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Article

Prognostic value of 25-hydroxyvitamin D3 levels at diagnosis and during follow-up in melanoma patients

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List of abbreviations:

25 hydroxy-vitamin D3: 25(OH)D3

American Joint committee on cancer: AJCC

Body mass index: BMI

Disease free survival: DFS

Hazard ratio: HR

Overall survival: OS

Vitamin D: vitD

Vitamin D3: vitD3

Abstract

Background: A low 25-hydroxyvitamin D3 (25(OH)D3) serum concentration at melanoma diagnosis might be associated with worse survival. We prospectively studied the prognostic value of 25(OH)D3 at diagnosis and during follow-up.

Methods: MelanCohort is a cohort of invasive melanoma patients. Serum 25(OH)D3 was measured by mass spectrometry and standardized on month of blood drawn, age, sex and body mass index (BMI).

Role of 25(OH)D3 levels and risk of relapse was analyzed in a Cox proportional hazards model adjusting for age, sex, BMI and American Joint committee on cancer (AJCC) stage. All statistical tests were two-sided.

Results: 1171 patients were included. 25(OH)D3 levels at diagnosis (median: 49.0 nmol/L) were inversely correlated with prognostic factors such as American Joint Committee on Cancer (AJCC) stage (p<0.0001 Kruskal-Wallis), Breslow's thickness (p<0.0001 Spearman correlation), and ulceration (p=0.0008 Kruskal-Wallis),**[Please report these P values as <0.001, as per style and so they match what is in the main text results.]** but not with risk of relapse. Changes in 25(OH)D3 levels during follow-up were associated with worse prognosis: with a third quartile Q3 of average change per year (-0.30 to 4.60 nmol/L/Y) **[Please make sure this is explicitly stated in the main text. All data in the Abstract must be in the Main text Results. Being presented only in a Table is not sufficient.]** used as reference, HR for the first, second and fourth quarters were 1.94 (95%CI:1.36-2.76), 1.23 (95%CI:0.85-1.78), and 1.61 (95%CI:1.14-2.28) **[Please make sure this is explicitly stated in the main text. All data in the Abstract must be in the Main text Results. Being presented only in a Table is not sufficient.]**, respectively. In sensitivity analyses, no changes were observed either by AJCC stage, number of 25(OH)D3 measures performed, or by 25(OH)D3 level at baseline. No evidence of reverse causation was identified. Analyses performed on overall survival yielded similar results.

Conclusions: We show that 25(OH)D3 variation during follow-up is an independent melanoma prognostic marker, but not its level at diagnosis. Previously reported associations between low 25(OH)D3 level at diagnosis and poor prognosis seem to be due to insufficient adjustment for prognostic factors.

The pro-hormone vitamin D (vitD) has pleiotropic actions on human cells, including some that are relevant to cancer, notably on cell growth, differentiation or apoptosis, or on the immune system. VitD is mainly synthetized in the skin during ultraviolet-exposure or, to a lesser extent, is ingested from some foods or supplements. It is mainly transported to target cells after hydroxylation to 25 hydroxy-vitaminD3 (25(OH)D3), which is believed to represent its reservoir form. 25(OH)D3 acts after transformation in target cells into active compounds, the main being 1-25(OH)2D3, through interaction with the vitD receptor (1).

Several prospective population-based studies have shown higher 25(OH)D3 serum levels statistically significantly associated with decreased risk of later diagnosis of breast (2), colon (3), renal (4), hepatocellular (5), or tobacco-related cancers (6). However, randomized intervention studies of vitD supplementation failed to demonstrate any reduction of cancer development (7), suggesting a reverse causation association between low 25(OH)D3 serum levels and presence of cancer.

Several cohort studies have shown statistically significant associations between low 25(OH)D3 serum levels at diagnosis and presence of poor prognosis criteria, decreased cancer-specific or overall survival (OS) in patients with colon (8), breast (2, 9), prostate (10), bladder (11), or all cancers (7, 12). However, when multivariate adjustments with other prognostic criteria, cancer therapy, or body mass index (BMI) were made, the association vanished, as for breast cancer (13). Few study investigated the prognostic value of variations of 25(OH)D3 serum level during follow-up of cancer patients. Post-diagnosis vitD intake or high 25(OH)D3 serum level were not associated with any improvement of all-cause or disease-specific mortality in prospective cohorts of colon (14), breast (13), or prostate (15) cancer patients. Similarly, no impact of vitD supplementation was demonstrated on prostate specific antigen level in black men with prostate cancer (16).

