**Title:**

# Vitamin D levels and Cancer Incidence in 217.244 individuals from Primary Health Care in Denmark

**Short title:**

Vitamin D and Cancer Incidence in Primary Health Care in Denmark

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**Key words:** vitamin D levels, cancer incidence, primary health care

Abbreviations: vitamin D: 25-hydroxy vitamin D; HR: Hazard ratio; IQR: Interquartile range; CI: confidence interval.

Article category: Cancer epidemiology

**Novelty and impact**

In this large observational cohort study of 217,244 individuals from primary care, vitamin D levels are not associated with the incidence of several major cancer types such as breast-, colorectal and urinary cancers, but higher levels are significantly associated with a higher incidence of skin, prostate, and hematological cancers as well as a lower incidence of lung cancer. These results do not support an overall protective effect against cancer by vitamin D.

In recent years, interest in the role of vitamin D in health has increased, and more blood tests have included vitamin D analysis. Taking advantage of these data, the authors here investigated the link between vitamin D and cancer incidence in a large Danish cohort. They found no association between circulating vitamin D and most cancers they studied. Higher levels of vitamin D did correlate with reduced risk of lung cancer, they found, and

also with increased risk of skin cancers, prostate cancer, non-Hodgkin's lymphoma, and hematologic cancers.

**Abstract**

Vitamin D has been linked to cancer development in both pre-clinical and epidemiological studies. This study examines the association between serum levels of vitamin D and cancer incidence in the Capital Region of Denmark. Individuals who had vitamin D analyzed at The Copenhagen General Practitioners Laboratory between April 2004 and January 2010 were linked to Danish registries with end of follow-up date at Dec 31st 2014, excluding individuals with pre-existing cancer. Cox regression models adjusted for age in one-year intervals, sex, month of sampling, and Charlson Comorbidity Index were applied. The study population of 217,244 individuals had a median vitamin D level of 46 nmol/L (IQR 27-67 nmol/L). Non-melanoma skin cancer was the most frequent form of cancer, followed by breast-, lung-, and prostate cancers. No associations were found between increments of 10nmol/L vitamin D and incidence of breast, colorectal, urinary, ovary or corpus uteri cancer. However, higher levels of vitamin D were associated with higher incidence of non-melanoma (HR 1.09 [1.09-1.1]) and melanoma skin cancer (HR 1.1 [1.08-1.13]) as well as prostate (HR 1.05 [1.03-1.07]) and hematological cancers (HR 1.03 [1.01-1.06]), but with lower incidence of lung cancer (HR 0.95 [0.93-0.97]). In this study, vitamin D levels are not associated with the incidence of several major cancer types, but higher levels are significantly associated with a higher incidence of skin, prostate, and hematological cancers as well as a lower incidence of lung cancer. These results do not support an overall protective effect against cancer by vitamin D.

## Introduction

25-hydroxy vitamin D (vitamin D) is a precursor for the steroid hormone calcitriol that besides having a central role in calcium homeostasis, has shown antineoplastic effects in cellular and animal studies [1]. In humans, exposure of the skin to sunlight largely determines the level of circulating vitamin D with a smaller contribution from dietary intake. In the past years, several epidemiological studies and meta-analyses have examined the association between vitamin D and cancer reporting inconsistent results [2]. The incidence of some cancer types such as colorectal cancer has generally shown inverse associations with vitamin D [3] while for other cancer types such as prostate cancer, positive associations have been seen [4]. Besides the role in calcium homeostasis, one of the biological functions of vitamin D is involvement in immune modulation. The active metabolite calcitriol has immune regulatory effects, mimicking what is seen in the immune regulatory environment in cancer [5]. Furthermore, calcitriol can also modulate the effect of known external carcinogenic substances in some cancers, e.g. by counteracting the effects of tobacco smoking on bronchial epithelium [6]. However, the effects of calcitriol might be dependent on the physiological concentration and has a biphasic effect on cancer cell growth in vitro [7].

