



# Pre-diagnostic 25-hydroxyvitamin D levels and survival in cancer patients

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## Abstract

**Purpose** Our main aim was to explore whether pre-diagnostic circulating levels of 25-hydroxyvitamin D (25(OH)D) among older individuals with cancer were associated with overall and cancer-specific survival after diagnosis.

**Design** We used data from the Reykjavik-AGES Study on participants ( $n=4,619$ ) without cancer at entry, when blood samples were taken for 25(OH)D standardized measurements. The association with cancer risk, all-cause- and cancer-specific mortality was assessed among those later diagnosed with cancer, comparing four 25(OH)D categories, using 50–69.9 nmol/L as the reference category.

**Results** Cancer was diagnosed in 919 participants on average 8.3 years after blood draw. No association was observed between the reference group and other 25(OH)D groups and total cancer incidence. Mean age at diagnosis was 80.9 ( $\pm 5.7$ ) years. Of those diagnosed, 552 died during follow-up, 67% from cancer. Low pre-diagnostic levels of 25(OH)D  $< 30$  nmol/L were significantly associated with increased total mortality (HR: 1.39, 95% CI 1.03, 1.88) and non-significantly with cancer-specific mortality (HR: 1.33, 95% CI 0.93, 1.90). Among patients surviving more than 2 years after diagnosis, higher pre-diagnostic 25(OH)D levels ( $\geq 70$  nmol/L) were associated with lower risk of overall (HR: 0.68, 95% CI 0.46, 0.99) and cancer-specific mortality (HR: 0.47, 95% CI 0.26, 0.99).

**Conclusions** Among elderly cancer patients, low pre-diagnostic serum 25(OH)D levels ( $< 30$  nmol/L) were associated with increased overall mortality.

**Keywords** Vitamin D · Nutrition · Cancer · Survival · Older individuals

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## Abbreviations

AGES Age, gene/environment susceptibility  
BMI Body mass index

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CI	Confidence interval
HR	Hazard ratio
25(OH)D	25-Hydroxyvitamin D

## Introduction

During the last decades, vitamin D status has been studied extensively in relation to various health outcomes including cancer. Yet, many questions remain unanswered especially with regard to prognosis [1]. While higher vitamin D levels—generally measured as serum 25-hydroxyvitamin D (25(OH)D)—might be associated with reduced incidence of several malignancies [1–6], the evidence is strongest for colorectal cancer [1, 4, 7]. However, a recent Mendelian randomisation study did not confirm these findings [8]. Vitamin D status might also affect cancer prognosis, possibly through inhibition of invasion, metastasis, angiogenesis [3] and improved immune function [9]. Higher vitamin D levels measured around the time of diagnosis are associated with overall longer survival in patients [10], but also with site-specific survival such as ovarian [11], breast [12], and some other cancers [13]. Reverse causality is, however, a concern when levels of 25(OH)D are measured at the time of diagnosis or later [14].

Some studies have assessed survival among cancer patients specifically using data on pre-diagnostic vitamin D status. The NHANES III survey was, to our knowledge, one of the first prospective population-based studies to examine this and found no association between pre-diagnostic circulating 25(OH)D levels and total cancer mortality, but an inverse association with colorectal and breast cancer mortality [15]. Studies have subsequently shown higher pre-diagnostic 25(OH)D levels to be associated with improved survival in patients with colorectal [16–18], prostate [6, 19–21], kidney [21], melanoma [21], and breast cancer [22] but poorer survival in patients with lung cancer [21]. Furthermore, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study with 4616 cancer patients has also reported overall lower cancer mortality among male smokers with higher pre-diagnostic 25(OH)D levels [21]. Meta-analyses of cohort studies have reported inverse association between higher 25(OH)D levels and all-cause mortality [23, 24] as well as cancer-specific mortality [23–25]. Additionally, a meta-analysis of four randomized controlled trials with supplemental vitamin D use for 2–7 years showed reduced total cancer mortality among supplement users [26]. Another meta-analysis of 22 randomized controlled trials only observed a reduced overall mortality among elderly participants taking vitamin D<sub>3</sub> supplements [24].

Living just south of the arctic circle, Icelanders largely depend on dietary and supplemental sources of vitamin D, resulting in minimal seasonal variations in 25(OH)D levels

among our study group [27]. In this population-based study, we explored whether pre-diagnostic standardized serum levels of 25(OH)D among older individuals living in Iceland were associated with survival after cancer diagnosis. We also assessed 25(OH)D levels in relation to subsequent cancer incidence.

