxyvitamin D.

Table 1.

Baseline characteristics among colorectal cancer patients by quartile of plasma vitamin D binding protein

	Vitamin D binding protein			
Characteristic	**Quartile 1**	**Quartile 2**	**Quartile 3**	**Quartile 4**
No. of patients	149	151	152	151
Age at blood collection, mean (SD), years	61.2 (7.8)	62.3 (8.1)	60.9 (8.3)	60.9 (8.5)
Age at diagnosis, mean (SD), years	71.3 (7.6)	71.9 (8.5)	71.0 (8.4)	70.9 (8.8)
Time from blood collection to diagnosis, mean (SD), years	10.1 (4.9)	9.7 (4.6)	10.1 (4.7)	9.9 (5.1)
Sex, No. (%)				
Female	89 (59.7)	89 (58.9)	90 (59.2)	89 (58.9)
Male	60 (40.3)	62 (41.1)	62 (40.8)	62 (41.1)
Body mass index, mean (SD), kg/m^2	26.9 (5.1)	25.8 (3.7)	25.6 (4.3)	25.2 (3.6)
Physical activity, mean (SD), MET-h/wk	22.1 (21.0)	26.4 (36.8)	24.5 (33.2)	25.7 (29.8)
Cancer stage, No. (%)				
I	39 (26.2)	40 (26.5)	39 (25.7)	41 (27.2)
II	39 (26.2)	37 (24.5)	34 (22.4)	38 (25.2)
III	33 (22.1)	30 (19.9)	30 (19.7)	31 (20.5)
IV	16 (10.7)	21 (13.9)	30 (19.7)	21 (13.9)
Unknown	22 (14.8)	23 (15.2)	19 (12.5)	20 (13.2)
Grade of tumor differentiation, No. (%)				
Well differentiated	16 (10.7)	14 (9.3)	17 (11.2)	17 (11.3)
Moderately differentiated	86 (57.7)	90 (59.6)	89 (58.6)	84 (55.6)
Poorly differentiated	24 (16.1)	19 (12.6)	18 (11.8)	25 (16.6)
Unknown	23 (15.4)	28 (18.5)	28 (18.4)	25 (16.6)
Location of primary tumor, No. (%)				
Proximal colon	66 (44.3)	57 (37.7)	75 (49.3)	72 (47.7)
Distal colon	42 (28.2)	43 (28.5)	36 (23.7)	42 (27.8)
Rectum	32 (21.5)	38 (25.2)	33 (21.7)	27 (17.9)
Unknown	9 (6.0)	13 (8.6)	8 (5.3)	10 (6.6)
Year of diagnosis, No. (%)				
1991–2000	68 (45.6)	78 (51.7)	66 (43.4)	67 (44.4)
2001–2011	81 (54.4)	73 (48.3)	86 (56.6)	84 (55.6)
Season of blood collection, No. (%)				
Summer (June, July, August)	45 (30.2)	46 (30.5)	48 (31.6)	53 (35.1)
Fall (September, October, November)	41 (27.5)	47 (31.1)	43 (28.3)	41 (27.2)
Winter (December, January, February)	33 (22.1)	26 (17.2)	28 (18.4)	26 (17.2)
Spring (March, April, May)	30 (20.1)	32 (21.2)	33 (21.7)	31 (20.5)

Abbreviations: MET, metabolic equivalent; SD, standard deviation.

Table 2.

Hazard ratios for overall mortality, colorectal cancer-specific mortality, and 5-year overall mortality among colorectal cancer patients by quartile of plasma vitamin D binding protein

	Hazard ratio (95% confidence interval)				
	Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**	P_{trend}
Mean (SD), μg/mL	125.2 (41.2)	213.5 (34.8)	274.6 (46.6)	383.5 (79.9)	
No. of patients	149	151	152	151	
Overall mortality					
No. of events	81	84	76	70	
Base model[a]	Referent	0.93 (0.68–1.26)	0.89 (0.65–1.21)	0.76 (0.55–1.04)	0.08
Multivariable model[b]	Referent	0.77 (0.57–1.06)	0.69 (0.50–0.96)	0.58 (0.41–0.80)	0.001
Model further adjusted for total 25(OH)D[c]	Referent	0.78 (0.57–1.06)	0.70 (0.50–0.96)	0.57 (0.41–0.79)	0.001
Colorectal cancer-specific mortality					
No. of events	45	48	50	39	
Base model[a]	Referent	1.02 (0.68–1.53)	1.08 (0.72–1.62)	0.80 (0.52–1.23)	0.39
Multivariable model[b]	Referent	0.76 (0.50–1.15)	0.73 (0.48–1.11)	0.58 (0.37–0.91)	0.02
Model further adjusted for total 25(OH)D[c]	Referent	0.75 (0.49–1.15)	0.73 (0.48–1.12)	0.56 (0.36–0.88)	0.02
5-year overall mortality					
No. of events	53	54	51	39	
Base model[a]	Referent	0.96 (0.66–1.40)	0.95 (0.65–1.40)	0.68 (0.45–1.02)	0.08
Multivariable model[b]	Referent	0.74 (0.50–1.10)	0.68 (0.46–1.01)	0.50 (0.32–0.76)	0.001
Model further adjusted for total 25(OH)D[c]	Referent	0.74 (0.50–1.09)	0.69 (0.46–1.02)	0.49 (0.32–0.75)	0.001

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; SD, standard deviation.