Cutaneous melanoma is a severe cancer once spread to distant organs, with main prognostic factors being Breslow's thickness, ulceration and mitotic rate of the primary lesion and status of the draining lymph nodes (17). In vitro studies have shown growth inhibition of malignant melanoma cell lines by 1,25(OH)2D3 (18). In patients with newly diagnosed melanoma, low 25(OH)D3 serum level was associated with worse prognostic factors, such as Breslow's thickness (19), higher American Joint committee on cancer (AJCC) melanoma stage (20) and with worse survival independently of Breslow's thickness in one prospective study (21).

Studies on 25(OH)D3 and cancer occurrence or prognosis were often flawed by various biases, such as lack or incomplete handling of factors that control its serum level, like seasonality of blood drawing, body mass index (BMI), age, and sex. In addition, no study ever evaluated the prognostic role of 25(OH)D3 serum level measured during the follow-up of melanoma patients. We aimed, using a prospective cohort of melanoma patients with multiple assessment of 25(OH)D3 serum levels and where most factors that control 25(OH)D3 serum levels were recorded, 1)to confirm the prognostic value of 25(OH)D3 serum level collected at diagnosis and, 2)for the first time, to evaluate whether changes during follow-up of 25(OH)D3 serum levels could be associated with modifications of disease free survival (DFS) or OS.

Patients and methods

Study population

MelanCohort has been described previously (22). Briefly, a cohort of adults diagnosed with skin melanoma was set up from great Paris university hospitals (APHP) between September 2003 and December 2008 and followed-up prospectively. Cases had confirmed melanoma by central pathology review and were included within 3 months after curative surgery of primary melanoma or of invaded

nodes or within one month after diagnosis of distant metastasis. This study was registered in ClinicalTrials.gov database (NCT00839410). All patients gave written informed consent and ethical committee CPP IDF 8 approved study. Follow-up visits were recorded prospectively every two visits required by the French Guidelines (twice yearly for stage I patients, 4 times yearly for stage II-III)(23). Melanoma prognostic criteria were collected prospectively (Breslow thickness, ulceration, nodal status, mitotic rate, age, sex, location of the primary, AJCC stage). At inclusion and during prescheduled follow-up visits, blood was drawn and serum samples were aliquoted and stored at -80 Celsius degrees in centralized biobank. Using central APHP Diagnosis Related-Groups system, we estimated that roughly 50% of melanoma patients presenting to APHP during study period were included in MelanCohort.

The follow-up was conducted until June 2013 for this study, allowing OS to be scarcely influenced by new efficient treatments of metastatic melanoma such as vemurafenib, which was launched in French pharmacies in February 2013. This date constituted the right censoring date for the follow-up. Detailed information on follow-up was collected, such as the last contact date, living status and date of death if any. Living status was confirmed by cross-checking individual status in the national register for cause of death (CepiDC, http://www.cepidc.inserm.fr). Information on relapse was also collected prospectively.

For this study, we analyzed patients with only one invasive melanoma. 25(OH)D3 serum level was measured, blinded to clinical information, by mass spectrometry coupled to high-performance liquid chromatography, with a precision of 5%, and expressed as nmol/L (see **Supplementary Methods**).

Statistical methods

To enable comparisons of survival according to serum level of 25(OH)D3 (initial value at diagnosis and values recorded during follow-up), we produced standardized 25(OH)D3 levels adjusted for month

of sampling, age, sex, and BMI, using a generalized linear model restricted to patients for whom 25(OH)D3 was measured within 6 months after primary melanoma diagnosis (**Fig 1**, **supplementary table 1**). Because of seasonal variations of 25(OH)D3, sensitivity analyses were conducted by repeating this standardization restricted to patients for whom the 25(OH)D3 was measured within 90 or 30 days after primary melanoma diagnosis.

For each individual, the yearly change of 25(OH)D3 level was computed based on a linear regression of the standardized 25(OH)D3 levels upon the years following diagnosis. The linear trend could not be computed for patients with only one 25(OH)D3 measure performed, for whom reverse causation bias was likely (death before having at least two measurements). A sensitivity analysis was conducted restricted to individuals with at least 4 measurements. To evaluate the assumption of linearity of the yearly trend of 25(OH)D3, student t-test was performed on the residuals of the linear regressions testing departure from zero.