## Methods

### Study population

In 1967, all 30,795 men and women living in the Reykjavik area of Iceland and born between 1907 and 1935 were invited by the Icelandic Heart Association to join a prospective cohort study. Approximately 19,000 attended the clinic at least once and became participants of the Reykjavik Study. Subsequently, in 2002, a random sample of the cohort comprising 5,764 men and women who were still alive in Iceland and aged 66–96 years entered the Ages Gene/Environment Susceptibility (AGES)—Reykjavik Study [28]. Serum 25(OH)D was measured in 5,519 of all 5,764 (96%) participants at study entry, from 2002 to 2006. The present study protocol was approved by the Icelandic Ethical Review Board (VSNb2007120014/03-7) and the Icelandic Data Protection Authority. All participants gave written informed consent.

### Measurement of serum 25(OH)D

As described previously [27], fasting serum samples were kept frozen at  $-80^{\circ}\text{C}$ . Total 25(OH)D (D2 and D3) was measured by direct, competitive chemiluminescence immunoassay (CLIA), using the LIAISON 25 OH Vitamin D Total assay (DiaSorin, Inc., Stillwater, Minnesota) [27]. Serum 25(OH)D values were standardized according to the NIH International Vitamin D Standardization Program [29] to facilitate comparison across different studies [30, 31]. Based on cut-points for deficient ( $\leq 30$  nmol/L) and sufficient ( $> 50$  nmol/L) levels of 25(OH)D, according to the Nordic Nutritional Recommendations [32], we classified levels of 25(OH)D into four groups:  $< 30$ , 30–49.9, 50–69.9, and  $\geq 70$  nmol/L. Season of blood draw was classified as summer (June–August), autumn (September–November), spring (March–May), and winter (December–February).

### Follow-up

We ascertained first incident cancer diagnoses through linkage to the nationwide Icelandic Cancer Registry which is virtually complete [33]. Information on cause of death was obtained from the Icelandic Directorate of Health. At study entry, 900 of the otherwise 5519 eligible participants were

already diagnosed with cancer, leaving 4,619 participants in our analytic cohort with information on vitamin D status but no diagnosed cancer at baseline. For analysis of cancer risk, all participants ( $n=4,619$ ) were followed from the study entry until cancer diagnosis, death, or end of the observation period whichever occurred first.

For mortality analyses, patients were followed from date of diagnosis until death or censored at end of the follow-up (December 31, 2014). We also individually analyzed the mortality risk among the four most common cancer types of lethal malignancies in Iceland (<http://www.krabbameinsskra.is>): lung, breast, prostate, and colorectal cancer. With regard to prostate cancer, no organized PSA-screening program has been introduced in Iceland [34]. However, PSA testing is widely used. Information on Gleason score at diagnosis was available for 93% of participants diagnosed during the follow-up (2002–2014). Gleason score was combined into four categories: <7, 3+4, 4+3, and 8–10. For breast cancer, information on tumor estrogen (ER) and progesterone (PR) receptor status (yes/no) was available for 84% of the cases. For colorectal cancer, information on TNM stage (I, II, III, and IV) was available for 79% of the cases. We had no information on stage for lung cancer cases.

## Covariates

Weight and height was measured at study entry and a trained interviewer collected data on smoking habits, education, food, dietary supplements, and alcohol intake using a structured questionnaire. Physical activity was also assessed at study entry by self-reported questionnaire. Participants were asked how many hours per week they participated in moderate or vigorous physical activities during the past 12 months. Predefined responses were never, rarely, weekly but less than 1 h (occasionally), 1–3 h per week, 4–7 h per week and more than 7 h per week. In the final analyses, the physical activity categories were combined into 2 categories: never, rarely, or occasionally (i.e., < 1 h per week); and moderate or high (i.e.,  $\geq 1$  h per week).

## Statistical analyses

We used Cox proportional hazards models to estimate hazard ratios (HR) and 95% confidence intervals (CI) of risk of cancer diagnosis and survival after cancer diagnosis by 25(OH)D levels. 25(OH)D levels were modeled categorically with categories <30, 30–49.9, 50–69.9, and  $\geq 70$  nmol/L, using levels between 50 and 69.9 nmol/L as a reference category, as levels above 50 nmol/L are considered sufficient levels according to the Nordic Nutritional Recommendations [32]. We also tested for trend in the hazard ratios for the categories relative to the first category, using polynomial contrasts.

