ORIGINAL ARTICLE

Determinants of 25-hydroxyvitamin D Status in a Cutaneous Melanoma Population

Julie DE SMEDT[1], Sofie VAN KELST[1], Laudine JANSSEN[1], Vivien MARASIGAN[1,2], Veerle BOECXSTAENS[3], Marguerite STAS[3], Dirk VANDERSCHUEREN[4], Ipek GULER[5], Kris BOGAERTS[5], Katleen VANDENBERGHE[6], Oliver BECHTER[7], Jaak BILLEN[8], Arjen NIKKELS[9], Tine STROBBE[10], Gabriella EMRI[11], Diether LAMBRECHTS[12,13] and Marjan GARMYN[1]

[1]Laboratory of Dermatology, Department of oncology, KU Leuven, Leuven, Belgium, [2]Department of Surgery, South Infirmary Victoria University Hospital, Cork, Ireland, [3]Oncological and vascular access surgery, Department of Surgical Oncology, KU Leuven, Leuven, [4]Clinical and experimental Endocrinology, Department Chronical illness and Metabolism, [5]Leuven Biostatistics and Statistical Bioinformatics Centre (L-BioStat), [6]Department of Cardiovascular Sciences, [7]Laboratory of Experimental Oncology (LEO), Department of Oncology, [8]Clinical and Experimental Endocrinology, Department of Chronic Diseases, KU Leuven, Leuven, [9]CHU Sart Tilman, University of Liège, Department of Dermatology, Liège, [10]Department of Dermatology, Imelda ziekenhuis, Bonheiden, Belgium, [11]Department of Dermatology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary, [12]Laboratory for Translational Genetics, Department of Oncology, KU Leuven, and [13]Center for cancer Biology (VIB), Leuven, Belgium

Vitamin D status is influenced by well-known determinants, but factors associated with low 25-hydroxyvitamin D levels in the cutaneous melanoma population are not well defined. The aim of this study was to confirm the well-known determinants and to assess new determinants for 25-hydroxyvitamin D levels in a cutaneous melanoma population. In a prospectively included cohort of 387 patients with cutaneous melanoma the association of 25-hydroxyvitamin D levels with sex, age, body mass index, time of blood withdrawal, Fitzpatrick phototype, vitamin D supplementation, score for intensity of lifetime sun exposure, smoking, education level, hair and skin colour, eye colour, total number of benign naevi, freckles and parameters of chronic sun damage was investigated. In addition, 25-hydroxyvitamin D levels were correlated with pathological parameters of the primary tumour and melanoma stage (8th edition of the American Joint Committee on Cancer (AJCC). Univariate and multivariate logistic regressions were performed using R software. The following factors had a significant effect on vitamin D status: body mass index, seasonal time of blood sampling, vitamin D supplementation, and a subtype of skin, and hair colour.

Key words: melanoma; vitamin D; body mass index.

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Corr: Julie De Smedt, School of Biomedical sciences, Department of Oncology, Catholic University of Leuven, Herestraat 49, BE-3000 Leuven, Belgium. E-mail: marjan.garmyn@uzleuven.be

SIGNIFICANCE

Low levels of 25-hydroxyvitamin D are associated with a worse outcome in patients with melanoma. Vitamin D status in healthy persons is influenced by well-known determinants. This study investigated the determinants of 25-hydroxyvitamin D levels in patients with cutaneous melanoma. The results showed a significant effect of the body mass index, seasonal time of blood sampling, use of vitamin D suppelements and a subtype of skin and hair color on 25-hydroxyvitamin D levels in melanoma patients. Current trials investigating the benefit of vitamin D supplementation in patients with melanoma, must take overweight and obesity into account.

Cutaneous melanoma (CM) is a form of skin cancer with increasing incidence rates worldwide. In 2020, an estimated 9,272 deaths in central and eastern Europe were caused by CM (1). According to the National Cancer Institute in the USA, CM was responsible for 5.6% of all new cases of cancer and 1.2% of all cancer deaths in 2021 (2).

