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Vitamin D status after colorectal cancer diagnosis and patient survival according to immune response to tumour

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Use of standardised official symbols: We use HUGO (Human Genome Organisation)-approved official symbols (or root symbols) for genes and gene products, including BRAF, CACNA1G, CD3, CD8, CD274, CDKN2A, CRABP1, FOXP3, IGF2, KRAS, MAPK, MLH1, NEUROG1, NFKB, PDCD1, PIK3CA, PTGS2, PTPRC, RUNX3, SOCS1, and VDR; all of which are described at www.genenames.org. The official symbols are italicised to differentiate from non-italicised colloquial names that are used along with the official symbols. This format enables readers to familiarise themselves with the official symbols for genes and gene products together with common colloquial names.

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Abstract

Background: High-level plasma 25-hydroxyvitamin D [25(OH)D] has been associated with lower colorectal cancer incidence and mortality. Considering evidence indicating immunomodulatory effects of vitamin D, we hypothesised that survival benefits from high systemic vitamin D level might be stronger for colorectal carcinoma with lower immune response to tumour.

Methods: Using 869 colon and rectal cancer cases within the Nurses' Health Study and Health Professionals Follow-up Study, we assessed the prognostic association of postdiagnosis 25(OH)D score [derived from diet and lifestyle variables to predict plasma 25(OH)D level] in strata of levels of histopathologic lymphocytic reaction. The Cox proportional hazards regression model was adjusted for potential confounders, including microsatellite instability, CpG island methylator phenotype, LINE-1 methylation, *PTGS2* (cyclooxygenase-2) expression, and *KRAS*, *BRAF*, and *PIK3CA* mutations.

Results: The association of postdiagnosis 25(OH)D score with colorectal cancer-specific mortality differed by levels of peritumoural lymphocytic reaction ($p_{\text{interaction}} = 0.001$).

Multivariable-adjusted mortality hazard ratios for a quintile-unit increase of 25(OH)D score were 0.69 [95% confidence interval (CI), 0.54–0.89] in cases with negative/low peritumoural lymphocytic reaction, 1.08 (95% CI, 0.93–1.26) in cases with intermediate peritumoural reaction, and 1.25 (95% CI, 0.75–2.09) in cases with high peritumoural reaction. The survival association of the 25(OH)D score did not significantly differ by Crohn's-like lymphoid reaction, intratumoural periglandular reaction, or tumour-infiltrating lymphocytes.

Conclusions: The association between the 25(OH)D score and colorectal cancer survival is stronger for carcinomas with lower peritumoural lymphocytic reaction. Our results suggesting interactive effects of vitamin D and immune response may contribute to personalised dietary and lifestyle intervention strategies.

Keywords

Clinical outcome; Immunology; Molecular pathological epidemiology; Precision medicine; Tumour microenvironment

1. Introduction

In colorectal cancer, high levels of lymphocytic reaction to tumour have been associated with prolonged patient survival [1–5]. Evidence supports the effectiveness of therapeutic antibodies that target immune checkpoint proteins such as *PDCD1* (programmed cell death 1, PD-1) and *CD274* (*PDCD1* ligand 1, PD-L1) in various cancers, including microsatellite instability (MSI)-high colorectal carcinoma [6–8]. Colorectal cancer consists of heterogeneous groups of neoplasms with varying sets of genetic and epigenetic alterations that are influenced by exogenous and endogenous factors [9–12]. A better understanding of inter-individual differences in anti-tumour effects of immunomodulatory factors would help develop personalised immunotherapeutic strategies [13].

High levels of plasma 25-hydroxyvitamin D [25(OH)D] are associated with lower incidence and mortality of colorectal cancer [14–19]. Vitamin D is hydroxylated in the liver to produce 25(OH)D, and plasma 25(OH)D level serves as a standard indicator of vitamin D activity. It is then hydroxylated further in the kidneys to produce a hormonally active metabolite, 1,25-dihydroxyvitamin D (also known as calcitriol) [20]. Some immune cells can also enzymatically convert 25(OH)D to calcitriol [21]. Experimental evidence suggests that calcitriol may modulate the innate and adaptive immunity [22,23], and can activate T lymphocyte-mediated anti-tumour immune response, thereby suppressing tumour progression [24]. Thus, we hypothesised that the association of vitamin D levels with colorectal cancer survival might be stronger for tumours with lower lymphocytic response than for tumours with higher lymphocytic response.

To test our hypothesis, we conducted this study based on two U.S. large prospective cohort studies. We utilised predicted 25(OH)D score derived from dietary and lifestyle data, which comprehensively takes into account both endogenous and exogenous sources of vitamin D, and estimates long-term plasma 25(OH)D levels [25,26].

2. Methods

2.1. Study population and data collection

We used two prospective cohort studies in the U.S., the Nurses' Health Study (NHS, 121,701 women aged 30–55 years followed since 1976) and the Health Professionals Follow-up Study (HPFS, 51,529 men aged 40–75 years followed since 1986) [27]. Study participants have been sent questionnaires biennially to update information on lifestyle factors and newly-diagnosed diseases. The follow-up rate has been over 90% for each biennial questionnaire cycle. Additional lethal colorectal cancer cases were identified using the National Death Index.