We examined the association between pre-diagnostic 25(OH)D and cancer risk (data reported in supplementary tables). The association was first assessed in a model adjusted only for age at entry. In the final multivariate model, we further adjusted for known cancer risk factors [35] and lifestyle factors that were statistically different across exposure groups: sex, BMI, smoking status, alcohol intake, multivitamin supplements, calcium supplements, other supplements, frequent aspirin use, type 2 diabetes, physical activity, and season of blood sample (see legend of Supplementary Table 2 for further details).

In addition to risk of total cancer, we also analyzed the risk of each of the four most common cancer types: lung, breast, prostate, and colorectal cancer. We used the same statistical models for each cancer type, except for lung cancer, we also included information on number of cigarettes [(1)  $\leq$  half a pack per day, (2) > half a pack up to one per day, (3) > one pack per day] among former and current smokers. Current or former smokers, who did not answer the question on number of cigarettes per day, were placed in the most common category, which was half a pack per day or less.

The associations between pre-diagnostic 25(OH)D levels and total survival, cancer survival, and non-cancer survival among cancer patients were assessed in 2 models. In the first model, we adjusted for age at diagnosis. In the fully adjusted model, we further included factors known previously to influence both vitamin D status in this cohort [27] and mortality. The covariates included were sex, BMI, education, smoking status, alcohol intake, physical activity, and season of blood sample (see legend of Table 2 for further details). In the multivariable model, data were missing for physical activity ( $n=32$ ), smoking status ( $n=22$ ), and alcohol consumption ( $n=22$ ). The outcomes were not materially altered when missing cases were placed into the most frequent category for each covariate (data not shown). Frequency of fish oil consumption was not included as a potential confounder in the main model, since it was considered a significant source of vitamin D in the Icelandic diet, with 71% of the cancer patients reporting consumption of fish oil.

We further undertook an analysis to examine potential effect of preclinical disease, i.e., presence of undiagnosed cancer that might have led to low vitamin D levels and subsequently also to low survival. Therefore, we additionally examined vitamin D levels and survival in all cancer patients within 2 years after diagnosis as well as among those surviving more than 2 years from cancer diagnosis.

For all statistical analysis, we used SPSS software, version 24.0 (SPSS Inc.; <http://www.spss.com>).

## Results

### Characteristics of the study cohort

The mean ( $\pm$  SD) serum 25(OH)D level for the 4,619 participants without diagnosed cancer at study entry was 57.1 ( $\pm$  18.0) nmol/L and the mean age was 76.5 ( $\pm$  5.6) years. Age categories were as follows: 66 to 69 (10.3%), 70 to 74 (30.5%), 80 to 84 (22.5%), and 85 years of age or older (8.3%). One out of every 12 participants (8%) had 25(OH)D below 30 nmol/L and 22% had values equal or greater than 70 nmol/L. The majority of study participants (68%) reported taking fish liver oil rich in vitamin D at baseline of the study. Those taking fish liver oil were more likely to also take multivitamins and calcium supplements, to report frequent physical activity, and were less likely to be obese and have DM2 compared to those not consuming fish liver oil. No difference was observed between fish oil users and non-users with regard to education and smoking habits.

Supplementary Table 1 summarizes characteristics of the study population who had not been diagnosed with cancer at study entry.

During 8,295 person-years of follow-up, 919 participants were diagnosed with cancer. Mean follow-up from blood sample collection to the end of follow-up was 8.3 ( $\pm$  3.3) years.

Table 1 summarizes baseline characteristics of participants who were diagnosed with cancer during follow-up. Mean age at cancer diagnosis was 80.9 ( $\pm$  5.7) years. Compared with those with low 25(OH)D levels, participants in the highest category ( $>$  50 nmol/L) were more likely to be physically active, have university education, and take food supplements and fish oil. Those in the 25(OH)D deficiency group ( $\leq$  30 nmol/L) were more likely to smoke, have type 2 diabetes, and be women (72%).

### Serum 25(OH)D and cancer risk

As seen in Supplementary Table 2, we found no significant association between baseline 25(OH)D concentrations and total cancer incidence (HR per 25 nmol/L increase: 1.04, 95% CI 0.95, 1.14) nor any association with any of the four most commonly diagnosed cancers, although there was a significantly increased risk of lung cancer in the lowest 25(OH)D category compared to the reference (HR = 2.21, 95% CI 1.26, 3.88). Among those diagnosed with lung cancer, 10% were never and 40% were current smokers.