Vitamin D (VD) is a secosteroid produced mainly in the human skin upon ultraviolet B (UVB) radiation. The other source of VD is exogenous via dietary intake or supplements. Metabolization of VD is required to form biologically active products. The classical metabolic pathway is a 2-step process with a 25-hydroxylation in the liver to form 25-hydroxyvitamin D (25OHD), followed by 1-alpha hydroxylation in the kidney to form 1-alpha,25-dihydroxyvitamin D (3). An alternative metabolic pathway of VD is via cytochrome P450 side-chain cleavage enzyme (CYP11A1), whereby multiple hydroxymetabolites of VD are produced (4–8). Measurement of the level of 25OHD in blood serum is the best index of VD stores (9, 10). However, this may have some limitations, since not all metabolites are measured. 25OHD levels in the general population are influenced by well-known determinants, such as body mass index (BMI), age, gender, season of blood sampling, sun protection (clothing and use of sunscreen), incidence of several chronic illnesses, and skin type (11). Levels of 25OHD are also influenced by genetic variants of VD pathway genes (12). Levels of 25OHD below 20 ng/ml are associated with the greatest risk of cancer, infections, cardiovascular and metabolic diseases (13).

Besides its well-known function in calcium/phosphate homeostasis, VD has also pleiotropic anti-cancer effects (3). *In vitro* and *in vivo* studies have demonstrated an anti-melanoma activity of VD, with effects on cellular

growth, differentiation, apoptosis, malignant cell invasion, and metastasis (14, 15).

Various observational studies have shown a beneficial effect of higher 25OHD levels on overall survival, relapse-free survival, and prognostic pathology parameters, such as Breslow thickness and ulceration of the primary tumour (16–22). In molecular and clinicopathological studies, defects in VD signalling pathways are correlated with CM progression and reduced disease outcome (23).

Given the association of VD status and clinical outcome, the question arises as to whether VD supplementation is useful in the (adjuvant) treatment of patients with CM. If VD supplementation proves useful, as currently assessed in ongoing trials, one question will be which patients with CM are prone to have low 25OHD levels, and hence would potentially benefit more from supplemental therapy with VD. The aim of this study is to define which factors are associated with 25OHD levels <20 ng/ml. Thus, in a well-defined prospective population of patients with CM, levels of 25OHD lower than 20 ng/ml were assessed, and 25OHD levels were correlated with basic patient demographics. A further aim was to investigate the relationship between 25OHD levels, tumour-node-metastasis (TNM) stage (8th AJCC) (24) and histopathological parameters in the study population.

MATERIALS AND METHODS

Study population, recruitment and study procedure

This study is part of a multicentre randomized double-blind placebo-controlled phase III trial investigating the effect of VD supplementation on melanoma outcome (ViDMe trial). The full protocol of the ViDMe trial has been published (25). Briefly, patients with stage IB to III CM (according to the 7th AJCC staging), age 18–80 years, were randomized in the ViDMe trial at the University Hospitals Leuven, the University Hospitals of Antwerp, the Centre Hospitalier Universitaire de Liège in Belgium and at the Clinical Center at the University of Debrecen in Hungary if the only treatment was surgery. Most patients were recruited in the study centres in Belgium, only 4 patients were recruited in Hungary. For inclusion and exclusion criteria we refer to the published study protocol (25). The ViDMe trial, which is still ongoing, was approved by the local ethics committees, and written informed consent was obtained from all participants. A total of 387 patients prospectively recruited from Q4 2012 to Q1 2020 with a baseline serum sample available were included in this study.

Determination of clinical parameters

Basic demographic data on age, sex, tumour site and clinical stage were collected. At randomization, patients were questioned on ethnicity, current smoking status, highest education level, personal medical history, previous and current sun exposure. Current (concomitant) drug intake, VD supplementation (via over-the-counter supplements before randomization) and calcium supplementation were registered. A full skin examination was performed by a physician to assess skin phototype, naevus phenotype, presence of freckles, and signs of chronic sun damage. The latter was determined by the presence of actinic keratosis, solar lentigines and guttate hypomelanosis. The patient's height and weight were measured in order to estimate BMI.