We analysed 869 cases with available data on postdiagnosis predicted 25(OH)D score, tumour tissue, and survival from participants diagnosed with colorectal cancer up to 2008 (Fig. 1 and Table 1). We included cases with colon and rectal carcinoma based on the colorectal continuum model [28]. We excluded patients who had been preoperatively treated. Patients were followed until death or end of follow-up (1 January 2014 for the HPFS; 30 June 2014 for the NHS), whichever came first. Causes of death were determined by study physicians based on a review of medical records. Formalin-fixed paraffin-embedded (FFPE) tissue blocks of surgically-resected colorectal carcinomas were collected from hospitals throughout the U.S.. A single pathologist (S.O.), who was unaware of other data, reviewed haematoxylin and eosin-stained tissue sections and recorded pathological features including tumour differentiation and four components of lymphocytic reaction, namely, Crohn's-like lymphoid reaction, peritumoural lymphocytic reaction, intratumoural periglandular reaction, and tumour-infiltrating lymphocytes (TIL) [29]. Each lymphocytic reaction component was graded as negative/low, intermediate, or high. A subset of cases ($n = 398$) were independently reviewed by a second pathologist (J.N. Glickman) with a good inter-observer correlation as previously described [29]. Tumour differentiation was categorised as well to moderate or poor (> 50% vs. ≤ 50% gland formation, respectively).

Informed consent was obtained from all participants. This study was approved by the institutional review boards at Harvard T.H. Chan School of Public Health, and Partner's Healthcare (Boston, MA, USA).

2.2. Predicted 25(OH)D score

The prediction model for plasma 25(OH)D level was described elsewhere [25]. Briefly, linear regression analysis was performed on 1,095 cancer-free male participants with available plasma 25(OH)D levels from the HPFS. The model identified race, region of residence, physical activity, body mass index (BMI), and dietary and supplementary vitamin D intake as independent predictors of plasma 25(OH)D level. The derived regression coefficients were used to estimate plasma 25(OH)D level. In an independent sample of 542 men with available plasma 25(OH)D levels from the HPFS [25], plasma 25(OH)D level increased according to the increase in deciles of predicted 25(OH)D score ($p_{trend} < 0.001$). The difference in the mean plasma 25(OH)D level between extreme deciles was 10.0 ng/mL, similar to the difference of 11.1 ng/mL in the derivation cohort. A similar approach was used to derive predicted 25(OH)D scores in the NHS [26]. We calculated postdiagnosis predicted

25(OH)D score using the earliest questionnaire returned between 6 and 48 months after colorectal cancer diagnosis.

2.3. Immunohistochemistry

We constructed tissue microarrays to include up to four cores from colorectal cancer and up to two cores from normal tissue blocks. We performed immunohistochemistry for *CD3*, *CD8*, CD45RO (one of *PTPRC* protein isoforms), and *FOXP3* as previously described [30]. We used an automated scanning microscope and the Ariol image analysis system (Genetix, San Jose, CA, USA) to measure densities (cells/mm^2) of *CD3*$^+$ cells, *CD8*$^+$ cells, CD45RO$^+$ cells, and *FOXP3*$^+$ cells in colorectal cancer tissue [30]. We conducted immunohistochemical analysis for *PTGS2* (cyclooxygenase-2) using an anti-*PTGS2* antibody (Cayman Chemical, Ann Arbor, MI, USA) [31].

2.4. Analyses of tumour molecular markers

DNA was extracted from FFPE tissue blocks. MSI status was determined using 10 microsatellite markers (D2S123, D5S346, D17S250, BAT25, BAT26, BAT40, D18S55, D18S56, D18S67, and D18S487), and MSI-high was defined as presence of instability in ≥ 30% of the markers [28]. Using bisulphite-treated DNA, methylation status of eight CpG island methylator phenotype (CIMP)-specific promoters (*CACNA1G, CDKN2A, CRABP1, IGF2, MLH1, NEUROG1, RUNX3,* and *SOCS1*) and long interspersed nucleotide element-1 (LINE-1) was analysed [28]. CIMP-high was defined as methylation in ≥ 6 of eight promoters [28]. Polymerase chain reaction and pyrosequencing were performed for *KRAS* (codons 12, 13, 61, and 146), *BRAF* (codon 600), and *PIK3CA* (exons 9 and 20) [28].

2.5. Statistical analysis

All statistical analyses were performed using SAS software (version 9.4; SAS Institute, Cary, NC, USA), and all *p* values were two-sided. In our primary hypothesis testing, we examined the statistical interaction between postdiagnosis predicted 25(OH)D score (cohort-specific quintiles, ordinal) and each lymphocytic reaction component (three-tiered, ordinal) using the Wald test in the multivariable-adjusted Cox proportional hazards regression model for colorectal cancer mortality. In addition, we assessed the interaction between postdiagnosis predicted 25(OH)D score and the density (ordinal quartile variable) of *CD3*$^+$ cells, *CD8*$^+$ cells, CD45RO$^+$ cells, or *FOXP3*$^+$ cells. In our primary hypothesis testing on new discoveries, we used the α level of 0.005 [32]. All other analyses represented secondary analyses, and we used the α level of 0.005. We estimated hazard ratio for a quintile-unit increase of postdiagnosis predicted 25(OH)D score in strata of levels of lymphocytic reaction components using a re-parameterisation of the interaction term in a single regression model [33]. In the Cox regression model, survival time was left-truncated at the date of return of the first postdiagnosis questionnaire. In colorectal cancer-specific mortality analyses, participants were censored at the time of deaths from other causes.