**Table 1** Characteristics of incident cancer patients according to 25(OH)D levels at entry in the AGES-Reykjavik study ( $n=919$ )

	Serum 25(OH)D level (nmol/L)			
	<30	30–49.9	50–69.9	$\geq$ 70
Sample size, $n$	80	213	399	227
Age at entry (year)	76.7 $\pm$ 6.0 <sup>a</sup>	75.9 $\pm$ 5.3	75.9 $\pm$ 5.1	76.7 $\pm$ 5.4
Age at diagnosis (year)	81.1 $\pm$ 6.0	80.6 $\pm$ 5.6	80.6 $\pm$ 5.6	81.6 $\pm$ 5.7
BMI at entry (kg/m <sup>2</sup> )	27.0 $\pm$ 5.9	28.0 $\pm$ 5.1	27.2 $\pm$ 4.1	26.2 $\pm$ 3.9
BMI in midlife (kg/m <sup>2</sup> )	25.4 $\pm$ 4.5	26.0 $\pm$ 4.2	25.1 $\pm$ 3.2	25.0 $\pm$ 3.0
Sex (%)				
Men	32.5	47.9	62.1	51.6
Women	67.5	52.1	37.9	48.4
Education (%)				
Primary and secondary	82.5	85.0	79.7	78.4
College (postsecondary)	13.8	11.3	13.0	13.2
University	3.8	3.8	7.3	8.4
Regular health check-up in midlife (%)	17.5	19.2	25.8	18.5
Type 2 diabetes (%)	20.0	13.6	13.3	14.5
Smoking status (%)				
Current	29.9	16.0	13.3	12.1
Alcohol intake (%)				
0 g per week	44.2	37.9	30.4	33.2
1–10 g per week	32.5	34.5	36.6	39.9
$>$ 10 g per week	23.4	27.7	33.0	26.9
Fish oil intake (%)				
Yes	43.2	61.7	79.9	88.8
Fish meals (%)				
$>$ 2 meals per week	51.9	68.0	68.8	73.0
Multivitamin supplements (%)				
Yes	12.5	20.2	35.8	34.4
Calcium supplements (%)				
Yes	5.0	7.5	20.6	18.9
Other supplements (%)				
Yes	40.0	60.1	80.2	81.9
Frequent aspirin users (%)				
Yes	32.5	27.7	34.1	42.7
Physical activity (%)				
Moderate/high	14.3	22.5	32.6	41.1
Season of blood sample				
Winter	25.0	30.0	25.3	21.6
Spring	26.3	26.3	24.1	33.0
Summer	10.0	9.9	14.0	16.7
Fall	38.8	33.8	36.6	28.6

<sup>a</sup>Mean  $\pm$  SD (all such values)

## Serum 25(OH)D and cancer survival

Among those with a cancer diagnosis, 552 (60%) died during follow-up (mean  $3.3 \pm 3.0$  years; median 2.5 years). The primary cause of death was cancer (67%). We found a significant association between low 25(OH)D levels ( $< 30$  nmol/L) and poorer overall survival (HR = 1.39, 95% CI 1.03, 1.88) compared to levels of 25(OH)D between 50 and 69.9 nmol/L in those with diagnosed cancer ( $p_{\text{trend}} = 0.01$ ) (Table 2). Similar results were seen for cancer-related mortality (371 events) when we only adjusted for age at diagnosis (HR = 1.43, 95% CI 1.00–2.03,  $p_{\text{trend}} < 0.001$ ). However, the association for cancer-related mortality was non-significant in the multivariable adjusted model, (357 events) (HR = 1.33, 95% CI 0.93, 1.90,  $p_{\text{trend}} = 0.06$ ) (Table 2). When we adjusted additionally for the main food source for vitamin D, i.e., fish oil, as well as other nutrients (e.g., omega-3 fatty acids), the risk estimate for overall survival was similar to our findings without adjusting for fish oil consumption (HR = 1.38, 95% CI 1.00, 1.90).

Age-stratified analysis showed that the lowest levels of 25(OH)D were significantly associated poorer survival only

in the age groups between 70 and 74 years (HR: 1.91, 95% CI 1.10, 3.30) as well as 75–79 years of age (HR: 1.90, 95% CI 1.10, 3.31). Sex-stratified analysis showed similar, yet statistically non-significant estimates given the small numbers, results as was seen for the total group for the lowest levels of 25(OH)D with poorer survival for men (263 events, HR: 1.51, 95% CI 0.90, 2.52) and women (262 events, HR: 1.42, 95% CI 0.97, 2.08).