Determination of pathological parameters

Pathological staging of the primary tumour was performed at the Department of Pathology of the participating centres and is based on the Breslow thickness of the tumour, mitotic rate and presence or absence of ulceration. In addition, the following histological parameters were registered: histological subtype, Clark level, Breslow thickness, ulceration, mitosis, vascular invasion, microsatellites, tumour infiltrating lymphocytes (TILs) and perineural invasion. Nodal micrometastasis were detected by routine H&E staining, supplemented by immunohistochemistry with a cocktail of antibodies directed against melan-A, tyrosinase and gp100 (HMB45). Processing of the sentinel node and reporting of the results was performed according to *European Organisation for Research and Treatment of Cancer* (EORTC) guidelines.

Determination of 25-hydroxyvitamin D serum levels

Upon inclusion in the ViDMe trial, serum was collected for determination of baseline 25OHD levels following standard procedures using liquid chromatography/tandem mass spectrophotometry in the diagnostic laboratory of Leuven University Hospital (26). This analytically complex method can be considered the gold standard for measurement of 25OHD, but is not available in most routine clinical laboratories. If accurate measurement or differentiation of VD metabolites is desired, this is the method of choice.

Statistical analyses

Clinical and pathological parameters were represented as means and standard deviations (SD) for continuous variables, and frequencies or proportions (%) for categorical variables between group A (25OHD <20 ng/ml) and group B (25OHD ≥20 ng/ml).

Initial melanoma stage (AJCC 7th edition) was recalculated to AJCC 8th edition for statistical analysis of the pathological parameters. Univariate logistic regression analysis and multivariate logistic regressions were performed in order to assess the associations between VD status and the clinical parameters.

The variable selection for the final multivariate model was carried out using a forward stepwise variable selection based on Akaike Information Criterion (AIC) using the data omitting missing values (n=357). The model with the lowest AIC was choosen as a final model and re-fitted with the whole dataset (n=387). Multicollinearity was checked by variance inflation factors for each variable with a threshold <5. The results of the logistic regression models were represented as odds ratios (ORs) on 25OHD levels <20 ng/ml and 95% confidence intervals (95% CI). All analyses were performed with R software (version 4.0.3).

RESULTS

In total, 387 serum samples were analysed. Of these, 155 (40%) had 25OHD levels <20 ng/ml and 232 (60%) had 25OHD levels ≥20 ng/ml. Mean 25OHD plasma concentration for the whole study population was 23.33 (SD 8.77) ng/ml.

Clinical characteristics of group A (25OHD <20 ng/ml) vs group B (25OHD ≥20 ng/ml)

Median age was 56 years in group A and 54 years in group B. In general, inclusion in the ViDMe study occurred more in the winter period (December to February). In group A, most patients (46%) had a Fitzpatrick phototype III and, in group B, most patients (42%) had

a Fitzpatrick phototype II. The majority of the general population (86%) was not taking VD supplements at the time of inclusion in the ViDMe study. Fifty-six percent of patients in group A and 52% in group B indicated having normal sun exposure in work and in their free time. The majority of patients in both groups were not smoking at the time of inclusion in the ViDMe study or had smoked in the past. In group A the majority (35%) of patients completed secondary school, compared with in group B the majority (32%) completed vocational university. In both groups, most patients had a light skin, blond or light-brown hair and blue/green/grey eye colour. Upon physical examination in both groups, most patients had a total number of benign naevi <25, no presence of actinic keratosis, presence of solar lentigines (head and neck region, on the back of the hands and on the shoulders), no freckles, and no guttate hypomelanosis (**Table I**).