The Copenhagen General Practitioners Laboratory serviced general practitioners from the Greater Copenhagen area with approximately 1.1 million inhabitants (first quarter of 2010). A database (CopD) included measurements of vitamin D from this laboratory in the period from 2004 to 2010. A reverse J-shaped association between serum levels of vitamin D and all-cause mortality has been reported based on the CopD database indicating an upper beneficial limit of vitamin D [8]. This finding was corroborated

by an analysis of the Third National Health and Nutrition Examination Survey, NHANES III [9]. In Denmark, like in other Western countries, awareness of the role of vitamin D in general health has increased in recent years, and a large part of the Danish population takes dietary vitamin D supplements on a regular basis [10]. In the period 2004 to 2010, the amount of vitamin D analyses performed at the Copenhagen General Practitioners Laboratory grew exponentially, and 14% of all requests for blood sampling included a vitamin D analysis [8]. Thus, almost one quarter of the inhabitants had their blood analyzed for vitamin D, suggesting a broad indication for vitamin D analysis. Therefore, the CopD database provides a unique opportunity to assess associations between vitamin D levels and disease outcomes in a regional population. The aim of the present study was to test the hypothesis that the level of vitamin D could be associated with both a lower and a higher incidence of cancer, depending on the type of cancer examined.

## Materials and methods

### Study population

The CopD database included measurements of vitamin D levels extracted from The Copenhagen General Practitioners Laboratory [8]. The laboratory served all general practitioners in the Copenhagen Municipality and the former Copenhagen County in Denmark. The population of the Copenhagen area was predominantly of Danish descent (83%) or non-Danish European descent (9%) in 2008 as defined by Statistics Denmark (Statistics Denmark http://www.statbank.dk/10021, accessed May 2018).

Our study population comprised 247,574 individuals who had at least one measurement of serum vitamin D in the period from April 2004 to January 2010. In the present study, the first measurement of vitamin D from each individual from the CopD

database was linked at individual-level to The Danish Cancer Registry [11], The National Patient Registry [12], and the Danish Civil Registration System [13]; with date of last follow-up as of December 31st 2014. We only included the first measurement to exclude bias by possible supplementation initiated after the first measurement. Firstly, we excluded individuals with replacement social security numbers since they are temporary until a valid social security number is provided (N=716). Then, individuals with a pre-existing cancer diagnosis from the Danish Cancer Registry (N=26,644) at the time of the first vitamin D measurement were excluded. Thereafter, remaining individuals who had a cancer diagnosis in the National Patient Registry (N=2,970) at the time of the first vitamin D measurement were excluded to reach a final cohort size of 217,244 individuals.

## Analysis of vitamin D

Vitamin D levels were assessed in serum using two different commercially available assays: LIAISON 25(OH)D assay (Diasorin, Italy); and OCTEIA 25(OH)D3 and 25(OH)D2 (Immunodiagnostic Systems, UK) quantifying both 25-hydroxy vitamin $D_2$ and 25-hydroxy vitamin $D_3$ as described in detail by Durup et al [8]. Quantitative values less than 10 nmol/L were set to 10 nmol/L due to the lower limit of the assays.

## Confounding variables

Besides age, sex and month of sampling, comorbidity was assessed by the Charlson Comorbidity Index [14, 15] using the International Classification of Diseases (ICD-10) codes. The Charlson Comorbidity Index was included at time of vitamin D analysis with all available diagnoses registered in the National Patient Registry since 1977.

Study endpoints

Information about incident cancers was obtained from The Danish Cancer Registry based on ICD-10 codes and grouped as shown in supplementary Table S1. Non-Hodgkin lymphoma was examined as a subgroup of hematological cancers as well as included in the hematological cancers. Each type of cancer with number of events above 200 in the follow-up period was included as outcome variables. Information on emigration and vital status was obtained from the Danish Civil Registration System with the date of last follow-up being December 31st 2014.