## Short versus long follow-up after cancer diagnosis

As shown in Table 2, the follow-up time was lowest for those with pre-diagnostic 25(OH)D levels below 30 nmol/L. Therefore, we examined the association for two time periods ( $\leq 2$  and  $> 2$  years after diagnosis). Table 3 shows that for the shorter follow-up time after diagnosis ( $\leq 2$  years), the HR between low 25(OH)D levels ( $< 30$  nmol/L) and all-cause mortality was 1.53 (95% CI 1.08, 2.17) and 1.51 for cancer mortality (95% CI 1.00, 2.29).

For those who survived longer than 2 years, there was no association between low 25(OH)D levels ( $< 30$  nmol/L) and total mortality (HR: 0.99, 95% CI 0.54, 1.82). However, the highest serum 25(OH)D levels ( $\geq 70$  nmol/L) before cancer

**Table 2** Hazard ratios (HR) and 95% confidence intervals (CI) of the association between pre-diagnostic plasma 25(OH)D levels and overall and cause-specific survival among cancer patients

25(OH)D (nmol/L)	Deaths (%)	Follow-up time <sup>a</sup> (25th–75th percentile)	HR (95% CI)
All-cause mortality <sup>b</sup>	<i>n</i> = 552/919		<i>n</i> = 525
< 30	59/80 (74)	0.91 (0.28, 3.32)	1.39 (1.03, 1.88)
30–49.9	131/213 (62)	2.75 (0.43, 5.41)	0.98 (0.79, 1.23)
50–69.9	235/399 (59)	3.44 (0.67, 5.89)	1 (ref.)
$\geq 70$	127/227 (56)	2.70 (0.55, 6.21)	0.90 (0.72, 1.13)
<i>P</i> <sub>lineartrend</sub>			0.01
HR per 25 nmol/L increase			0.94 (0.84, 1.05)
Cancer mortality <sup>b</sup>	<i>n</i> = 371/919		<i>n</i> = 357
< 30	39/80 (49)	0.44 (0.11, 0.85)	1.33 (0.93, 1.90)
30–49.9	92/213 (43)	0.76 (0.13, 2.42)	0.99 (0.76, 1.29)
50–69.9	158/399 (40)	0.80 (0.18, 2.77)	1 (ref.)
$\geq 70$	82/227 (36)	0.52 (0.14, 1.47)	0.91 (0.67, 1.20)
<i>P</i> <sub>lineartrend</sub>			0.06
HR per 25 nmol/L increase			0.93 (0.81, 1.07)
Non-cancer mortality <sup>b</sup>	<i>n</i> = 181/919		<i>n</i> = 168
< 30	20/80 (25)	1.65 (0.32, 3.16)	1.49 (0.86, 2.57)
30–49.9	39/213 (18)	3.27 (1.83, 4.40)	0.99 (0.66, 1.49)
50–69.9	77/399 (19)	2.60 (1.22, 5.10)	1 (ref.)
$\geq 70$	45/227 (20)	3.27 (0.99, 5.24)	0.84 (0.57, 1.24)
<i>P</i> <sub>lineartrend</sub>			0.10
HR per 25 nmol/L increase			0.93 (0.75, 1.15)

<sup>a</sup>Median time in years

<sup>b</sup>Adjusted for age (year; continuous) at diagnosis, sex, BMI (in kg/m<sup>2</sup>; continuous), education (primary and secondary; college; university), smoking status (never smoker; former smoker; current smoker), alcohol intake (0 g/week; 1–10 g per week;  $> 10$  g per week), physical activity (never, rarely or occasionally; moderate or high), and season of blood sampling (winter; spring; summer; fall)

**Table 3** Hazard ratios (HR) and 95% confidence intervals (CI) for survival by pre-diagnostic plasma 25(OH)D by different follow-up times ( $\leq 2$  and  $> 2$  years) after cancer diagnosis