Pathological characteristics of group A (25OHD < 20 ng/ml) vs group B (25OHD ≥20 ng/ml)

In both groups the most dominant histological subtype was superficial spreading melanoma, with 58% of patients in group A and 39% of patients in group B having this subtype. Regarding primary tumour thickness, the mean Breslow thickness for group A was 1.48 mm and for group B 1.30 mm, and most tumour tissue in both groups was Clark level 4. For other pathological parameters, both groups showed the same trend, with, mostly, no presence of ulceration, mitosis, vascular invasion, or microsatellites, but presence of TILs. For perineural invasion, information on primary tumour tissue was, in the majority, unknown or missing. In the group with 25OHD levels <20 ng/ml, most patients were stage IB followed by stage III. In comparison in the group with 25OHD levels ≥20 ng/ml, most patients were stage IB, followed by stage IA (Table SI).

Univariate analysis between 25OHD status and clinical parameters

Having a high BMI ≥25–30 kg/m^2 or ≥30 kg/m^2 was a significant risk factor for 25OHD levels <20 ng/ml compared with patients with CM with a normal BMI (≥18.5–<25 kg/m^2) (OR 2.21, 95% CI 1.36–3.63, $p=0.002$ and OR 5.03, 95% CI 2.73–9.48, $p<0.001$, respectively). Patients included during winter and spring had a significantly higher risk for 25OHD levels <20 ng/ml than patients included during summertime (OR 2.14, 95% CI 1.20–3.91, $p=0.011$; OR 2.09, 95% CI 1.11–4.02, $p=0.024$, respectively) (**Table II**).

Patients with CM with light skin and blond/light-brown hair had a higher risk of 25OHD levels ≥20 ng/ml compared with patients with CM with light skin and dark-brown/black hair (OR 0.47, 95% CI 0.29–0.77, $p=0.003$). Compared with patients with idiopatic guttate hypomelanosis as a sign of chronic sun damage, patients

Table I. Clinical characteristics of the study population (N=387)

Characteristics	Group A 25(OH)D3 <20 ng/ml *n* = 155 (39.7%)	Group B 25(OH)D3 ≥20 ng/ml *n* = 232 (59.4%)
Sex, *n* (%)		
Male	78 (50)	95 (41)
Female	77 (50)	137 (59)
Age, mean (SD)	56 (47, 66)	54 (45, 64)
<40 years	16 (10)	37 (16)
40–60 years	79 (51)	118 (51)