Statistical analysis

The association between vitamin D levels and the incidence of each type of cancer was estimated using a Cox proportional hazards model with time since first vitamin D measurement as the underlying timescale. All subjects were followed to the time of first cancer (any cancer diagnosis, N=18,359), time of emigration (N=5,583), time of death (N=24,087), or end of follow-up (by December 31st 2014), whichever came first. The associations were presented as hazard ratios (HRs) with corresponding 95% confidence interval (CI) from crude models as well as from models adjusted for potential confounding effects from comorbidity as the Charlson Comorbidity Index, age at blood sampling in one-year intervals, sex, and month of blood sampling. All potential confounder variables were included as strata variables to allow for different underlying hazards in the strata.

The assumption of proportional hazards for the vitamin D variable in the Cox regression analysis was checked graphically using Schoenfeld residual plots. To exclude

prevalent cases, we also computed the effects with delayed entry of one year as well as 5 years. Furthermore, we performed sensitivity analyses for sex specific effects and analyses with vitamin D levels at the lower limit of the assay of 10 nmol/L set to 5 nmol/L. Also, we analyzed individuals with blood samplings from April 2005 and onwards to exclude bias from measurements performed from April 2004 to April 2005 that were not the first measurement of vitamin D in the individual.

To detect non-linear associations between vitamin D levels and the incidence of cancer, we fitted associations of vitamin D levels and incidence of cancer for each cancer type using restricted cubic splines with 4 knots placed at 12.5, 25, 75, and 100 nmol/L vitamin D, using 50 nmol/L as the reference. The lower knots were derived from the cut-offs used in clinical practice in Europe [16]. Graphical presentations of estimates together with 95% pointwise CI for all cancer types were reviewed but only those that had significant findings by Cox regression analysis were displayed as figures. Since the study was register based there were no missing data or loss to follow-up.

The statistical analyses were performed in the SAS 9.3 software (SAS institute, Cary, USA). Descriptive analyses of quantitative variables were reported as medians with interquartile ranges (IQR), and the categorical variables as numbers and percentages of total (%). All HRs were computed with 95% CI. P-values were two-sided and considered statistically significant if less than 0.05.

**Results**

The study population of 217,244 individuals had a median level of vitamin D of 46 nmol/L (IQR 27-67 nmol/L), a median age of 48.8 years (IQR 33.5-64.1 years), female

predominance (65.3%), and a low comorbidity burden (Charlson Comorbidity Index of 0 in 79.5%) with pulmonary disease being the most frequent comorbidity. Vitamin D levels were grouped by clinical cut-offs into severe deficiency (<12.5 nmol/L), moderate deficiency (12.5-25 nmol/L), insufficiency (25-50 nmol/L), sufficiency (50-75 nmol/L), moderately high levels (75-100 nmol/L), high levels (100-125 nmol/L), and very high levels (≥125 nmol/L) for tabulation of baseline characteristics by the level of vitamin D (Table 1).

A total of 18,359 individuals were diagnosed with an incident cancer (8.5% of the population) during the follow-up period. Non-melanoma skin cancer (N=5,045) was the most frequent incident cancer followed by breast cancer (N=2,167), lung cancer (N=1,707), prostate cancer (N=1,470), and colon-rectosigmoidal cancers (N=1,108). The median level of vitamin D differed depending on cancer type ranging from 47 nmol/L in individuals developing an incident lung or rectum cancer to 58 nmol/L in individuals developing a non-melanoma skin cancer (Table 2).

