Serum levels of vitamin D are not associated with future risk of venous thromboembolism

The Tromsø Study

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Summary

Previous studies have provided indirect evidence for a possible association between vitamin D status and risk of venous thromboembolism (VTE). However, no study has so far investigated the association between serum levels of 25-hydroxyvitamin D (25(OH)D), the biomarker of vitamin D status, and risk of VTE. The aim of our study was to investigate whether high levels of 25(OH)D were associated with decreased risk of VTE in a prospective population-based study. Serum levels of 25(OH)D were measured in 6,021 men and women, aged 25–84 years, who participated in the Tromsø Study in 1994–1995. Incident VTE-events were registered from date of inclusion through the end of follow-up, September 1, 2007. Cox-regression models were used to calculate hazard ratios (HR) with 95% confidence interval (CI) for VTE. There were 201 incident VTE-events during a median of 10.7 years of follow-up. The risk of VTE did not decrease per one standard deviation (SD) (19.8 nmol/l) increase in serum 25(OH)D (multivariable HR 1.02; 95% CI 0.91–1.22). Moreover, subjects with serum 25(OH)D ≥ 70 nmol/l (upper quartile) did not have decreased risk of VTE compared to those ≤ 44 nmol/l (lower quartile) in age- and sex-adjusted analysis (HR 0.91, 95% CI: 0.60–1.37, p for trend across quartiles 0.9) or multivariable analysis adjusted for age, sex, body mass index, smoking, and physical activity (HR 0.76, 95% CI: 0.45–1.28, p for trend across quartiles 0.9). Subgroup analyses showed no associations between serum levels of 25(OH)D and unprovoked or provoked VTE. In conclusion, in our study, normal serum levels of 25(OH)D were not associated with future risk of VTE, suggesting that vitamin D status does not play an important role in the pathogenesis of VTE. However, our findings did not apply to subjects with vitamin D deficiency (< 30 nmol/l) due to lack of statistical power among these subjects.

Keywords

Deep-vein thrombosis, epidemiological studies, hormones

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Introduction

Venous thromboembolism (VTE), including deep-vein thrombosis (DVT) and pulmonary embolism (PE), is a common disease with serious short- and long-term complications and potential fatal outcome (1, 2). The incidence of VTE is 1-3 per 1,000 person-years, with a steep incline with age. Even though many environmental and inherited predisposing factors have been associated with VTE (1-5), still 30–50% of the events have no obvious provoking factors (6-8). Thus, it is important to identify biomarkers and risk behaviours of VTE that could be subject to modification in order to minimise the disease burden.

Some prospective cohort studies have provided indirect evidence for an inverse association between the vitamin D status and risk of VTE. In a recent population-based study, women with active sun exposure habits had 30% lower risk of VTE than women with low sun exposure (9), presumably mediated via higher vitamin D levels (9). This study is supported by experimental and clinical studies showing that vitamin D may exert antithrombotic effects through inhibition of tissue factor (TF) in monocytes (10), induction of t-PA secretion and downregulation of PAI-1 expression (11) and plasma levels (12), and by enhancement of platelet aggregation in vitamin D receptor (VDR) knock-out mice (10). Thus, it is likely to assume that high levels of vitamin D are associated with lower risk of VTE.

Serum 25-hydroxyvitamin D (25(OH)D) provides an overall estimate of vitamin D status as it integrates vitamin D derived from endogenous production and dietary intake (13). To date, no study has been conducted to investigate the association between serum levels of 25(OH)D and future risk of VTE. The aim of our study was to investigate whether serum levels of 25(OH)D were associated with risk of VTE in a large, prospective, population-based study.

Methods

Study population

Participants were recruited from the fourth survey of the Tromsø Study (conducted in 1994-1995), a single-centre prospective, population-based study, with repeated health surveys of the inhabitants of Tromsø, Norway. All inhabitants aged >24 years were invited, and 27,158 (77% of the eligible population) participated. The participants aged 55–74 years and 5–10% of the other birth cohorts (n=9,057) were invited to a more extensive visit 3–12 weeks later, and 75% (n=6,889) attended. Subjects who did not consent to medical research (n=23), subjects not officially registered inhabitants of the municipality of Tromsø at baseline (n=16), subjects with a previous history of VTE (n=18), and subjects with missing values of serum 25(OH)D (n=811) were excluded from the study. Thus, 6,021 subjects were included in the study, and incident VTE events among the study participants were recorded from the date of enrolment to the end of follow-up, 1st of September 2007. The study was approved by the Regional Committee for Medical and Health Research Ethics, and all participants gave written informed consent to participate.