25(OH)D (nmol/L)	All-cause mortality (%) <i>n</i> = 342/919	HR <sup>a</sup> (95% CI) <i>n</i> = 327/919	Cancer deaths (%) <i>n</i> = 300/919	HR <sup>a</sup> (95% CI) <i>n</i> = 262/919
Follow-up $\leq 2$ years				
< 30	45/80 (56)	1.53 (1.08, 2.17)	36/80 (45)	1.51 (1.00, 2.29)
30–49.9	77/213 (36)	1.01 (0.76, 1.36)	75/213 (35)	1.08 (0.78, 1.49)
50–69.9	136/399 (34)	1 (Ref.)	113/399 (28)	1 (Ref.)
$\geq 70$	84/227 (37)	1.25 (0.94, 1.67)	76/227 (33)	1.26 (0.91, 1.75)
<i>P</i> <sub>lineartrend</sub>		0.30		0.38
HR per 25 nmol/L increase		0.97 (0.84, 1.13)		0.99 (0.84, 1.16)
	<i>n</i> = 210/505	<i>n</i> = 198	<i>n</i> = 99/505	<i>n</i> = 95
Follow-up $> 2$ years				
< 30	14/31 (45)	0.99 (0.54, 1.82)	6/31 (19)	0.94 (0.39, 2.23)
30–49.9	54/123 (44)	0.98 (0.69, 1.34)	27/123 (22)	0.87 (0.53, 1.42)
50–69.9	99/224 (44)	1 (Ref.)	53/224 (24)	1 (Ref.)
$\geq 70$	43/127 (34)	0.68 (0.46, 0.99)	13/127 (10)	0.47 (0.25, 0.87)
<i>P</i> <sub>lineartrend</sub>		0.29		0.24
HR per 25 nmol/L increase		0.94 (0.75, 1.17)		0.95 (0.70, 1.29)

<sup>a</sup>Adjusted for age (y; continuous) at diagnosis, sex, BMI (in kg/m<sup>2</sup>; continuous), education (primary and secondary; college; university), smoking status (never smoker; former smoker; current smoker), alcohol intake (0 g/week; 1–10 g per week; >10 g per week), physical activity (never, rarely or occasionally; moderate or high), and season of blood sampling (winter; spring; summer; fall)

diagnosis showed both significantly lower all-cause mortality and cancer mortality (HR: 0.68, 95% CI 0.46, 0.99 and HR: 0.47, 95% CI 0.25, 0.87, respectively).

### Serum 25(OH)D and site-specific cancer survival

As we observed higher survival among those with high 25(OH)D levels in the longer follow-up group, we also explored the association between pre-diagnostic vitamin D status and mortality among patients with the four most common cancer types (Table 4). During the follow-up, 172 were diagnosed with prostate cancer, 89 with breast cancer, 118 with colorectal cancer, and 124 with lung cancer. Mean age at diagnosis was 79.2 ( $\pm 5.3$ ) years for prostate cancer, 80.8 ( $\pm 6.1$ ) years for breast cancer, 81.4 ( $\pm 5.1$ ) years for colorectal cancer, and 79.8 ( $\pm 5.0$ ) years for lung cancer. More women were diagnosed with colorectal (56%) and lung cancer (56%) in the study group.

Among men with prostate cancer (HR = 0.43, 95% CI 0.22, 0.84) and women with breast cancer (HR = 0.28, 95% CI 0.08, 0.94), those with the highest serum 25(OH)D levels ( $\geq 70$  nmol/L) had lower all-cause mortality rates compared with the reference levels (50–69.9 nmol/L) (Table 4). When we additionally adjusted for Gleason grade among prostate cancer patients and receptor status among breast cancer patients, the HR for the highest category became 0.35 (95% CI 0.17, 0.75) for overall mortality among those with

prostate cancer and 0.28 (95% CI 0.08, 0.94) for breast cancer. Moreover, when also adjusting for fish oil, the association between pre-diagnostic vitamin D levels and survival was not altered among prostate and breast cancer patients.

A positive significant association between low pre-diagnostic vitamin D status and all-cause mortality was observed among those with colorectal cancer (HR = 2.89, 95% CI 1.28, 6.56, *p*<sub>trend</sub> = 0.02) compared with the reference levels (50–69.9 nmol/L) (Table 4). When additional adjustments were made for TNM stage among participants with colorectal cancer (52 events), the lowest category (< 30 nmol/L) was also associated with poorer overall survival (HR = 3.07, 95% CI 1.09, 8.62). No association was observed between pre-diagnostic 25(OH)D levels and survival among those with lung cancer.

When examining the association between 25(OH)D levels and cancer-specific mortality for the four most common cancer types, we observed similar results as for total mortality—yet with less statistical power.