In the unadjusted Cox regression analysis, increments of 10 nmol/L in the level of vitamin D were significantly positively associated with almost all incident cancer types (Table 2). However, when adjusting for the potential confounding variables, no associations were found for breast-, urinary-, colon-rectosigmoidal-, rectum-, ovary, corpus uteri or cancers of the central nervous system. However, non-melanoma (HR 1.09 [1.09-1.1]) and melanoma skin cancer (HR 1.1 [1.08-1.13]), as well as prostate (HR 1.05 [1.03-1.07]) and hematological cancers (HR 1.03 [1.01-1.06]) retained a significant positive association with vitamin D levels, while lung cancer showed a negative association (HR 0.95 [0.93-0.97]) (Table 2). Applying delayed entry of one year or five years produced similar results (Figure 1, Table 3). For melanoma skin cancer, the hazard ratio seemed to peak around a vitamin D level of 80 nmol/L (Figure 2A) while it further increased for non-

melanoma skin cancer (Figure 2B). For individuals with an incident prostate cancer, the rate of increase in hazard ratio was largest below a vitamin D level of 80 nmol/L (Figure 3A) in contrast to individuals with incident hematological cancers or incident non-Hodgkin lymphomas where the largest rate of increase in hazard ratio was seen at the level above 80 nmol/L and 100 nmol/L, respectively (Figure 3B and 3C). Finally, for individuals with an incident lung cancer, the strongest association was for vitamin D levels below 30 nmol/L (Figure 3C). The sensitivity analyses yielded no differences when examining the effect of sex, except for non-Hodgkin lymphoma, where the association was only significant in women (Table S2); when only examining individuals who had their blood analyzed for vitamin D from April 2005 and onwards (Table S3-4); nor when calculating with the lower limit of vitamin D set to 5 nmol/L (data not shown).

## Discussion

We examined associations between the level of vitamin D and the incidence of several cancer types in a large population from general practice in Denmark. We found that the level of vitamin D showed no association with most cancer types, while in some cancer types, higher levels of vitamin D were associated with both higher and lower incidence of cancer depending on the type of cancer examined.

The CopD study was observational and thus we could not examine causality due to possible residual confounding and reverse causation. Furthermore, a general problem of observational cohort studies is that participants tend to be healthier than their source population. The opposite could be an issue for the present study since the request for vitamin D analysis, and thereby the study participants, was selected by general

practitioners. The generalizability to the entire population may therefore be a concern. However, in 2010 the background population serviced by the Copenhagen General Practitioners Laboratory was comprised of approximately 1.1 million people and almost one quarter thereof had vitamin D assessed in the period from 2004 to 2010, enhancing the likelihood of generalizability of this study. The cancer incidence of the CopD study population was higher than expected. We observed 18,359 incident cancer diagnoses, whereas an age- and sex-standardized calculation based on the cancer incidence rates for the period 2004 to 2014 in the entire Danish population revealed an expected number of 11,733 (5.4%). The major difference was seen for non-melanoma skin cancer with 5045 cancers observed versus 643 expected. This was even more clear if calculated with delayed entry of 5 years where there was a total of 3,664 observed cancers versus 2,698 expected and the numbers for non-melanoma skin cancer were 1,062 observed cancers versus 145 expected. This points to, that the CopD population may be more closely followed in the health system compared with the general population. Another concern is the skewed sex distribution with an overweight of women, especially in the groups with high levels of vitamin D, reflecting the well-known higher frequency of health concern in women. This could be the reason for the higher numbers of non-Hodgkin lymphomas in women found in our study compared to what is seen in the general population where these diseases have male predominance. As revealed in the sensitivity analysis, the association of non-Hodgkin lymphoma with vitamin D was only significant in women. However, the associations in men and women were not significantly different by interaction analysis.

Since the study was register based, we had complete follow-up but no available information on smoking habits, alcohol consumption, obesity or exercise. Confounding by these factors can therefore neither be evaluated nor ruled out. Inclusion of

the Charlson Comorbidity Index [14] was done to account for diseases that arise from these exposures. Outcomes (cancer incidence, emigrations, and overall mortality) were obtained by registry linkage. The Danish Cancer Registry and The Danish Civil Registration system both have very high validity, and the risk of misclassification is considered minimal. Misclassification could be of greater concern as to the vitamin D exposure assessment. We only included one sampling per participant and the assays used are known to have some variability. However, all measurements were performed at the same laboratory with well documented and internationally approved quality assured assays to reduce this variability [8]. The potential errors related to vitamin D assessment would most likely be non-differential and thereby lead to an underestimation of the associations.