Measurements

Height and weight were measured with subjects wearing light clothing and no shoes. Body mass index (BMI) was calculated as weight in kilograms, divided by the square of height in meters (kg/m^2). Information on the amount of moderate and high intensity physical activity (vigorous enough to work up a sweat and cause shortness of breath) carried out during leisure time per week during the previous year was collected by a self-administered questionnaire. Information on self-reported diabetes, current smoking (pipe/cigar/cigarettes) and intake of fish oil was also collected through a self-administered questionnaire.

Serum levels of 25(OH)D, were measured in sera stored at −70°C for a median of 13 years. $25(OH)D_3$ was determined by immunometry (ECLIA) using an automated clinical chemistry analyzer (Modular E170, Roche Diagnostics, Mannheim, Germany). The total analytical precision of the assay had a coefficient of variation ≤7.8% for any of three different concentrations (48.6, 73.8, and 177.0 nmol/l) according to the manufacturer. The cross-reactivity with $25(OH)D_2$ was <10%, and the analytical sensitivity was 10 nmol/l. Five subjects had 25(OH)D below the detection limit, and their values were set to 5 nmol/l. The manufacturer provides a population-based reference range of 27.7–107.0 nmol/l for serum concentrations of $25(OH)D_3$ in adults. We revealed that this particular assay artificially measured 15–20% higher serum 25(OH)D levels in smokers than in non-smokers (14). Serum parathyroid hormone (PTH) was analyzed with an immunoassay using an automated clinical chemical analyser (Immunlite 2000, Siemens Diagnostics, Los Angeles, CA, USA). The assay measures intact PTH, with a reference range of 1.1–6.8 pmol/l for those ≤ 50 years and 1.1–7.5 pmol/l for those above 50 years. Serum calcium was analysed using an automated analyser (Hitachi 917, Roche, Basel, Switzerland) with reagents from Boehringer Mannheim, reference range 2.15–2.55 mmol/l.

VTE ascertainment

All first lifetime events of VTE during follow-up were identified as previously described (15) by searching the hospital discharge diagnosis registry, the autopsy registry, and the radiology procedure registry at the University Hospital of North Norway.

The medical records for each potential VTE case were reviewed by trained personnel. For subjects derived from the hospital discharge diagnosis registry and the radiology procedure registry, an episode of VTE was verified and recorded as a validated outcome when all four of the following criteria were fulfilled: (i) objectively confirmed by diagnostic procedures (compression ultrasonography, venography, spiral-computed tomography [CT], perfusion-ventilation scan, pulmonary angiography or autopsy); (ii) the medical record indicated that a physician had made a diagnosis of DVT or PE; (iii) signs and symptoms consistent with DVT or PE were present; (iv) therapy with anticoagulants (heparin, warfarin, or similar agent) thrombolytics, or vascular surgery was required. For subjects derived from the autopsy registry, a VTE event was recorded as an outcome when the autopsy record indicated VTE as a cause of death or as a significant condition.

Based on the presence of provoking factors at the time of diagnosis, the VTE event was classified as unprovoked (no provoking factors) or provoked (≥1 provoking factors). Major surgery, trauma, or acute medical condition (acute myocardial infarction, ischaemic stroke, or major infectious disease) within eight weeks before the event, active cancer at time of event, marked immobilisation (bed rest for longer than three days, wheelchair, or long distance travel exceeding 4 hours (h) within the last 14 days before event) were considered provoking factors.

Statistical analyses

Statistical analysis was carried out using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Participants were allocated into quartiles based on serum 25(OH)D concentrations within each month to account for the seasonal variation (16). Baseline characteristics of participants were compared using a Chi^2 test for categorical variables and one-way ANOVA for continuous variables. Cox-proportional hazards regression models were used to estimate hazard ratios (HR), with 95% confidence interval (CI), for unprovoked-, provoked- and total VTE by quartiles of serum 25(OH)D. In the Cox-models, the lowest quartile of serum 25(OH)D was used as reference group. HRs for the associations between serum 25(OH)D and risk of VTE were primarily adjusted for age and sex, and subsequently for additional potential confounders such as BMI, smoking, physical activity and intake of fish oil. The proportional hazard assumption was verified by evaluating the parallelism between the curves of the log-log survivor function for quartiles of serum 25(OH)D. Separate analyses were also performed for non-smokers and smokers due to the artificial effect of smoking

Table 1: Baseline characteristics across quartiles of serum 25(OH)D. The Tromsø Study 1994-2007. Values are means with standard deviations (SD) in brackets for continuous variables and percentages with numbers in brackets for dichotomised variables.