[a]Adjusted for age at diagnosis (continuous).

[b]Addtionally adjusted for sex, body mass index (continuous), physical activity (continuous), cancer stage (I to IV or unknown), grade of tumor differentiation (well differentiated, moderately differentiated, poorly differentiated, unknown), location of primary tumor (proximal colon, distal colon, rectum, unknown), and year of diagnosis (continuous).

[c]Additionally adjusted for total 25-hydroxyvitamin D levels (quartiles).

Table 3.

Multivariable hazard ratios for overall mortality, colorectal cancer-specific mortality, and 5-year overall mortality among colorectal cancer patients by quartile of plasma vitamin D binding protein and time interval from blood collection to diagnosis

	Quartile 1		Quartile 2		Quartile 3		Quartile 4		
	No. of patients/ events	HR (95% CI)[a]	No. of patients/ events	HR (95% CI)[a]	No. of patients/ events	HR (95% CI)[a]	No. of patients/ events	HR (95% CI)[a]	P_{trend}
Overall mortality									
≥2 years	149/81	Referent	151/84	0.77 (0.57–1.06)	152/76	0.69 (0.50–0.96)	151/70	0.58 (0.41–0.80)	0.001
≥3 years	138/72	Referent	141/80	0.83 (0.60–1.15)	141/71	0.72 (0.51–1.01)	140/61	0.61 (0.43–0.87)	0.004
≥4 years	130/66	Referent	131/74	0.84 (0.59–1.19)	134/65	0.72 (0.51–1.03)	131/58	0.63 (0.44–0.91)	0.01
≥5 years	124/60	Referent	125/71	0.87 (0.61–1.24)	125/59	0.77 (0.53–1.11)	125/56	0.68 (0.47–1.00)	0.04
Colorectal cancer-specific mortality									
≥2 years	149/45	Referent	151/48	0.76 (0.50–1.15)	152/50	0.73 (0.48–1.11)	151/39	0.58 (0.37–0.91)	0.02
≥3 years	138/40	Referent	141/45	0.76 (0.49–1.19)	141/46	0.73 (0.46–1.13)	140/33	0.60 (0.37–0.96)	0.04
≥4 years	130/35	Referent	131/42	0.77 (0.48–1.24)	134/41	0.74 (0.46–1.19)	131/30	0.58 (0.35–0.97)	0.05
≥5 years	124/35	Referent	125/38	0.70 (0.43–1.14)	125/37	0.76 (0.47–1.23)	125/29	0.58 (0.35–0.98)	0.07
5-year overall mortality									
≥2 years	149/53	Referent	151/54	0.74 (0.50–1.10)	152/51	0.68 (0.46–1.01)	151/39	0.50 (0.32–0.76)	0.001
≥3 years	138/46	Referent	141/51	0.79 (0.52–1.20)	141/47	0.71 (0.47–1.08)	140/35	0.54 (0.34–0.85)	0.007
≥4 years	130/42	Referent	131/48	0.78 (0.50–1.20)	134/43	0.71 (0.46–1.10)	131/32	0.50 (0.31–0.80)	0.005
≥5 years	124/42	Referent	125/45	0.72 (0.46–1.13)	125/39	0.71 (0.46–1.12)	125/30	0.47 (0.29–0.77)	0.004

Abbreviations: CI, confidence interval; HR, hazard ratio.

[a]Adjusted for age at diagnosis (continuous), sex, body mass index (continuous), physical activity (continuous), cancer stage (I to IV or unknown), grade of tumor differentiation (well differentiated, moderately differentiated, poorly differentiated, unknown), location of primary tumor (proximal colon, distal colon, rectum, unknown), and year of diagnosis (continuous).

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Table 4.