Several other studies, both separate studies and meta-analyses, have examined the association between vitamin D and cancer incidence [2, 17]. Like the present study, Skaaby et al. showed a significantly higher incidence of non-melanoma skin cancers with higher levels of vitamin D in approximately 12,000 individuals pooled from 3 population based studies carried out in Denmark from 1993 to 2008 [18]. Thus, it is likely there is confounding by sun exposure for non-melanoma skin cancer as these cancers are induced by exposure of the skin to the ultraviolet light from the sun [19]. Furthermore, an inverse association of vitamin D levels with lung cancer was shown by Afzal et al. in more than 9000 individuals from The Copenhagen City Heart Study [20]. Of note, their findings were unaffected by smoking habits.

For several cancer types, we did not find any association with the level of vitamin D, in contrast to other studies. For example, a recent meta-analysis performed in the elderly compiled from several population based cohorts in Europe showed an

association of higher risk of breast cancer with higher vitamin D levels [21]. However, individuals in the CopD study cohort were younger, and therefore the results are not directly comparable. Also, a large case-control study based on pooling of 17 cohorts showed a protective effect of higher vitamin D levels with regards to the risk of colon-rectosigmoidal cancers in women [22]. We do not find incident colon-rectosigmoidal cancers to be associated with the level of vitamin D, neither in women only. In that respect confounding needs to be considered. Information on important lifestyle risk factors as smoking, obesity, unhealthy diet and physical inactivity were not available in the current study and confounding by these factors are thereby possible. However, these factors are most often found related to lower blood levels of vitamin D [23, 24]. It is therefore more likely that adjustment for these factors would lead to the opposite finding in our study, i.e. that higher levels of vitamin D were associated with higher incidence of colon-rectosigmoidal cancers. Also, recent data from a randomized calcium and vitamin D supplementation trial suggested that supplementation increased the risk of subsequent serrated polyps of the colon, but as a late effect [25]. We do not have information on the intake of calcium or vitamin D supplements in this study, but in Denmark, there is generally a high consumption of supplements in the Danish population (60% of females and 51% of males aged 18 to 75 years) with a relatively low median level of dietary intake of 7.8-8.4 µg for users of dietary supplements versus 2.0-2.9 µg in non-users [10]. Furthermore, recent studies have examined vitamin D receptor polymorphisms and risk of colorectal diseases and found different results for different disease entities [26], and a large mendelian randomization study provides no evidence for a causal relationship between vitamin D and risk of colorectal cancers, although small effect sizes and non-linear relationships cannot be ruled out [27]. Like our study, none of these studies had information on potential

confounding factors such as smoking habits, alcohol consumption, body fatness, physical activity or family history of cancer. Thus, the possible association between vitamin D levels and colorectal cancers warrants further large studies including information on potential confounders and genetic susceptibility as well as more specific disease entities as outcome.

For prostate cancer, Schenk et al showed no association of vitamin D levels with total prostate cancer risk in a nested case control study of 1700 cases and controls from the Prostate Cancer Prevention Trial [28]. However, when accounting for Gleason score, there was a modest increase in the risk of prostate cancer with a Gleason score of 2-6, with increasing vitamin D levels, but a decrease in risk of prostate cancer with a Gleason score 8-10. Thus, our results could be biased by a predominance of low Gleason score groups identified through screening using Prostate Specific Antigen. In Denmark, the incidence of prostate cancer rose by 2.5 fold in the late 1990's, largely due to an increase in the diagnosis of locally advanced prostate cancers [29]. In parallel to our results, a large Norwegian population based nested case control study showed a positive association between a 30 nmol/L increase in the vitamin D level and the risk of prostate cancer, not depending on the stage of the disease at diagnosis [30]. However, staging was not done by Gleason score in their study.