>60 years	60 (39)	77 (33)
Body mass index, *n* (%)		
<18.5 kg/m^2	1 (0.6)	4 (1.7)
≥18.5–<25 kg/m^2	34 (22)	101 (44)
≥25–<30 kg/m^2	75 (49)	101 (44)
≥30 kg/m^2	44 (29)	26 (11)
Missing	1	0
Season of blood draw, *n* (%)		
Winter (Dec–Feb)	62 (40)	73 (31)
Spring (Mar–May)	39 (25	47 (20)
Summer (Jun–Aug)	23 (15)	58 (25)
Autumn (Sept–Nov)	31 (20)	54 (23)
Fitzpatrick phototype, *n* (%)		
I[a]	17 (11)	26 (11)
II[b]	51 (33)	96 (42)
III[c]	72 (46)	77 (33)
IV + V + VI[d]	15 (9.7)	32 (14)
Missing	0	1
Vitamin D supplementation, *n* (%)		
No	150 (97)	184 (79)
Yes	5 (3.2)	48 (21)
Score for intensity of lifetime sun exposure, *n* (%)		
Normal sun exposure	87 (56)	120 (52)
Low sun exposure	51 (33)	89 (39)
High sun exposure	17 (11)	22 (9.5)
Missing	0	1
Smoking, *n* (%)		
Never smoked	64 (41)	100 (43)
Current smoking	28 (18)	35 (15)
Ex-smoker	63 (41)	97 (42)
Education level, *n* (%)		
Primary school	7 (4.5)	9 (3.9)
Secondary school	55 (35)	67 (29)
Vocational training	35 (23)	43 (19)
Vocational university	34 (22)	74 (32)
University graduated	23 (15)	38 (16)
Other	1 (0.6)	1 (0.4)
Hair colour + skin colour, *n* (%)		
Light skin, red or red-blond hair	15 (9.7)	14 (6.1)
Light skin, blond or light-brown hair	69 (45)	137 (59)
Light skin, brown or black hair	52 (34)	49 (21)
Medium tone skin, brown or black hair or brown skin, dark-brown or black hair or black skin, dark-brown or black hair	19 (12)	31 (13)
Missing	0	1
Eye colour, *n* (%)		
Brown	29 (19)	39 (17)
Blue/green/grey	109 (70)	165 (71)
Hazel (brownish green)	16 (10)	25 (11)
Missing	0	1
Total number of benign naevi, *n* (%)		
<25	71 (46)	97 (42)
25–49	32 (21)	58 (25)
25–100	29 (19)	47 (20)
>100	22 (14)	30 (13)
Missing	1	0
Solar lentigines, *n* (%)		
Head–neck region		
No presence	38 (25)	59 (25)
Presence	117 (75)	173 (75)
Back of the hands		
No presence	42 (27)	68 (29)
Presence	113 (73)	164 (71)
Shoulders		
No presence	27 (17)	47 (20)
Presence	128 (83)	185 (80)
Freckles, *n* (%)		
No presence	128 (83)	186 (80)
Presence	27 (17)	46 (20)
Guttate hypomelanosis, *n* (%)		
No presence	143 (92)	198 (85)
Presence	12 (7.7)	34 (15)
Actinic keratosis, *n* (%)		
No presence	132 (85)	204 (88)
Presence	23 (15)	28 (12)