Hazard ratios for overall mortality, colorectal cancer-specific mortality, and 5-year overall mortality among colorectal cancer patients by quartile of plasma total 25-hydroxyvitamin D, bioavailable 25-hydroxyvitamin D, and free 25-hydroxyvitamin D

	Hazard ratio (95% confidence interval)				
	Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**	P_{trend}
Total 25-hydroxyvitamin D					
Mean (SD), ng/mL	15.6 (4.3)	23.7 (3.7)	29.4 (3.8)	40.5 (9.0)	
No. of patients	155	158	158	156	
Overall mortality					
No. of events	74	88	89	77	
Base model[a]	Referent	1.19 (0.87–1.63)	1.22 (0.89–1.67)	0.93 (0.67–1.30)	0.70
Multivariable model[b]	Referent	1.18 (0.84–1.65)	1.13 (0.80–1.59)	0.72 (0.49–1.05)	0.09
Model further adjusted for VDBP[c]	Referent	1.18 (0.84–1.65)	1.14 (0.81–1.61)	0.73 (0.50–1.07)	0.11
Colorectal cancer-specific mortality					
No. of events	38	51	57	41	
Base model[a]	Referent	1.29 (0.85–1.98)	1.47 (0.97–2.23)	0.97 (0.61–1.53)	0.97
Multivariable model[b]	Referent	1.22 (0.77–1.93)	1.45 (0.92–2.30)	0.57 (0.34–0.97)	0.08
Model further adjusted for VDBP[c]	Referent	1.23 (0.78–1.96)	1.51 (0.95–2.40)	0.60 (0.35–1.01)	0.12
5-year overall mortality					
No. of events	49	53	58	43	
Base model[a]	Referent	1.00 (0.68–1.48)	1.12 (0.76–1.65)	0.74 (0.48–1.12)	0.25
Multivariable model[b]	Referent	0.92 (0.60–1.40)	1.05 (0.69–1.59)	0.48 (0.30–0.78)	0.01
Model further adjusted for VDBP[c]	Referent	0.92 (0.60–1.41)	1.08 (0.71–1.65)	0.50 (0.31–0.81)	0.02
Bioavailable 25-hydroxyvitamin D[d]					
Mean (SD), ng/mL	1.8 (0.7)	2.9 (0.7)	3.9 (0.8)	6.5 (2.3)	
No. of patients	146	154	151	152	
Overall mortality					
No. of events	65	85	84	77	
Base model[a]	Referent	1.22 (0.88–1.69)	1.25 (0.90–1.74)	1.16 (0.83–1.63)	0.41
Multivariable model[b]	Referent	1.11 (0.78–1.59)	1.12 (0.78–1.61)	1.19 (0.82–1.73)	0.39
Colorectal cancer-specific mortality					
No. of events	38	50	47	47	
Base model[a]	Referent	1.21 (0.79–1.85)	1.14 (0.74–1.76)	1.17 (0.76–1.81)	0.59
Multivariable model[b]	Referent	1.07 (0.66–1.71)	1.01 (0.61–1.65)	1.26 (0.77–2.06)	0.43
5-year overall mortality					
No. of events	41	54	53	49	

	Hazard ratio (95% confidence interval)				
	Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**	P_{trend}
Base model[a]	Referent	1.19 (0.79–1.79)	1.16 (0.77–1.76)	1.10 (0.72–1.69)	0.72
Multivariable model[b]	Referent	1.07 (0.68–1.69)	1.03 (0.65–1.63)	1.14 (0.71–1.82)	0.66
Free 25-hydroxyvitamin D[d]					
Mean (SD), pg/mL	4.5 (1.6)	7.0 (1.6)	9.4 (1.7)	15.8 (5.5)	
No. of patients	147	151	153	152	
Overall mortality					
No. of events	66	81	79	85	
Base model[a]	Referent	1.18 (0.85–1.63)	1.14 (0.82–1.58)	1.33 (0.96–1.85)	0.12
Multivariable model[b]	Referent	1.18 (0.82–1.69)	1.11 (0.77–1.59)	1.36 (0.94–1.95)	0.15
Colorectal cancer-specific mortality					
No. of events	38	49	44	51	
Base model[a]	Referent	1.21 (0.79–1.86)	1.04 (0.67–1.62)	1.31 (0.85–2.01)	0.35
Multivariable model[b]	Referent	1.18 (0.73–1.90)	1.05 (0.64–1.70)	1.35 (0.83–2.18)	0.32
5-year overall mortality					
No. of events	42	50	48	57	
Base model[a]	Referent	1.09 (0.72–1.65)	1.01 (0.67–1.54)	1.28 (0.85–1.92)	0.29
Multivariable model[b]	Referent	1.03 (0.65–1.64)	0.97 (0.61–1.53)	1.29 (0.82–2.02)	0.31

Abbreviations: SD, standard deviation; VDBP, vitamin D binding protein.

[a]Adjusted for age at diagnosis (continuous) and season of blood collection (summer, fall, winter, spring).

[b]Addtionally adjusted for sex, body mass index (continuous), physical activity (continuous), cancer stage (I to IV or unknown), grade of tumor differentiation (well differentiated, moderately differentiated, poorly differentiated, unknown), location of primary tumor (proximal colon, distal colon, rectum, unknown), and year of diagnosis (continuous).

[c]Additionally adjusted for vitamin D binding protein levels (quartiles or missing).

[d]Calculated by total 25-hydroxyvitamin D, vitamin D binding protein, and albumin levels and the constant affinity of vitamin D binding protein and albumin for 25-hydroxyvitamin D (6×10^5 and 7×10^8, respectively).