The positive association between higher vitamin D levels and the incidence of non-Hodgkin lymphomas is corroborated by a recent meta-analysis in finding no protective effect of higher vitamin D levels in relation to the risk of developing non-Hodgkin lymphoma [31]. Several cancers are associated with immune suppression, and non-Hodgkin lymphomas occur more often in patients with non-melanoma skin cancer [32].

This mutual association with immune suppression could explain that we find both types of cancers to be positively associated with the level of vitamin D.

For both skin cancers as well as for prostate and hematological cancers, and especially non-Hodgkin lymphomas we found that higher levels of vitamin D were associated with higher incidence, while we found an inverse association with lung cancer. The active metabolite of vitamin D, calcitriol, has several effects on both cancer cells and immune cells and can also mediate the effects of tobacco carcinogens [6]. Moreover, UV-radiation can affect CD4+ T-cells towards a more immune suppressive environment [33]. Thus, it is possible that the blood level of vitamin D could be related to the evolution and progression of cancer. However, it is also likely that vitamin D is a surrogate marker for the function of the immune system at the individual level. This might explain why studies conducted in cohorts from countries at different latitudes find differences in 'optimal' levels of vitamin D such as studies conducted in the USA versus in countries from northern Europe [34]. These hypotheses remain to be tested.

To conclude, vitamin D levels are not associated with the incidence of several major cancers such as breast, urinary, and colon-rectosigmoidal cancers in a population from primary care in Denmark, but higher vitamin D levels are associated with a higher incidence of skin, prostate, hematological cancers, and non-Hodgkin lymphomas solely as well as a lower incidence of lung cancer. These results should be interpreted in the light of the representativeness of the cohort as well as the known limitation of registry studies in lack of information on potential confounding factors. Our study results do not support an overall protective effect against cancer by vitamin D.

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**Data availability statement**

The data from this study are deposited at Statistics Denmark (ref no 706282 [35]) and can only be accessed with a secure access granted by Statistics Denmark.

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## Tables

Table 1: Baseline characteristics by the level of 25-hydroxy vitamin D.