### Discussion

Our data show that low pre-diagnostic serum 25(OH)D levels (< 30 nmol/L) are associated with lower overall survival among elderly cancer patients. Also, individuals with the highest pre-diagnostic 25(OH)D levels ( $\geq 70$  nmol/L) who

**Table 4** Hazard ratios (HR) and 95% confidence intervals (CI) for survival among site-specific cancer patients by pre-diagnostic plasma 25(OH)D

	25(OH)D (nmol/L)	All-cause mortality (%)	HR (95% CI)	Cancer deaths (%)	HR (95% CI)
<b>Prostate cancer<sup>a</sup></b>					
	<i>n</i> = 72/172		<i>n</i> = 69	<i>n</i> = 30	<i>n</i> = 28
< 30	3/7 (43)		1.74 (0.52, 5.86)	1/7 (14)	1.63 (0.20, 13.43)
30–49.9	12/30 (40)		0.86 (0.44, 1.69)	7/30 (23)	1.59 (0.60, 4.20)
50–69.9	41/83 (50)		1 (Ref.)	14/83 (17)	1 (ref.)
≥ 70	16/52 (31)		0.43 (0.22, 0.84)	6/52 (12)	0.69 (0.23, 2.07)
<i>P</i> <sub>lineartrend</sub>			0.10		0.56
<b>Breast cancer<sup>a</sup></b>					
	<i>n</i> = 37/89		<i>n</i> = 37	<i>n</i> = 17/89	<i>n</i> = 17
< 30	3/9 (33)		0.25 (0.06, 1.08)	1/11 (9)	0.30 (0.03, 3.03)
30–49.9	11/21 (52)		1.15 (0.47, 2.77)	8/21 (38)	1.89 (0.57, 6.25)
50–69.9	18/42 (43)		1 (Ref.)	8/42 (19)	1 (ref.)
≥ 70	5/17 (29)		0.28 (0.08, 0.94)	0/17 (0)	0
<i>P</i> <sub>lineartrend</sub>			0.05		0.36
<b>Colorectal cancer<sup>b</sup></b>					
	<i>n</i> = 77/118		<i>n</i> = 70	<i>n</i> = 40/118	<i>n</i> = 37
< 30	13/13 (100)		2.89 (1.28, 6.56)	7/13 (54)	2.70 (0.96, 7.57)
30–49.9	22/33 (67)		0.62 (0.32, 1.21)	12/33 (36)	0.65(0.27, 1.55)
50–69.9	29/47 (62)		1 (Ref.)	15/47 (32)	1 (Ref.)
≥ 70	13/25 (53)		0.68 (0.32, 1.46)	6/25 (24)	0.66 (0.22, 1.96)
<i>P</i> <sub>lineartrend</sub>			0.02		0.046
<b>Lung cancer<sup>b</sup></b>					
	<i>n</i> = 103/124		<i>n</i> = 101	<i>n</i> = 91/124	<i>n</i> = 106
< 30	20/22 (91)		1.23 (0.68, 2.22)	17/22 (77)	1.12 (0.60, 2.24)
30–49.9	23/27 (85)		1.28 (0.73, 2.23)	20/27 (74)	1.23 (0.68, 2.23)
50–69.9	36/45 (80)		1 (Ref.)	32/45 (71)	1 (Ref.)
≥ 70	24/27 (89)		1.03 (0.58, 1.82)	22/27 (82)	1.06 (0.58, 1.93)
<i>P</i> <sub>lineartrend</sub>			0.79		0.92

<sup>a</sup>Adjusted for age (year; continuous) at diagnosis, BMI (in kg/m<sup>2</sup>; continuous), education (primary and secondary; college; university), smoking status (never; former; current smoker), alcohol intake (0 g/week; 1–10 g per week; >10 g per week), physical activity (never, rarely or occasionally; moderate or high), and season of blood sampling (winter; spring; summer; fall)

<sup>b</sup>Adjusted for same variables as in model<sup>a</sup> as well as sex

survived at least 2 years after diagnosis had 32% lower risk of death and 53% lower risk of cancer death. The Icelandic study population living at high latitude is dependent on dietary sources to maintain adequate vitamin D status as suggested by a study looking at the impact of low ultraviolet B availability on 25(OH)D status in Europe [36].

We observed no statistically significant association between 25(OH)D levels and cancer diagnosis except an increased risk of lung cancer was observed among those with the lowest 25(OH)D levels. Some studies have reported an inverse association between higher 25(OH)D levels and breast cancer incidence [5], colorectal cancer [4, 7], advanced prostate cancer [19, 37], and lung cancer [2]. Additionally, positive associations have been reported for total prostate cancer, though this might reflect more PSA testing in men with high 25(OH)D levels [38, 39]. However, a study in people 50–84 years old in three large European population-based cohorts (CHANCES Consortium) reported no association between pre-diagnostic 25(OH)D levels and total cancer incidence [40]. Nevertheless, a recent study

based on pre-diagnostic 25(OH)D levels from 17 studies reported 31% increased risk of colorectal among those with low 25(OH)D levels (< 30 nmol/L) compared with levels between 50 and < 62.5 nmol/L [7].