When continuous variables were converted into categorical data, the quartiles of the distribution of the variable were used as cut-point. For the trend of 25(OH)D3, an additional variable was computed based on every 10% of the distribution. The association between two variables was tested by non-parametric methods: Kruskal-Wallis for a continuous variable and a categorical variable, Spearman correlation for two continuous variables; chi-square test was used for categorical variables.

The prognostic role of 25(OH)D3 level at inclusion and change along time was analyzed in a Cox proportional hazards model adjusting for age, sex, BMI and AJCC stage. Proportional hazards hypothesis was verified by investigating Schoenfeld residuals. Primary and secondary endpoints were DFS and OS, respectively. DFS was defined as the time from first melanoma pathological diagnosis to first progression (occurrence of regional or first distant metastasis) or death. For Stage IV patients, only date of death

was used for DFS. OS was defined as the time from melanoma diagnosis to death. The SAS software version 9.3 for PC (SAS Institute, Cary, NC) was used.

All tests of statistical significance were two-sided. Power calculations have been performed considering a 0.7 hazard ratio (HR) against DFS or OS for the upper 25-OHD3 quartile versus the three lowest quartiles. A minimal follow-up time of 3 years was estimated mandatory to gain 80% power with a two-sided type I error of 5%. An objective of 0.70 HR seemed adequate as in the Newton-Bishop study [21], the HRs for risk of relapse were 0.70 (95%CI, 0.42-1.14) and 0.57 (95%CI, 0.33-0.97) for the upper two 25(OH)D3 level tertiles, respectively. This project required follow-up of all MelanCohort patients until at least June 2011, and 4094 serum samples were expected. A P value of less than 0.05 was considered statistically significant.

Results

For 31 individuals, no adequate serum sample was available. Overall, 1171 individuals (579 men and 592 women; mean age 54.2 years, interquartile range 41-67) with at least one 25(OH)D3 measurement, were included, and 1008 had a first 25(OH)D3 level measured within 6 months after melanoma diagnosis. Median follow-up was overall 4.5 years. Forty-one individuals were lost to follow-up. Average BMI was 25.2 kg/m^2 (interquartile range 22.0-27.3, 11 individuals with missing information). Overall, 3728 25(OH)D3 serum level measurements were available (mean 3.25/individual, range 1-13, interquartile range 1-4). Sentinel lymph node biopsy has been performed in 408 patients, with clear nodal invasion in 102.

Table 1 shows descriptive statistics of patients included and corresponding standardized 25(OH)D3 levels at baseline. Median 25(OH)D3 at diagnosis was 49.0 nmol/L (interquartile range 35.7-64.9). The median delay between diagnosis and first 25(OH)D3 measurement was 39 days (inter-quartile range: 24-77). 25(OH)D3 levels at diagnosis were inversely associated with melanoma prognostic factors, such as

AJCC stage (*P*<.001 Kruskal-Wallis test), Breslow's thickness in mm (*P*<.001 Kruskal-Wallis test), and ulceration (*P*<.001 Kruskal-Wallis test).

During follow-up, 411 individuals experienced relapse, and 303 died. **Table 2** presents the Cox model on risk of relapse, adjusting on age, sex, BMI, AJCC stage, and 25(OH)D3 level at diagnosis. When adjusting for known melanoma prognostic factors, the standardized baseline 25(OH)D3 serum level was not associated with prognosis.

Overall 856 individuals had at least 2 values of serum 25(OH)D3 level measured, allowing to calculate an annual trend of variation of 25(OH)D3 serum level (**Supplementary figure 1**). Visual inspection of the 25(OH)D3 and residuals of the predicted values at individual level did not show departure from the linear assumption (*P*=0.40). The annual variations of standardized 25(OH)D3 serum level over time, either increasing or decreasing, were statistically significantly associated with worse prognosis (**Table 2**). Individuals who experienced increases above 4.60 nmol/L/year or decreases below 5.25 nmol/L/year had a worse DFS in the Kaplan Meier curve (**Figure 2**). **Figure 3** presents the same analysis as **Table 2** but with more categories of 25(OH)D3 trend. A change <0.5 nmol/L per year, either increasing or decreasing, was used as reference category. The figure is U-shaped, any variation above or under 0.5 nmol/L/year being associated with worse prognosis, with risk being even greater when variations were above or below 3 nmol/L/year.