| | | 25-hydroxy vitamin D (nmol/L) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **all** | **<12.5** | **12.5-25** | **25-50** | **50-75** | **75-100** | **100-125** | **≥125** |
| **N** | 217,244 | 12,268 (5.6%) | 36,770 (16.9%) | 71,762 (33.0%) | 58,073 (26.7%) | 26,109 (12.0%) | 8,175 (3.8%) | 4,087 (1.9%) |
| **Age** | 48.8 (33.5-64.1) | 43.0 (30.5-58.7) | 44.3 (31.5-59.8) | 48.9 (33.9-63.6) | 51.8 (35.5-66.0) | 52.0 (34.1-66.3) | 50.2 (31.9-65.8) | 48.8 (30.7-64.8) |
| **Male sex** | 78,414 (34.7%) | 5,047 (41.1%) | 14,553 (39.6%) | 27,882 (38.9%) | 19,710 (33.9%) | 7,898 (30.2%) | 2,274 (27.8%) | 1,050 (25.7%) |
| **Comorbidity** | | | | | | | | |
| **Charlson Comorbidity Index of zero** | 172,735 (79.5%) | 9,535 (77.7%) | 28,922 (78.7%) | 57,019 (79.5%) | 46,619 (80.3%) | 20,947 (80.2%) | 6,423 (78.6%) | 3,270 (80%) |
| **Diabetes** | 16,944 (7.8%) | 1,384 (11.3%) | 3,789 (10.3%) | 5,951 (8.3%) | 3,747 (6.5%) | 1,444 (5.5%) | 423 (5.2%) | 206 (5.0%) |
| **Heart disease** | 20,918 (9.6%) | 1,277 (10.4%) | 3,622 (9.9%) | 6,942 (9.7%) | 5,426 (9.3%) | 2,431 (9.3%) | 832 (10.2%) | 388 (9.5%) |
| **Pulmonary disease** | 24,180 (11.1%) | 1,584 (12.9%) | 4,414 (12.0%) | 7,922 (11.0%) | 6,055 (10.4%) | 2,764 (10.6%) | 947 (11.6%) | 494 (12.1%) |
| **Neurological disease and dementia** | 23,460 (10,8%) | 1,326 (10.8%) | 3,841 (10.5%) | 7,652 (10.7%) | 6,218 (10.7%) | 2,890 (11.1%) | 1,027 (12.6%) | 506 (12.4%) |
| **Liver disease** | 5,080 (2.3%) | 492 (4.0%) | 1,150 (3.1%) | 1,664 (2.3%) | 1,061 (1.8%) | 462 (1.8%) | 157 (1.9%) | 94 (2.3%) |
| **Renal disease** | 3,272 (1.5%) | 217 (1.8%) | 628 (1.7%) | 1,039 (1.5%) | 842 (1.5%) | 360 (1.4%) | 124 (1.5%) | 63 (1.5%) |
| **Ulcer** | 7,337 (3.4%) | 592 (4.8%) | 1,433 (3.9%) | 2,474 (3.5%) | 1,704 (2.9%) | 728 (2.8%) | 263 (3.2%) | 143 (3.5%) |
| **Reumatological disease** | 5,404 (2.5%) | 181 (1.5%) | 688 (1.9%) | 1,574 (2.2%) | 1,569 (2.7%) | 870 (3.3%) | 331 (4.1%) | 191 (4.7%) |
| **HIV** | 119 (0.1%) | 5 (0.0%) | 25 (0.1%) | 38 (0.1%) | 30 (0.1%) | 15 (0.1%) | 5 (0.1%) | <5 (0.0%) |

Age is shown as median with inter quartile ranges (IQR). All other variables are shown as numbers and percentages (%).

Table 2: Results from cox regression analysis.

| | Cases | 25-hydroxy vitamin D, nmol/L | Unadjusted | | | Adjusted [#] | | |
|---|---|---|---|---|---|---|---|---|
| **CANCER** | **N** | **Median (IQR)** | **HR** | **95% CI** | **p** | **HR** | **95% CI** | **p** |
| **Non-melanoma skin cancer** | 5,045 | 58 (40-78) | 1.11 | 1.10-1.12 | <0.0001 | 1.09 | 1.09-1.10 | <0.0001 |
| **Breast** | 2,167 | 51 (33-70) | 1.05 | 1.03-1.06 | <0.0001 | 1.00 | 0.99-1.02 | 0.6 |
| **Lung** | 1,707 | 47 (28-66) | 1.00 | 0.98-1.01 | 0.8 | 0.95 | 0.93-0.97 | <0.0001 |
| **Prostate** | 1,470 | 51 (35-70) | 1.05 | 1.03-1.07 | <0.0001 | 1.05 | 1.03-1.07 | <0.0001 |
| **Colon-rectosigmoidal** | 1,108 | 49 (32-69) | 1.03 | 1.01-1.05 | 0.0004 | 0.98 | 0.96-1.00 | 0.1 |
| **Urinary** | 1,016 | 48 (30-66) | 1.02 | 1.00-1.04 | 0.2 | 0.99 | 0.96-1.01 | 0.3 |
| **Hematological** | 968 | 53 (32-74) | 1.06 | 1.04-1.08 | <0.0001 | 1.03 | 1.01-1.06 | 0.004 |
| **Non-Hodgkin lymphoma** | 425 | 53 (31-73) | 1.03 | 0.99-1.06 | 0.1 | 1.03 | 1.00-1.07 | 0.07 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Central nervous system** | 689 | 49 (31-69) | 1.03 | 1.00-1.05 | 0.03 | 1.00 | 0.98-1.03 | 0.8 |
| **Melanoma of the skin** | 684 | 57 (41-75) | 1.10 | 1.08-1.13 | <0.0001 | 1.10 | 1.08-1.13 | <0.0001 |
| **Rectum** | 461 | 47 (32-67) | 1.01 | 0.98-1.04 | 0.6 | 0.98 | 0.94-1.01 | 0.2 |
| **Corpus-uteri** | 347 | 52 (33-69) | 1.04 | 1.01-1.08 | 0.01 | 0.99 | 0.96-1.03 | 0.7 |
| **Ovary, fallopian tube and broad ligament** | 254 | 52 (34-69) | 1.05 | 1.01-1.09 | 0.01 | 0.99 | 0.94-1.03 | 0.6 |