In all survival analyses, the inverse probability weighting (IPW) method was applied to reduce the potential bias due to the availability of postdiagnosis questionnaire data [34,35]. Cumulative survival probabilities were estimated using the IPW-adjusted Kaplan-Meier

method, and a linear trend in survival probabilities across ordinal categories of postdiagnosis predicted 25(OH)D score was assessed using the weighted log-rank test for trend. The multivariable IPW-adjusted Cox regression model initially included the variables described in Table 2, and a backward elimination with a threshold p of 0.05 was used to select variables for the final models. The Cox regression model was stratified by the time between colorectal cancer diagnosis and the first questionnaire return (≤ 1 year vs. 1.1–2.0 years vs. 2.1–3.0 years vs. 3.1–4.0 years). Cases with missing data were assigned to the majority category of a given categorical covariate: tumour differentiation (0.7%), MSI status (9.9%), CIMP status (14%), *PTGS2* expression (11%), *KRAS* mutation (11%), *BRAF* mutation (9.1%), and *PIK3CA* mutation (16%). Cases with missing data on prediagnosis predicted 25(OH)D score (6.1%) were included in the middle quintile. For cases with missing data on LINE-1 methylation level (12%), we assigned a separate indicator variable. We confirmed that excluding cases with missing data on any of the covariates did not substantially alter our results (data not shown). The Cox regression model without IPW yielded similar results to the IPW-adjusted model (Supplementary Table 1). The assumption of proportional hazards was generally satisfied using the assessment of a time-varying covariate; i.e., the cross-product of postdiagnosis predicted 25(OH)D score and log-transformed survival time in strata of each lymphocytic reaction component ($p > 0.05$).

3. Results

We included 869 colorectal cancer cases (Fig. 1 and Table 1). Postdiagnosis predicted 25(OH)D score modestly correlated with prediagnosis predicted 25(OH)D score (Spearman $r = 0.68$). During the median follow-up time of 13.3 years (interquartile range, 9.8–17.8 years) for censored cases, there were 480 all-cause deaths, including 122 colorectal cancer-specific deaths.

The association of postdiagnosis predicted 25(OH)D score with colorectal cancer-specific mortality statistically significantly differed by levels of peritumoural lymphocytic reaction ($p_{\text{interaction}} = 0.001$; with the α level of 0.005; Table 2 and Supplementary Table 2). The multivariable-adjusted HRs for colorectal cancer-specific mortality for a quintile-unit increase in postdiagnosis predicted 25(OH)D score were 0.69 [95% CI (confidence interval), 0.54–0.89] in patients with negative to low peritumoural lymphocytic reaction, 1.08 (95% CI, 0.93–1.26) in patients with intermediate peritumoural reaction, and 1.25 (95% CI, 0.75–2.09) in patients with high peritumoural reaction. In Kaplan-Meier survival analyses, a trend towards lower colorectal cancer-specific mortality associated with higher postdiagnosis predicted 25(OH)D score was observed in tumours with negative to low peritumoural lymphocytic reaction, but did not reach statistical significance ($p = 0.032$; with the α level of 0.005; Fig. 2). In contrast, no such trend was observed in tumours with intermediate to high peritumoural lymphocytic reaction ($p = 0.33$, Fig. 2). We did not observe a statistically significant interaction of postdiagnosis predicted 25(OH)D score with other lymphocytic reaction components ($p_{\text{interaction}} > 0.006$). We did not observe a statistically significant interaction between postdiagnosis predicted 25(OH)D score and lymphocytic reaction in relation to overall mortality ($p_{\text{interaction}} > 0.3$).

Considering that predicted 25(OH)D level might reflect any of other factors used in the prediction model, we included postdiagnosis BMI or postdiagnosis physical activity level as an additional covariate in the multivariable models. We observed a similar differential prognostic association of postdiagnosis predicted 25(OH)D score according to peritumoural lymphocytic reaction ($p_{interaction} = 0.001$).

In secondary analyses, we did not observe a significant differential association of postdiagnosis predicted 25(OH)D score with colorectal cancer mortality according to the density of any of T cell populations ($p_{interaction} > 0.05$, Supplementary Table 3).

4. Discussion

We found that the beneficial survival association of postdiagnosis predicted 25(OH)D score appeared stronger for colorectal cancer with lower peritumoural lymphocytic reaction. In contrast, we did not observe such a differential association for overall mortality, and therefore, a further investigation is warranted considering causes of deaths other than colorectal cancer. Our findings provide evidence for inter-personal heterogeneity of anti-tumour effects of vitamin D according to anti-tumour immune response, potentially contributing to development of tailored dietary and lifestyle intervention strategies for cancer patients.