[a]Always burns, never get darks: very light, caucasian type. [b]Always burns, then gets darker/tans sometimes after continued sun exposure: light skin, caucasian. [c]Always burns and then always gets darker/tans after continued sun exposure: medium skin, usually caucasian. [d]Rarely burns, gets darker/tans easily after sun exposure (olive skin) + never burns, skin gets darker after sun exposure (brown to dark-brown skin) + never burns, no darkening of the skin after sun exposure (black skin).
SD: standard deviation; 25(OH)D3: 25-hydroxyvitamin D3.

Table II. Associations between 25-hydroxyvitamin D (25OHD) status and clinical parameters, univariate analysis

	25OHD <20 ng/ml vs ≥20 ng/ml			
Characteristics	OR	95% CI	*p*-value	*p*-global
Sex (*n* = 387)				0.069
Male (ref)	–	–		
Female	0.68	0.45–1.03	0.070	
Age, years (*n* = 387)	1.02	1.00–1.03	0.061	0.059
Body mass index (*n* = 386)				< 0.001
<18.5 kg/m^2	0.74	0.04–5.24	0.793	
≥18.5–<25 (ref) kg/m^2	–	–		
≥25–<30 kg/m^2	2.21	1.36–3.63	0.002	
≥30 kg/m^2	5.03	2.73–9.48	< 0.001	
Season of blood draw (*n* = 387)				0.043
Winter (Dec–Feb)	2.14	1.20–3.91	0.011	
Spring (Mar–May)	2.09	1.11–4.02	0.024	
Summer (Jun–Aug) (ref)	–	–		
Autumn (Sept–Nov)	1.45	0.75–2.81	0.268	
Fitzpatrick phototype (*n* = 386)				0.063
I	1.39	0.59–3.35	0.451	
II	1.13	0.57–2.33	0.726	
III	1.99	1.01–4.07	0.051	
IV + V + VI (ref)	–	–		
Vitamin D supplementation (*n* = 387)				< 0.001
Yes (ref)	–	–		
No	7.83	3.33–23.0	< 0.001	
Score for intensity of lifetime sun exposure (*n* = 386)				0.520
0=normal sun exposure	0.94	0.47–1.89	0.856	
1=low sun exposure	0.74	0.36–1.54	0.416	
2=high sun exposure (ref)	–	–		
Smoking (*n* = 387)				0.739
Ex-smoker (ref)	–	–		
Current smoker	1.23	0.68–2.22	0.488	
Never smoked	0.99	0.63–1.54	0.948	
Education level (*n* = 387)				0.333
Primary (ref)	–	–		
Secondary school	1.06	0.37–3.13	0.920	
Vocational training	1.05	0.35–3.20	0.934	
Vocational university	0.59	0.20–1.78	0.334	
University graduated	0.78	0.25–2.44	0.659	
Other	1.29	0.05–36.6	0.867	
Hair colour + skin colour (*n* = 386)				0.013
Light skin, red or red-blond hair	1.01	0.44, 2.33	0.982	
Light skin, blond or light-brown hair	0.47	0.29, 0.77	0.003	
Light skin, brown or black hair (ref)	–	–	–	
Medium tone skin, brown or black hair or brown skin, dark-brown or black hair or black skin, dark-brown or black hair	0.58	0.29–1.15	0.120	
Eye colour (*n* = 386)				0.965
Brown (ref)	–	–		
Blue, green, grey	0.89	0.52–1.53	0.666	
Hazel (brownish green)	0.86	0.39–1.89	0.710	
Unknown	0.67	0.03–7.35	0.751	
Total number of benign naevi (*n* = 386)				0.722
< 25 (ref)	–	–		
25–49	0.75	0.44–1.27	0.295	
50–100	0.84	0.48–1.46	0.546	
> 100	1.00	0.53–1.89	0.995	
Solar lentigines (*n* = 387)				
Head–neck region				0.839
No presence	0.95	0.59–1.52	0.839	
Presence (ref)	–	–		
Back of the hands				0.636
No presence	0.90	0.57–1.41	0.636	
Presence (ref)	–	–		
Shoulders				0.485
No presence	0.83	0.49–1.39	0.487	
Presence (ref)	–	–		
Freckles (*n* = 387)				0.552
No presence	1.17	0.70–2.00	0.553	
Presence (ref)	–	–		
Guttate hypomelanosis (*n* = 387)				0.043
No presence	2.05	1.05–4.25	0.043	
Presence (ref)	–	–		
Actinic keratosis (*n* = 387)				0.432
No presence	0.79	0.44–1.44	0.431	
Presence (ref)	–	–		

Overall *p*-values are represented for each variable, *p*-values for comparisons of each category levels are represented only for those who have more than 2 levels. OR: odds ratio on 25OHD levels <20 ng/ml; CI: confidence interval; ref: reference.

with no such lesions were at risk of lower 25OHD levels (OR 2.05, CI 1.05–4.25, $p = 0.043$).

Patients with CM not taking VD supplements were at higher risk of low 25OHD levels compared with patients with CM taking VD supplements (OR 7.83, 95% CI 3.33–23.0, $p < 0.001$). None of the other investigated correlations of low VD levels with the other parameters were statistically significant.

Univariate analysis between 25OHD status, pathological parameters and TNM staging

Univariate analysis was performed with histological subtype, Clark level, mitosis, regression, micosatellites, vascular invasion, perineural invasion, TILs and pTNM 8th edition of AJCC staging. A significant correlation was found between higher tumour staging and probability of having low 25OHD levels (OR 1.14, 95% CI 1.01–1.29). No statistically significant correlation was found between other histopathological parameters and 25OHD levels (Table SII).