	Quartiles of serum 25(OH)D (nmol/l)				P
	≤ 44	45–56	57–69	≥ 70	
Participants, no	1524	1528	1527	1527	
Serum 25(OH)D (nmol/l)	36.3(8.2)	50.5(6.6)	61.9(6.9)	83.3(15.1)	<0.001
Age, years (SD)	62(10)	61(9)	60(10)	59(9)	<0.001
Male sex, % (n)	37(564)	45(687)	51(785)	48(735)	<0.001
Smoking, % (n)	8(121)	18(282)	35(537)	42(342)	<0.001
BMI, kg/m^2 (SD)	27.1(4.6)	26.3(4.1)	25.7(3.6)	24.9(3.4)	<0.001
Physical activity (h/week)	2.9(1.1)	3.0(1.1)	3.1(1.0)	3.1(1.0)	0.002
Serum PTH (pmol/l) (SD)	3.21(1.64)	2.86(1.38)	2.66(1.24)	2.40(1.23)	0.001
Serum calcium (mmol/l) (SD)	2.37(0.11)	2.38(0.10)	2.37(0.11)	2.39(0.11)	0.19
Intake of fish oil, % (n)	31(292)	39(408)	44(489)	48(556)	<0.001

BMI: body mass index, serum 25(OH)D: 25-hydroxyvitamin D (25(OH)D, PTH: parathyroidea hormone.

on the 25(OH)D measurements with uncertain impact of the risk estimates.

Results

There were 201 incident VTE events during a median of 10.7 years of follow-up. Time from serum 25(OH)D measurement to VTE event ranged from 0.1 to 12.9 years. The overall crude incidence rate of VTE was 2.7 per 1,000 person-years. As expected from ultraviolet (UV) radiation on latitude 69.6° N, serum 25(OH)D levels showed characteristic seasonal variations with highest levels in August (17). The mean serum 25(OH)D concentration was 58.1 ± 19.8 nmol/l (71.2 ± 19.5 nmol/l in smokers and 51.7 ± 16.6 nmol/l in non-smokers), and the overall prevalence of 25(OH)D deficiency (<30 nmol/l) was 5%.

Baseline characteristics of participants across quartiles of serum levels of 25(OH)D are shown in ▶Table 1. As expected, age, BMI and serum PTH levels decreased across quartiles of serum 25(OH)D, whereas proportions of males and smokers, increased significantly (all p-values for trend <0.001). Participants in the upper quartile of serum 25(OH)D had higher intake of fish oil, and spent more leisure time on moderate to high intensity physical activity than participants in the lower quartile (p-values for trend <0.002).

Characteristics of VTE patients at the time of the event are shown in ▶Table 2. Among the VTE patients, 62.3% had DVT and 38.7% had PE with or without concurrent DVT (▶Table 2). A total of 90 (40.1%) events were unprovoked. Cancer was the most common provoking factor (26.5% of the VTE patients had a cancer-related VTE event), followed by surgery (21.9%).

There was no significant association between serum 25(OH)D and future risk of VTE (▶Table 3). The HR of total VTE per one standard deviation (SD)(19.8 nmol/L) increase in serum 25(OH)D was 1.04 (95% CI 0.90–1.19) in age- and sex-adjusted analysis, and HR 1.02 (95% CI 0.91–1.22) in multivariable analysis. Similar risk estimates were found for unprovoked and provoked VTE. In quartile-based analyses, subjects with serum 25(OH)D ≥70 nmol/l (upper quartile) did not have reduced risk of VTE compared to those ≤44 nmol/l (lower quartile) in either age- and sex- adjusted

Table 2: Characteristics of VTE patients (n= 201), at the time of the VTE-event. The Tromsø Study, 1994-2007. Values are percentages with numbers in brackets.

Women	51(114)
Deep-vein thrombosis	62.3 (139)
Pulmonary embolism	38.7 (85)
Unprovoked VTE	40.1 (90)
Clinical risk factors:	
Estrogens*	5.8(14)
Heredity†	2.3 (5)
Pregnancy	0
Other medical condition‡	23 (53)
Provoking factors:	
Surgery	21.9 (47)
Trauma	5.2 (12)
Acute medical condition	15.2 (34)
Cancer	26.5 (59)
Immobilisation (bed rest>3 days, wheelchair)	9.6 (22)
Other§	3.9 (8)

*Hormone replacement therapy/oral contraceptives. †Heredity: Family history of VTE in first degree relative before the age of 60 years. ‡Other diseases within the previous year (myocardial infarction, ischaemic stroke, heart failure, inflammatory bowel disease, chronic infections, chronic obstructive pulmonary disease or myeloproliferative disorders). §Other factor specifically described as provoking in the medical record (e.g. intravascular catheter). VTE: venous thromboembolism.