Incident cancers, 25-hydroxy vitamin D levels, and hazard ratios (HR) from both unadjusted and adjusted cox regression analysis per 10 nmol/L increments in 25-hydroxy vitamin D. IQR: Interquartile range. CI: confidence intervals.
[#]adjusted for age in 1-year intervals, sex, month of sampling, and comorbidity.

Table 3: Results from analyses performed with delayed entry of 5 years.

| **Delayed entry 5y** | **N** | **HR** | **95% CI** | **p** |
|---|---|---|---|---|
| **Non-melanoma skin cancer** | 1,062 | 1.11 | 1.09-1.13 | <.0001 |
| **Breast** | 455 | 1.02 | 0.99-1.05 | 0.2 |
| **Lung** | 318 | 0.93 | 0.89-0.98 | 0.003 |
| **Prostate** | 221 | 1.09 | 1.04-1.15 | 0.0002 |
| **Colon-rectosigmoidal** | 222 | 0.97 | 0.93-1.03 | 0.3 |
| **Urinary** | 213 | 0.98 | 0.93-1.04 | 0.5 |
| **Hematological** | 181 | 1.08 | 1.03-1.13 | 0.002 |
| **Non-Hodgkin lymphoma** | 84 | 1.10 | 1.03-1.17 | 0.006 |
| **Central nervous system** | 144 | 1.00 | 0.95-1.06 | 0.9 |

| | | | | |
|---|---|---|---|---|
| **Melanoma of the skin** | 130 | 1.14 | 1.08-1.20 | <.0001 |
| **Rectum** | 88 | 0.98 | 0.91-1.06 | 0.7 |
| **Corpus-uteri** | 90 | 0.99 | 0.92-1.06 | 0.7 |
| **Ovary, fallopian tube and broad ligament** | 53 | 0.98 | 0.89-1.08 | 0.7 |

Incident cancers, 25-hydroxy vitamin D levels, and hazard ratios (HR) from adjusted[#] cox regression analysis per 10 nmol/L increments in 25-hydroxy vitamin D. CI: confidence intervals.

[#]adjusted for age in 1 year intervals, sex, month of sampling, and comorbidity.

**Figure legends**

Figure 1: Forest plot showing association with cancer incidence per 10 nmol/L increments of 25-hydroxy vitamin D. Hazard ratios (HR) are presented on logarithmic scale with 95% confidence intervals (CI). Analyses are performed with delayed entry of 1 year.

Figure 2: Restricted cubic spline curves for incident skin cancers by the level of 25-hydroxy vitamin D. Hazard ratios (HR) are presented on logarithmic scale with 95% pointwise confidence intervals (CI). Analyses are performed with delayed entry of 1 year. A: Melanoma. B: Non-melanoma.

Figure 3: Restricted cubic spline curves for incident cancers by the level of 25-hydroxy vitamin D. Hazard ratios (HR) are presented on logarithmic scale with 95% pointwise confidence intervals (CI). Analyses are performed with delayed entry of 1 year. A: Prostate cancer. B: Hematological cancer. C: Non-Hodgkin lymphoma. D: Lung cancer.