Multivariate analysis between 25OHD status and clinical parameters

The final multivariate model is represented in **Table III**. As all the variance inflation factors were <5, it is assumed that there is no multicollinearity. Higher BMI (≥25–30 kg/m^2 or >30 kg/m^2) was associated with higher risk of 25OHD levels <20 ng/ml compared with CM patients with normal BMI (≥18.5–25 kg/m^2) (OR 2.23, 95% CI 1.32–3.81 and OR 5.80, 95% CI 2.99–11.6, respectively). Patients whose serum was taken in wintertime for baseline 25OHD serum levels had a significantly higher risk of lower 25OHD levels compared with patients whose serum was taken in summertime (OR 2.74, 95% CI 1.44–5.38). Patients with CM taking no VD supplementation had a higher risk of low 25OHD levels compared with patients taking VD supplements (OR 8.69, 95% CI 3.53–26.4). Similarly to the univariate analysis, patients with light skin and blond or light-brown hair in the multivariate analysis had higher chances of normal 25OHD levels compared with patients with light skin and brown or black hair (OR 0.48, 95% CI 0.28–0.83) (Table III). Other parameters were not selected in the multivariate model.

DISCUSSION

This study investigated the correlation of socio-demographic variables (sex, age and educational level), clinical variables (BMI, Fitzpatrick phototype, hair/skin/eye colour, naevus phenotype, and signs of chronic sun damage), behavioural variables (smoking and sun exposure habits), season, and VD supplementation with 25OHD status in a well-defined carefully phenotyped and assessed CM population.

Table III. Associations between 25-hydroxyvitamin D (25OHD) status and clinicalparameters, the final multivariate model including the selected parameters (N=387)

	25OHD <20 ng/ml vs 25OHD ≥20 ng/ml			
Characteristics	OR	95% CI	*p*-value	*p*-global
VD supplementation				<0.001
Supplementation (ref)	–	–		
No supplementation	8.69	3.53, 26.4	<0.001	
Body mass index (kg/m^2)				<0.001
<18.5	0.69	0.03, 5.23	0.753	
≥18.5–<25 (ref)	–	–		
≥25–<30	2.23	1.32, 3.81	0.003	
≥30	5.80	2.99, 11.6	<0.001	
Season of blood draw				0.022
Winter (Dec–Feb)	2.74	1.44, 5.38	0.003	
Spring (Mar–May)	1.94	0.97, 3.93	0.063	
Summer (Jun–Aug) (ref)	–	–		
Autumn (Sept–Nov)	1.73	0.85, 3.58	0.134	
Hair colour + skin colour				0.012
Light skin, red or red-blond hair	1.37	0.56, 3.42	0.492	
Light skin, blond or light-brown hair	0.48	0.28, 0.83	0.008	
Light skin, brown or black hair (ref)	–	–	–	
Medium tone skin, brown or black hair or brown skin, dark-brown or black hair or black skin, dark-brown or black hair	0.54	0.24, 1.17	0.121	

OR: odds ratio on 25OHD levels < 20ng/ml ; CI: confidence interval; ref: reference.

To our knowledge, this is the first study to investigate a wide range of potential determinants of VD status (measured as 25OHD levels in serum) simultaneously in a prospectively recruited CM population.

Low 25OHD levels (<20 ng/ml) were found in 40% of patients with CM, and a mean 25OHD level of 23.33 ng/mL) in the CM population in this study. Low 25OHD levels (with the strongest association for levels <20 ng/ml) are associated with cancer in general (13), but also with CM, as established in previous studies that showed higher 25OHD levels in the serum of healthy controls than in patients at the time of CM diagnosis (27). Higher 25OHD levels at the time of diagnosis of the primary melanoma have been shown to predict a lower risk of relapse and increased survival, independent of Breslow thickness (16, 22). In contrast, in another study, a change in 25OHD levels during follow-up after CM diagnosis, and not the 25OHD level at diagnosis, was ascribed as contributing to the effect on CM survival (28). Further research is necessary in patients with CM to explore the utility of determination of 25OHD status and to decipher the impact of baseline 25OHD levels and VD dynamics.

In the current study population with CM, a clear and significant correlation of overweight (BMI ≥25–<30 and obesity BMI ≥30 kg/m^2; $p<0.001$) with 25OHD levels <20 ng/ml was observed. This is in line with the literature demonstrating an association between low VD status and obesity in the general population (29). An inverse correlation between serum 25OHD levels, BMI, body weight and fat mass is a consistent finding in both adults and childeren of different ethnicities in a range of geographical locations (30).