Calcitriol exerts anti-neoplastic effects by binding to *VDR* (vitamin D receptor) [20], which is prevalently expressed in intestinal epithelial cells and immune cells [18,21,36]. Experimental evidence suggests that the anti-inflammatory effects of vitamin D may occur via suppression of the *PTGS2* (cyclooxygenase-2), *MAPK*, and *NFKB* pathways as well as suppression of several cytokines in cancers [18,37,38]. In addition, the immunomodulatory effects of vitamin D have been proposed as an alternative mechanism through which tumour progression are suppressed [18,36,37]. Vitamin D modulates adaptive immunity by altering responses of B cells, helper T cells, and regulatory T cells [21,22,36], as well as cytotoxic T cells for immune surveillance of cancers [24]. Our study supports the role of the vitamin D-mediated pathway in suppression of human colorectal cancer progression through activation of anti-tumour immune response.

This study supports the potential of lymphocytic reaction status in colorectal cancer as a biomarker for the survival benefits associated with high-level vitamin D. Interestingly, our previous study has shown that the association of plasma 25(OH)D level with low colorectal cancer incidence is stronger for tumours with high intratumoural periglandular reaction [17]. We speculate that carcinomas which have evolved in the presence of a high abundance of lymphocytes may have acquired resistance to calcitriol activated by the lymphocyte-rich microenvironment. In contrast, carcinomas with little lymphocytic response may be more susceptible to immunomodulatory effects of calcitriol. In addition, the multifaceted effects of vitamin D on different tumour subtypes may change during tumour evolution in a continuously changing microenvironment consisting of extra-cellular matrix and non-neoplastic host cells [39].

We observed a trend towards higher colorectal cancer-specific mortality associated with higher postdiagnosis predicted 25(OH)D score in patients with tumours accompanying intermediate/high lymphocytic reaction. However, considering not only little or no evidence for adverse effect of vitamin D on colorectal cancer survival but also multiple comparisons behind the individual hazard ratio estimates, the observed trend might have occurred by chance.

The present study has limitations. First, the retrospective and hypothesis-generating nature of our analyses was a limitation of the current study, and our findings need to be validated in prospective trial studies. Second, data on cancer treatment were limited. However, the selection of cancer treatment was unlikely to be made based on anti-tumour immune response, because such data were not available for treating physicians. Third, the predicted 25(OH)D score inevitably has a measurement error. In addition, we cannot completely exclude the possibility that lower levels of postdiagnosis predicted 25(OH)D score might reflect patient characteristics associated with poor prognosis. Forth, data from postdiagnosis questionnaires used to calculate 25(OH)D score were not available for every colorectal cancer patient in the cohorts. Hence, we applied the IPW method to reduce this potential selection bias.

There are strengths of our current study. A major strength is the use of the molecular pathological epidemiology approach [39,40]. An integrated analysis incorporating prospectively-collected data on epidemiological exposures, clinicopathological features, and tumour molecular markers allowed us to comprehensively examine the interaction between the predicted 25(OH)D score and immune response to tumour. There might be a variety of confounding factors for the association between vitamin D status and colorectal cancer survival. Our results generally became stronger after adjustment for potential confounders. Notably, our study population was drawn from a large number of cases from hospitals throughout the U.S., which increases the generalisability of our findings.

In conclusion, the beneficial survival association of high postdiagnosis vitamin D level is stronger for colorectal carcinoma with lower-level peritumoural lymphocytic reaction than for carcinoma with higher-level reaction. Our study supports differential anti-tumour immunomodulatory effects of vitamin D according to host immune response to tumour. Immune checkpoint inhibition can be effective for treating MSI-high carcinomas but not non-MSI-high colorectal carcinomas. Based on our data supporting the anti-tumour immune-enhancing effects of vitamin D, it is worth examining whether vitamin D can enhance effects of immune checkpoint inhibitors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

25(OH)D	25-hydroxyvitamin D
BMI	body mass index
CI	confidence interval
CIMP	CpG island methylator phenotype
FFPE	formalin-fixed paraffin-embedded
HPFS	Health Professionals Follow-up Study
HR	hazard ratio
IPW	inverse probability weighting
LINE-1	long interspersed nucleotide element-1
MSI	microsatellite instability
NHS	Nurses' Health Study
SD	standard deviation
TIL	tumour-infiltrating lymphocytes