Quartile range serum 25(OH)D (nmol/l)	Subjects	VTE events	HR (95% CI)*	HR (95% CI)**
Total VTE				
≤ 44	1474	50	1.00 (reference)	1.00 (reference)
45–56	1470	58	0.92 (0.61–1.37)	0.72 (0.41–1.30)
57–69	1481	46	1.09 (0.74–1.61)	0.93 (0.55–1.50)
≥ 70	1480	47	0.91 (0.60–1.37)	0.76 (0.45–1.28)
P for trend			0.91	0.89
Per 1SD decrease in serum 25(OH)D		201	1.04 (0.90–1.19)	1.02 (0.91–1.22)
Provoked VTE				
≤ 44	1474	31	1.00 (reference)	1.00 (reference)
45–56	1470	35	0.76 (0.44–1.27)	0.79 (0.37–1.67)
57–69	1481	27	0.93 (0.80–1.53)	1.05 (0.55–2–04)
≥ 70	1480	30	0.79 (0.47–1.33)	0.85 (0.44–1.59)
P for trend			0.84	0.95
Per 1SD decrease in serum 25(OH)D		123	1.01 (0.81–1.21)	1.10 (0.80–1.41)
Unprovoked VTE				
≤ 44	1474	19	1.00 (reference)	1.00 (reference)
45–56	1470	23	0.73 (0.22–2.48)	0.59 (0.22–1.52)
57–69	1481	19	0.89 (0.28–2.81)	0.73 (0.30–1.74)
≥ 70	1480	17	0.85 (0.65–4.95)	0.60 (0.24–1.49)
P for trend			0.12	0.85
Per 1SD decrease in serum 25(OH)D		78	1.14 (0.81–1.21)	1.03 (0.93–1.25)

1SD = 19.8 nmol/l. *Age and sex-adjusted. **Multivariable model adjusted for age, sex, body mass index, smoking, physical activity. VTE: venous thromboembolism.

Table 3: Adjusted hazard ratios (HR) with 95% confidence interval (CI) for VTE by quartiles of serum 25(OH)D. The Tromsø Study 1994-2007.

analysis (HR 0.91, 95% CI: 0.61–1.37, p for trend across quartiles 0.91) or multivariable analysis adjusted for age, sex, BMI, smoking, physical activity and (HR 0.76, 95% CI: 0.45- 1.28, p for trend across quartiles 0.89). Similar risk estimates across quartiles of serum 25(OH)D were found for unprovoked and provoked VTE. Participants in the lowest quartile (≤ 44 nmol/l) did not have increased risk compared to participants in the higher quartiles (HR 0.99, 95% CI: 0.68-1.28)

The cumulative incidence of VTE by quartiles of 25(OH)D was plotted against time. The risk estimates did not change significantly over time (data not shown). Separate analyses for smokers (n=1,973) and non-smokers (n=4,133) showed similar effects of serum 25(OH)D on risk estimates of VTE (data not shown). Subjects with serum 25(OH)D deficiency (<30 nmol/l) (n=311) did not have significantly higher risk of VTE (HR 1.82, 95% CI 0.85-3.90) compared to those with normal serum 25(OH)D levels (≥30 nmol/l).

Discussion

Previous observational data, along with favourable antithrombotic properties of vitamin D, support the hypothesis of an inverse association between serum 25(OH)D and risk of VTE. We are, to the best of our knowledge, the first to provide actual data on the relation between serum 25(OH)D and risk of VTE. In the present population-based cohort study, we found no association between serum 25(OH)D and future risk of VTE, either in analysis where serum 25(OH)D was treated as a continuous variable, or in analysis where serum 25(OH)D was treated as a categorised variable. Vitamin D deficient subjects (<30 nmol/l), though a minor fraction of our population (5%), did not exhibit higher risk of VTE than those with normal vitamin D levels. Subgroup analyses revealed no associations between serum levels of 25(OH)D and unprovoked or provoked VTE.