It is not known if this association is causative, or is just a bystander effect (i.e. a not yet defined underlying condition may lead to low VD status), or if it simply originates from an association with common lifestyle factors (13, 29).

Multiple possible mechanisms are proposed as the cause of low 25OHD levels in obesity (30): lower sun exposure, low dietary intake, impaired synthesis in the skin, variance in VD metabolism (due to alterations in the vitamin D binding protein (VDBP) or more rapid metabolic clearance), or increased volumetric diluton into a larger tissue volume.

Sunlight habits differ geographically and between cultural groups, but previous studies did not show a difference in sunlight exposure and dietary intake between normal weight and obese adults (32, 33). A similar cutaneous production of VD was observed between normal weight and obese people (33).

Circulating VD is protein bound to VDBP and albumin. Alterations in the concentrations of these proteins and genetic variations in the binding affinity of VDBP affect 25OHD measurement. No differences were found between obese and normal weight people concerning those proteins, and no differences in VDBP genotypes were observed (31, 34).

VD is fat soluble and distributed in the liver, muscle, fat and, in smaller amounts, in other tissues. The volume of these compartments is increased in obese patients. A logical consequence of more widely distributed VD in a larger tissue volume is a lower level of distributed VD in the serum. The volumetric dilution has clinical implications if oral VD supplementation is given to obese patients. A smaller increase in 25OHD levels is seen in obese patients compared with normal weight patients, therefore loading doses of VD will need to be larger to achieve the same 25OHD levels (31).

In the current study population, intake of VD supplements was a significant determinant of 25OHD level ($OR_{\text{no VD supplementation}}=8.69$, p-value <0.001). Further research is required into the effect of VD supplementation on 25OHD levels and, subsequently, the effect on outcome in patients with CM, especially those in the obese and overweight group.

Univariate and multivariate analyses showed a significant correlation between seasonal collection of blood and 25OHD levels. Blood sampling in winter had a lower chance of normal 25OHD levels compared with the summer period (OR 2.74; $p=0.022$). This is in line with the fact that VD is produced in the skin upon UVB-exposure, which is more pronounced during the summer period.

In both univariate and multivariate analysis, a protective effect of having a light skin in combination with blond or light-brown hair was seen in terms of normal 25OHD levels, compared with patients with light skin and brown or black hair. We cannot explain this finding, nor have we found any similar documentation in the literature.

We further investigated sociodemographic, and other clinical and behavioural parameters as possible deter-

minants for 25OHD levels, but there were no statistically significant correlations with regard to sex, age, Fitzpatrick phototype, score for intensity of lifetime sun exposure, smoking, education level, eye colour, total number of benign naevi, solar lentigines, freckles, idiopathic guttate hypomelanosis, and actinic keratosis. In the univariate analysis, there was a significant correlation of idiopathic guttate hypomelanosis with 25OHD levels. Idiopathic guttate hypomelanosis is a marker of chronic sun damage and thus can be linked with higher 25OHD levels. However, the correlation with idiopathic guttate hypomelanosis was no longer significant in the multivariate analysis. Other parameters of chronic sun damage, such as solar lentigines and actinic keratosis, were not statistically significantly associated with 25OHD levels.

Univariate analysis demonstrated a statistically significant association between higher tumour stage and lower 25OHD levels, in line with previous studies (16, 17, 35, 36). We found no significant correlation between 25OHD level and histological subtype of primary tumour tissue, Clark level, mitosis, vascular invasion, perineural invasion, regression, or TILs.

In conclusion, this study found a clear significant association between 25OHD levels and the following factors: BMI, taking VD supplements, season of blood drawing, hair and skin colour. Low levels of 25OHD were associated with a higher tumour stage. Further research is ongoing to investigate whether VD supplementation after removal of the primary melanoma has a protective effect on melanoma outcome and could be used as adjuvant therapy in patients with CM. It will be of interest to investigate the benefit of VD supplementation in the overweight and obese CM population.

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