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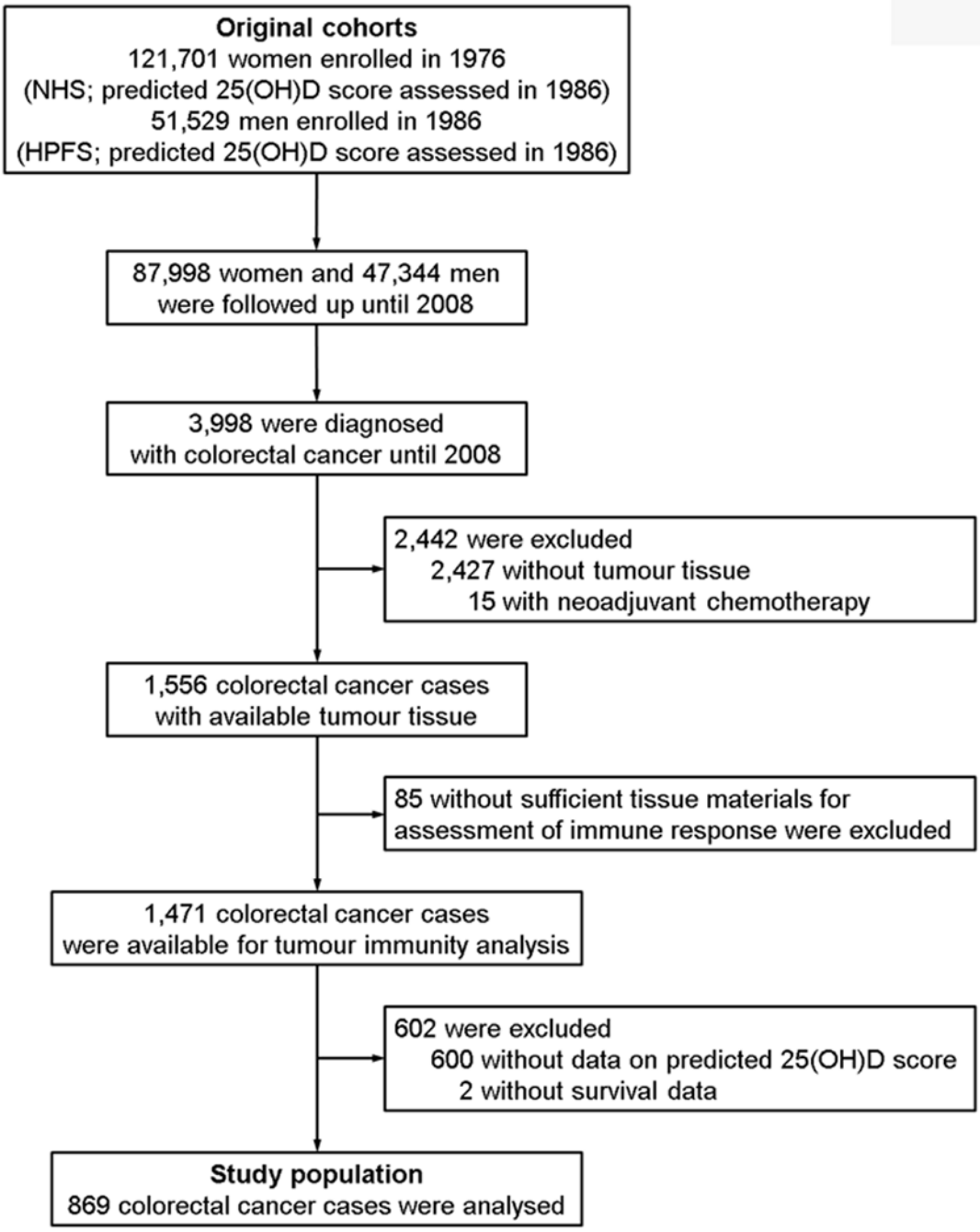

Fig. 1.

Flow diagram of the study population in the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS). 25(OH)D, 25-hydroxyvitamin D.