The Swedish MISS (Melanoma Inquiry of Southern Sweden) study, including 40,000 women with 11 years of follow-up, re-

ported that women with more active sun exposure habits had 30% reduced risk of VTE and that the incidence of VTE was 50% lower during the summer season (9). They suggested that both phenomena were attributed to vitamin D. The lack of association between serum 25(OH)D and future risk of VTE found in our study contradicts their hypothesis, but do not rule out that reduced risk of VTE among women with active sun exposure habits may be mediated by unrecognised confounders. Furthermore, there is not a consistent variability of VTE incidence rates during the seasons. Although a recent meta-analysis, including 35,000 patients with venous thrombosis (18), showed increased incidence of VTE during winter season, the meta-analysis did not include a large registry study of seven million individuals from the United States that failed to find a seasonal variability on incidence of VTE (19). Recently, a multicenter study on consecutive case series from Milan, Leiden and Tromsø showed similar moderately lowered incidence rates of VTE in the spring despite differences in temperature and seasonal variations in light exposure (20)

The seasonal variation of vitamin D status in Tromsø, located at latitude 69.6° N with large seasonal variation in UV exposure, is expected to vary tremendously. Even though a previous report from our present cohort confirmed seasonal variation in serum 25(OH)D status in Tromsø with peak levels in late summer/autumn (17), the seasonal variation in serum 25(OH)D status is diluted due to a generally high dietary intake of vitamin D provided by fish meals and fish oil supplementation in the Tromsø population, especially in winter (21). The average vitamin D intake provided by a single fish meal containing fish liver and fresh cod-liver oil is 73.3 μg, which is about 15-fold higher than the recommended daily intake (5.0 μg) (22, 23). In our study, participants reported on average three fish servings per week and 41% reported fish oil supplementation during the last 14 days before blood sampling. The high dietary intake of vitamin D may contribute to the low proportion of serum 25(OH)D deficiency and generally high levels of serum 25(OH)D (average 58 nmol/l) in the population.

Evidence from longitudinal studies imply that subjects with serum 25(OH)D levels below 30 nmol/l are at increased risk of cardiovascular diseases (CVD) (24, 25). These findings suggest the existence of threshold levels for serum 25(OH)D below which subjects are at increased risk of disease. However, our data did not support this notion. Even though only 5% of the population exhibited serum 25(OH)D deficiency (<30 nmol/l), we were not able to demonstrate significantly higher risk of VTE within this subpopulation, or among those with serum 25(OH)D in the lowest quartile of the population (<44 nmol/l). Our findings indicate that there is no threshold value for VTE risk by serum 25(OH)D, or that the threshold value is so low that it could not be detected in our population with generally high serum levels of 25(OH)D.

The main strengths of our study are the large number of participants and validated VTE events, the prospective design, and long-term follow-up. In addition, we used a direct, objective measure of vitamin D status (25(OH)D levels) rather than relying on self-reported vitamin D intake or sunlight exposure. The study has, however, some limitations. There is low statistical power to detect an 1.8 fold increased risk of VTE in subjects with serum 25(OH) D deficiency (5%, n=311), among which only seven subjects experienced a VTE event during follow-up,. Thus, it is likely that our non-significant finding among these subjects may be due to a type II error. Because of the known seasonal variation for 25(OH)D (26), we used intra-monthly vitamin D quartiles in the analyses. Individuals can be assumed to remain in the same 25(OH)D quartile throughout the year as a person's 25(OH)D levels tend to be consistent when measured 12 months apart, although the same person can have significantly higher 25(OH)D levels during summer than during winter (27, 28). However, outdoor exposure time, sun exposure, dietary vitamin D intake/supplements, and skin pigmentation may cause considerable individual variations (27, 28). Decreased outdoor exposure time among chronically ill or elderly homebound individuals may especially affect seasonal 25(OH)D variation, as shown by Melin *et al.* who found seasonal variation only among elderly individuals with outdoor exposure time >3 h/week (28).

In conclusion, serum levels of 25(OH)D were not found to be associated with future risk of VTE in our population-based cohort study. Our findings suggest that vitamin D levels within the normal range do not play an important role in the pathogenesis of VTE. However, our findings did not apply to subjects with vitamin D deficiency (< 30 nmol/l) due to lack of statistical power among these subjects. Further studies are warranted to investigate the impact of vitamin D deficiency on risk of VTE.

What is known about this topic?

- Some prospective cohort studies have provided indirect evidence. for an inverse association between vitamin D status and risk of venous thromboembolism (VTE).
- No study has previously investigated the association between serum levels of vitamin D and risk of VTE directly.

What does this paper add?

- Serum levels of vitamin D were not associated with future risk of VTE.
- Vitamin D status does not play an important role in the pathogenesis of VTE.

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Conflicts of interest

None declared.

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