We also observed that pre-diagnostic 25(OH)D levels might have a role in overall mortality and cancer mortality, which is in agreement with findings from a recent meta-analysis of cohort studies [23–25] and from studies of individuals with genetically low 25(OH)D concentrations [41, 42]. Meanwhile, our findings are not in line with the results of the NHANES III survey and a meta-analysis using eight cohort studies with 26,916 participants in Europe (age range 32–81 years) where no association was observed between serum 25(OH)D levels and cancer-related mortality [15, 43]. Likely explanation for this discrepancy could be that these studies did not have information on whether participants had cancer at the time of blood draw for 25(OH)D measurements.

In present study, the follow-up time was on average less than a year from diagnosis among those with

pre-diagnostic 25(OH)D levels below 30 nmol/L. In a population-based study aimed to examine whether low vitamin D status was mainly an indicator of poorer health, an association between vitamin D deficiency and all-cause mortality was observed despite adjustments for common morbidity factors [44].

Whether vitamin D deficiency is a causal factor in overall greater mortality risk among cancer patients or whether our findings can be explained by reverse causality or confounding by health in general or other factors remains open to speculation. However, studies of cell cultures and animal models support a causal role for vitamin D and calcitriol in delaying cancer development and progression through mechanisms such as inhibition of invasion, metastasis, and angiogenesis [3]. Furthermore, Mendelian Randomization studies suggest a causal role for low serum 25(OH)D levels on increased mortality risk [41, 42].

We observed an inverse association between high 25(OH)D levels and cancer-specific survival among patients with more than 2 years of follow-up after diagnosis. Considering the four most common cancer types, we observed that the highest pre-diagnostic 25(OH)D levels ( $\geq 70$  nmol/L) were associated with improved overall survival among women with breast cancer and men with prostate cancer, even though the reference group was within the level of what is defined as sufficient ( $> 50$  nmol/L) vitamin D status according to the Nordic Nutritional Recommendations [32]. These findings are also in agreement with previous studies [15–17, 19–22, 39].

The main strengths of our study is the population-based prospective design with assessment of many lifestyle factors associated with cancer and mortality risk as well as standardized values to VDSP were used for the exposure data on 25(OH)D levels [29]. Additionally, the data were linked to the Icelandic Cancer registry that covers 99% of all cancers diagnosed in Icelandic residents, so follow-up was virtually complete [33]. This feature allows for valid exclusion of all prevalent cancers cases. However, a limitation of the study is that we did not have information on tumor stage at diagnosis for all cancer cases in the main analysis of 25(OH)D levels and mortality risk. Nevertheless, with less statistical power, when focusing on the largest cancer groups with additional adjustment for Gleason score (prostate cancer), tumor receptor status (breast cancer) or TNM stage (colorectal cancer) yielded similar results. Another limitation of the study is the lack of participants with high levels of 25(OH)D ( $> 100$  nmol/L) to study possible adverse effect as has been detected among women with breast cancer [22]. Finally, we emphasize that our study lacked power to address the association between vitamin D and the most common cancer types, particularly cancer type-specific mortality. Our findings on possible improved survival among cancer patients with higher pre-diagnostic 25(OH)D levels needs to

be addressed in larger studies where cancer-specific mortality can also be examined for site-specific cancer.

In conclusion, our data suggest that low pre-diagnostic serum 25(OH)D levels ( $< 30$  nmol/L) may be associated with lower overall survival for cancer patients, possibly due to poorer health among those with lower serum 25(OH)D levels. For elderly patients with more than 2 years survival after diagnosis, our data suggest that higher pre-diagnostic serum 25(OH)D levels, including levels above the range considered sufficient, may be associated with improved survival.

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**Author contributions** The authors' responsibilities were as follows: VG, MFC, TBH, and LT provided essential materials for the study. JET, TA, UAV, LAM, ELG, MJS, and LS designed the research; JET performed the statistical analysis and drafted the manuscript; JET, TA, UAV, MFC, HOA, LAM, ELG, MJS, and LS interpreted the data and critically reviewed the manuscript; JET and LS had primary responsibility for the final content; and all authors read and approved the final manuscript.

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## Compliance with ethical standards

**Conflict of interest** None of the authors declared a conflict of interest.

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