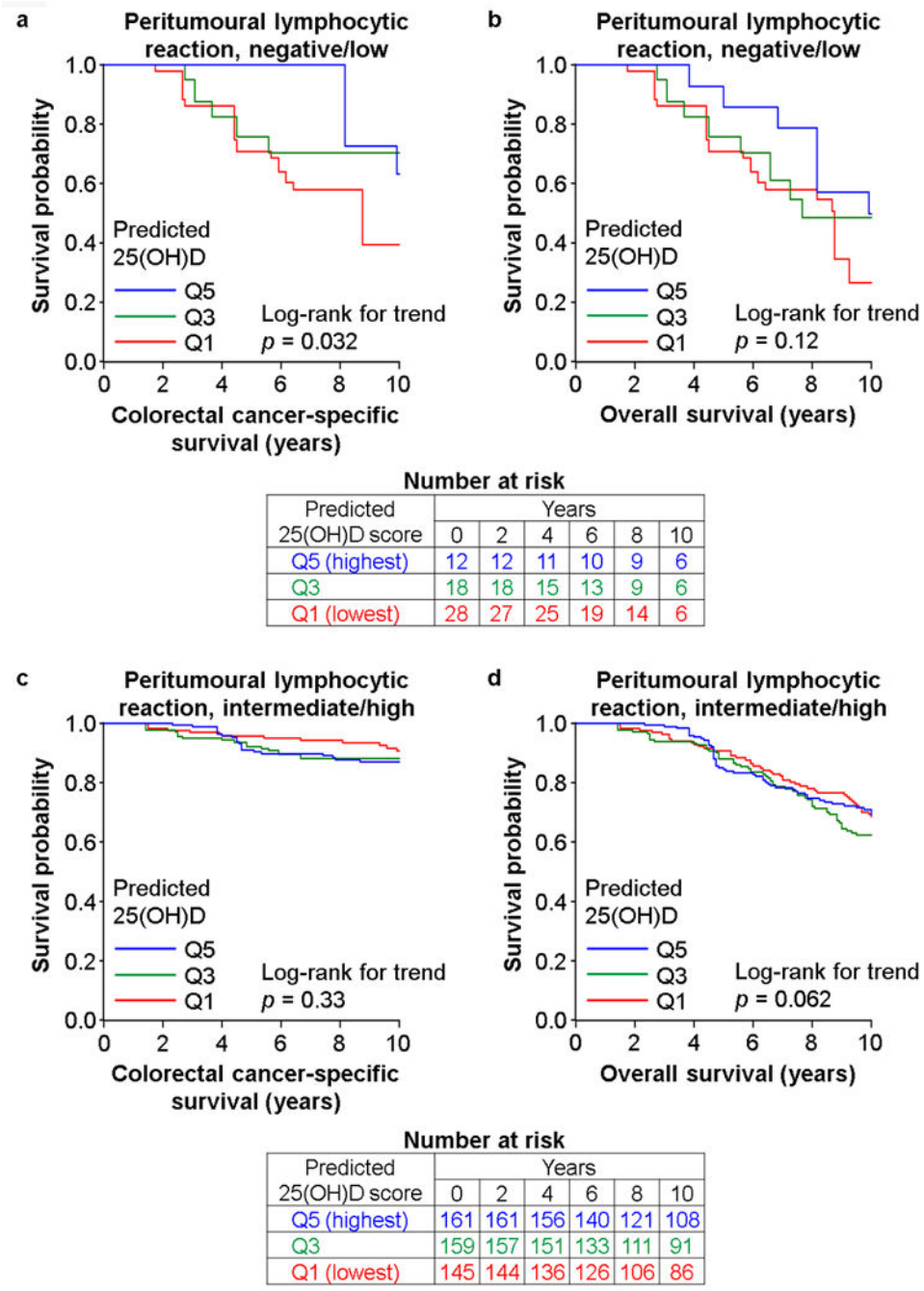

Fig. 2.

Inverse probability weighting (IPW)-adjusted Kaplan-Meier survival curves of colorectal cancer patients according to postdiagnosis predicted 25(OH)D score in strata of peritumoural lymphocytic reaction. The *p* values were calculated using the weighted log-rank test for trend (two-sided). **a** and **b**, colorectal cancer-specific survival and overall survival, respectively, among patients with tumours accompanying negative to low peritumoural lymphocytic reaction. **c** and **d**, colorectal cancer-specific survival and overall survival, respectively, among patients with tumours accompanying intermediate to high

peritumoural lymphocytic reaction. 25(OH)D, 25-hydroxyvitamin D; Q1, quintile 1; Q3, quintile 3; Q5, quintile 5.

Table 1.

Clinical, pathological, and molecular characteristics of colorectal cancer cases according to postdiagnosis predicted 25(OH)D score.

Characteristic[a]	All cases (*n* = 869)	Postdiagnosis predicted 25(OH)D score (ng/mL)					*p*[b]
		Quintile 1 (*n* = 173)	Quintile 2 (*n* = 171)	Quintile 3 (*n* = 179)	Quintile 4 (*n* = 172)	Quintile 5 (*n* = 174)	
Postdiagnosis predicted 25(OH)D score (ng/mL), median (range)							
Female (*n* = 454, NHS)	27.4 (18.3–35.3)	23.9 (18.3–25.2)	26.2 (25.3–27.0)	27.4 (27.0–28.4)	29.4 (28.4–30.4)	31.7 (30.4–35.3)	-
Male (*n* = 415, HPFS)	28.4 (20.5–36.0)	25.3 (20.5–26.4)	27.3 (26.4–28.0)	28.4 (28.0–29.2)	29.9 (29.2–30.9)	32.4 (30.9–36.0)	-
Mean age ± SD (years)	68.3 ± 8.6	69.2 ± 8.7	68.2 ± 8.8	68.4 ± 8.7	67.7 ± 8.3	67.8 ± 8.6	0.53
Year of diagnosis							0.25
1995 or before	347 (40%)	62 (36%)	76 (44%)	59 (33%)	71 (41%)	79 (45%)	
1996–2000	277 (32%)	55 (32%)	54 (32%)	66 (37%)	51 (30%)	51 (29%)	
2001–2008	245 (28%)	56 (32%)	41 (24%)	54 (30%)	50 (29%)	44 (25%)	
Family history of colorectal cancer in first-degree relative(s)							0.47
Absent	690 (79%)	132 (76%)	141 (82%)	140 (78%)	142 (83%)	135 (78%)	
Present	179 (21%)	41 (24%)	30 (18%)	39 (22%)	30 (17%)	39 (22%)	
Tumour location							0.78
Caecum	161 (19%)	31 (18%)	29 (17%)	40 (22%)	32 (19%)	29 (17%)	
Ascending to transverse colon	238 (27%)	45 (26%)	48 (28%)	48 (27%)	44 (26%)	53 (30%)	
Splenic flexure to sigmoid colon	284 (33%)	51 (29%)	61 (36%)	53 (30%)	59 (34%)	60 (34%)	
Rectum	186 (21%)	46 (27%)	33 (19%)	38 (21%)	37 (22%)	32 (18%)	
Tumour differentiation							0.92
Well to moderate	797 (92%)	158 (91%)	158 (93%)	167 (93%)	158 (93%)	156 (91%)	
Poor	66 (7.7%)	15 (8.7%)	12 (7.1%)	12 (6.7%)	12 (7.1%)	15 (8.8%)	
AJCC disease stage							0.53
I	245 (31%)	45 (28%)	51 (33%)	59 (36%)	44 (29%)	46 (28%)	
II	294 (37%)	60 (37%)	49 (32%)	60 (37%)	58 (38%)	67 (41%)	
III	220 (28%)	45 (28%)	48 (31%)	39 (24%)	42 (28%)	46 (28%)	
IV	36 (4.5%)	12 (7.4%)	7 (4.5%)	4 (2.5%)	7 (4.6%)	6 (3.6%)	
MSI status							0.26
Non-MSI-high	652 (83%)	126 (78%)	135 (87%)	137 (86%)	122 (82%)	132 (84%)	
MSI-high	131 (17%)	35 (22%)	21 (13%)	22 (14%)	27 (18%)	26 (16%)	
CIMP status							0.75
CIMP-low/negative	621 (83%)	119 (80%)	128 (84%)	123 (82%)	118 (84%)	133 (85%)	
CIMP-high	127 (17%)	30 (20%)	24 (16%)	27 (18%)	23 (16%)	23 (15%)	