In sensitivity analyses, HR for the effect of variation of standardized 25(OH)D3 serum level remained of similar magnitude when using different initial period for standardization or restricting the sample to individuals with a short delay between diagnosis and first 25(OH)D3 measurement, (**Supplementary Table 2**). The sensitivity analysis was also conducted by AJCC stage groups (**Supplementary Table 3**) or by number of 25(OH)D3 measures performed. Overall, 76 individuals (6.5%) had detectable serum level of 25(OH)D2, a source of some vitD supplementations in France, at some

point during follow-up. They were on average younger (median age: 49 years for those with detectable serum level of 25(OH)D2 vs 56 for others, *P*=0.004), more frequently women (7.77% vs 5.18% for men, *P*=0.07). No statistically significant association was found between having detectable 25(OH)D2 level and Breslow's thickness (*P*=0.24) or AJCC stage (*P*=0.72). Serum level of 25(OH)D2 was independent of the first 25(OH)D3 serum level (p=0.99) nor of any variation over time (*P*=0.93).

Reverse causation (24) could be a likely explanation between change in 25(OH)D3 and prognosis if a rapid change in 25(OH)D3 level occurred shortly before distant metastasis development, while we assumed a linear change in 25(OH)D3 level. Hence, the analysis was replicated excluding the last 25(OH)D3 measurement if occurring within 6 months before event or right censoring and provided similar results to the full analysis, without any modification of point estimates nor statistical significance (data not shown). Biases could also be caused by heterogeneity in measurements, with patients with short survival having 25(OH)D3 slopes estimated with less tests and larger standard errors than patients with longer survival. We thus replicated the analysis taking only the initial year of 25(OH)D3 measurement and individuals with at least one year of follow-up (**Supplementary Table 4**) and found statistically non-significant results, likely due to the low power of this analysis, the reference category for the trend being based on 26 individuals and 4 events. To circumvent this methodological problem, we replicated the main analysis but stratifying on the number of test per individuals (**Supplementary Table 5**). This analysis, for which only individuals with similar number of tests are compared in each stratum, resulted in similar results as the main analysis.

Analyses performed on OS yielded similar results, with no effect of baseline 25(OH)D3 on survival, but changes of 25(OH)D3 through time associated with worse prognosis (**Supplementary Table 6**).

Characteristics of individuals who experienced an important increase or decrease of 25(OH)D3 serum levels with time are given in **Supplementary Table 7**. No association was found with age or sex.

These individuals had slightly worse presentation, with more advanced AJCC stage, thicker or ulcerated primary melanoma. A correlation was observed between baseline 25(OH)D3 level and variation of 25(OH)D3 with time, which appeared artifactual, as individuals with already high 25(OH)D3 level at baseline have little chance to see its increase with time.

Because our results differed markedly from Julia Newton-Bishop et al ones (21), we applied to our data the same modeling (without the Townsend score, unrecorded in our study). When adjusting for age as continuous variable, sex, BMI, anatomical body site, and Breslow's thickness as continuous variable, statistically significantly improved prognosis was found with increased initial 25(OH)D3 levels (*P*=0.04). This yielded to an HR of 0.90 (95%CI 0.82-0.99) for each 20nmol/L increase. But, when adjusting for Breslow's thickness as categorical variable, the association with 25(OH)D3 was no longer statistically significant (*P*=0.19, HR=0.997; 95%CI 0.992-1.002 per 1nmol/L increase). It became even flat when adjusting for AJCC stage (*P*=0.75, HR=1.001; 95%CI 0.996-1.006 per 1nmol/L increase). A further analysis stratified on AJCC stage led to flat result (*P*=0.8, HR=1.001; 95%CI 0.996-1.006). **Figure 4** shows the HR on the risk of relapse for the same Cox model using Breslow's thickness either as a continuous variable, i.e. linear parameter, or as a categorical variable to account for non-linearity.