Characteristic[a]	All cases (*n* = 869)	Postdiagnosis predicted 25(OH)D score (ng/mL) Quintile 1 (*n* = 173)	Quintile 2 (*n* = 171)	Quintile 3 (*n* = 179)	Quintile 4 (*n* = 172)	Quintile 5 (*n* = 174)	*p*[b]
Mean LINE-1 methylation level ± SD (%)	62.8 ± 9.6	63.5 ± 10.2	61.4 ± 9.2	62.9 ± 10.3	63.3 ± 9.3	63.0 ± 9.1	0.35
KRAS mutation							0.056
Wild type	465 (60%)	106 (68%)	85 (54%)	98 (62%)	90 (62%)	86 (55%)	
Mutant	310 (40%)	49 (32%)	73 (46%)	61 (38%)	56 (38%)	71 (45%)	
BRAF mutation							0.85
Wild type	690 (87%)	139 (87%)	141 (89%)	136 (85%)	134 (89%)	140 (87%)	
Mutant	100 (13%)	21 (13%)	18 (11%)	24 (15%)	17 (11%)	20 (13%)	
PIK3CA mutation							0.74
Wild type	606 (83%)	124 (84%)	120 (82%)	126 (86%)	114 (81%)	122 (81%)	
Mutant	125 (17%)	24 (16%)	26 (18%)	20 (14%)	27 (19%)	28 (19%)	
PTGS2 (cyclooxygenase-2) expression							0.82
Negative	297 (38%)	62 (39%)	66 (42%)	57 (37%)	53 (36%)	59 (38%)	
Positive	476 (62%)	96 (61%)	91 (58%)	96 (63%)	96 (64%)	97 (62%)	
Crohn's-like lymphoid reaction							0.49
Negative/low	508 (72%)	99 (71%)	92 (69%)	115 (77%)	90 (69%)	112 (77%)	
Intermediate	130 (19%)	26 (19%)	27 (20%)	22 (15%)	28 (21%)	27 (18%)	
High	63 (9.0%)	15 (11%)	15 (11%)	13 (8.7%)	13 (9.9%)	7 (4.8%)	
Peritumoural lymphocytic reaction							0.15
Negative/low	92 (11%)	28 (16%)	19 (11%)	18 (10%)	15 (8.8%)	12 (6.9%)	
Intermediate	639 (74%)	121 (70%)	119 (70%)	131 (74%)	130 (76%)	138 (80%)	
High	133 (15%)	24 (14%)	33 (19%)	28 (16%)	25 (15%)	23 (13%)	
Intratumoural periglandular reaction							0.40
Negative/low	88 (10%)	24 (14%)	18 (11%)	18 (10%)	15 (8.7%)	13 (7.5%)	
Intermediate	662 (76%)	125 (72%)	123 (72%)	137 (77%)	136 (79%)	141 (82%)	
High	118 (14%)	24 (14%)	30 (18%)	23 (13%)	21 (12%)	20 (11%)	
Tumour-infiltrating lymphocytes							0.25
Negative/low	638 (73%)	122 (71%)	126 (74%)	120 (67%)	134 (78%)	136 (78%)	
Intermediate	128 (15%)	30 (17%)	22 (13%)	34 (19%)	19 (11%)	23 (13%)	
High	103 (12%)	21 (12%)	23 (13%)	25 (14%)	19 (11%)	15 (8.6%)	

25(OH)D, 25-hydroxyvitamin D; AJCC, American Joint Committee on Cancer; CIMP, CpG island methylator phenotype-specific promoters; HPFS, Health Professionals Follow-up Study; LINE-1, long interspersed nucleotide element-1; MSI, microsatellite instability; NHS, Nurses' Health Study; SD, standard deviation.

[a]Percentage indicates the proportion of cases with a specific clinical, pathological, or molecular characteristic in all cases or in strata of quintiles of postdiagnosis predicted 25(OH)D score.

[b]To compare characteristics between subgroups, we used the chi-square test for categorical variables, and the analysis of variance for continuous variables.

Table 2.

Colorectal cancer mortality according to postdiagnosis predicted 25(OH)D score in all cases or in strata of levels of lymphocytic reaction components.

		Colorectal cancer-specific mortality HR for a quintile-unit increase of postdiagnosis predicted 25(OH)D score			Overall mortality HR for a quintile-unit increase of postdiagnosis predicted 25(OH)D score		
	No. of cases	No. of events	Univariable HR[a] (95% CI)	Multivariable HR[a,b] (95% CI)	No. of events	Univariable HR[a] (95% CI)	Multivariable HR[a,b] (95% CI)
All colorectal cancer cases	869	122	0.95 (0.81–1.10)	1.06 (0.88–1.26)	480	0.92 (0.86–0.99)	0.94 (0.88–0.99)
Crohn's-like lymphoid reaction							
Negative/low	508	84	0.88 (0.74–1.04)	1.01 (0.83–1.25)	276	0.93 (0.85–1.02)	0.95 (0.87–1.02)
Intermediate	130	13	1.01 (0.67–1.53)	1.21 (0.83–1.76)	75	0.93 (0.78–1.10)	0.98 (0.86–1.12)
High	63	5	1.46 (0.90–2.34)	1.95 (1.01–3.77)	34	0.86 (0.69–1.07)	0.80 (0.64–1.01)
$p_{interaction}$[c]			0.13	0.092		0.59	0.39
Peritumoural lymphocytic reaction							
Negative/low	92	29	0.73 (0.53–1.02)	0.69 (0.54–0.89)	51	0.79 (0.61–1.03)	0.84 (0.68–1.03)
Intermediate	639	87	1.06 (0.90–1.25)	1.08 (0.93–1.26)	358	0.96 (0.89–1.04)	0.98 (0.91–1.05)
High	133	5	1.18 (0.73–1.91)	1.25 (0.75–2.09)	70	0.85 (0.72–1.01)	0.85 (0.74–0.99)
$p_{interaction}$[c]			0.022	0.001		0.54	0.98
Intratumoural periglandular reaction							
Negative/low	88	24	0.77 (0.53–1.12)	0.74 (0.57–0.96)	43	0.83 (0.62–1.11)	0.80 (0.64–0.99)
Intermediate	662	93	1.02 (0.88–1.19)	1.05 (0.91–1.21)	375	0.97 (0.90–1.04)	0.98 (0.92–1.06)
High	118	5	1.16 (0.73–1.83)	1.27 (0.77–2.08)	62	0.77 (0.65–0.91)	0.83 (0.72–0.94)
$p_{interaction}$[c]			0.10	0.007		0.64	0.98
Tumour-infiltrating lymphocytes							
Negative/low	638	102	0.88 (0.75–1.04)	0.98 (0.82–1.18)	347	0.91 (0.84–0.99)	0.93 (0.86–0.99)
Intermediate	128	15	1.34 (0.98–1.82)	1.64 (1.17–2.30)	74	1.02 (0.87–1.18)	1.00 (0.87–1.15)
High	103	5	1.23 (0.62–2.43)	1.66 (0.81–3.44)	59	0.84 (0.70–0.99)	0.91 (0.78–1.07)
$p_{interaction}$[c]			0.036	0.008		0.83	0.87

25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; HR, hazard ratio; IPW, inverse probability weighting.

[a] IPW was applied to reduce a bias due to the availability of questionnaire data after cancer diagnosis (see "Statistical analysis" subsection for details).

[b] The multivariable Cox regression model initially included sex (female vs. male), age at diagnosis (continuous), year of diagnosis (continuous), family history of colorectal cancer (absent vs. present), prediagnosis predicted 25(OH)D score (cohort-specific quintiles of cumulative average, ordinal), tumour location (proximal colon vs. distal colon vs. rectum), tumour differentiation (well to moderate vs. poor), disease stage (I-II vs. III-IV vs. missing), microsatellite instability status (high vs. non-high), CpG island methylator phenotype-specific promoter status (high vs. low/negative), long interspersed nucleotide element-1 methylation level (continuous), *KRAS* mutation (wild-type vs. mutant), *BRAF* mutation (wild-type vs. mutant), *PIK3CA* mutation (wild-type vs. mutant), and *PTGS2* (cyclooxygenase-2) expression (negative vs. positive). A backward elimination with a threshold p of 0.05 was used to select variables for the final models. The variables which remained in the final models for peritumoural lymphocytic reaction are described in Supplementary Table 2.

[c] $p_{interaction}$ (two-sided) was calculated using the Wald test for the cross-product of postdiagnosis predicted 25(OH)D score (ordinal quintile variable) and each of the lymphocytic reaction variables (ordinal) in the IPW-adjusted Cox regression model.