Discussion

The analysis of 25(OH)D3 serum level in this prospective cohort of carefully followed-up melanoma patients enabled us to confirm already described association between low 25(OH)D3 serum level at diagnosis and prognostic factors, such as higher Breslow's thickness (19) or higher AJCC melanoma stage (20) and, to the best of our knowledge for the first time, to investigate the role of its variations during follow-up. As expected by the associations at diagnosis, in crude analysis, low 25(OH)D3 levels were associated with poorer DFS and OS. However, as soon as proper adjustments were used for prognostic

factors such as AJCC stage, or stratifying the analysis on prognostic markers, this association totally disappeared. Thus, in our study, 25(OH)D3 serum level at diagnosis was not an independent melanoma prognostic marker.

These results strongly differ from J Newton-Bishop et al (21) ones. Our analysis showed that a statistically significant association could be evidenced only when poorly accounting for prognostic factors, such as in unadjusted analysis or when using Breslow's thickness as a continuous variable. This was further confirmed by a stratified analysis. It appeared from the comparison of both approaches (**Figure 4**) that Breslow's thickness used as raw continuous variable would be systematically under-adjusted for values above 2mm and over-adjusted for thin melanomas. Thus, the approach used by Julia Newton-Bishop et al likely led to biased results.

Noteworthy, our study had some methodological advantages: it followed the REMARK guidelines for tumor markers in prognostic studies (25), patient losses to follow-up were limited, serum samples were carefully handled and 25(OH)D3 serum levels were measured by mass-spectrometry, considered as the most accurate method (26). Some widely used techniques of 25(OH)D3 serum level measurement are easier, but are hampered by high inter-method variability (26). Our method of standardization of 25(OH)D3 serum level used adjustments to the main factors that influence its level in the normal population (27). Although we cannot exclude that unmeasured confounders biased our results, we believe this method of standardization as accurate.

Although the baseline value of standardized 25(OH)D3 serum level was not an independent prognostic marker, its variation through time was. The use of time-dependent variables as a prognostic marker is always a limitation because potential bias could arise, such as reverse causation. Our analysis did not indicate evidence of reverse causation in the analysis of changes in 25(OH)D3 serum level. Because of our careful standardization of 25(OH)D3 serum level, we think this result valuable.

Noteworthy, a U-shaped association between 25(OH)D3 serum levels at diagnosis and prognosis has been shown for prostate cancer, with both high and low levels at diagnosis being associated with higher grade disease (28).

The biological significance of our finding on the prognostic value of changes in 25(OH)D3 levels overtime is unclear. Decrease in 25(OH)D3 levels upon time may be associated with inflammation, and serum concentrations of tumor necrosis factor-α or C-reactive protein have been repeatedly reported inversely correlated with 25(OH)D3 concentrations (7). Driver mutations in melanoma cells increase secretion of pro-inflammatory cytokines such as interleukin-6 and -8 and there is also evidence that ulceration of the primary lesion, a potent poor prognostic factor for melanoma, is associated with inflammation (29). Alternatively, progression is associated with poorer global health, thus limiting sun-exposure, the main source of vitD. It is more difficult to understand the cause of increase of 25(OH)D3 upon time. VitD3 supplementation may be an explanation, but we did not recorded prospectively use of vitD supplements. However, we measured vitD2 levels, a method of vitD supplementation used in France, and failed to show that vitD2 supplementation had any impact on variation over time of 25(OH)D3 levels. Nevertheless, we cannot exclude that some patients ingested vitD3 as supplements, nor did we measured patients' expositions to the sun. In fact, all patients were instructed to refrain from exposing their skin to the sun. Our results do not also favor the hypothesis that vitD might mediate the reported lowered risk of relapse for melanoma patients who have sunny holidays after melanoma diagnosis (30).

Because, a change of 25(OH)D3 serum level upon time in both directions was associated with worse prognosis in melanoma patients, it is unlikely a direct consequence of vitD biological actions. We postulate that fluctuation of 25(OH)D3 serum level reflected a global instability in patient's metabolisms, which finally impact 25(OH)D3 serum level by any of the multiple pathways in the vitD3 regulation (31). Although our results should be replicated, they add a new cautionary note to

widespread use of vitD3 supplementation in melanoma patients in order to improve survival. In fact, vitD3 supplementation failed to prevent melanoma in two prospective cohort studies in the general population (32, 33). In the J Newton-Bishop et al. study, reported vitD3 supplementation was not associated with improved survival (21). Results of ongoing clinical trial on vitD supplementation to prevent relapse in high-risk melanoma are awaited (34), as well as large supplementation trials in the general population (35).

Notes:

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Conflict of interest: We have no conflict of interest to declare.

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Table 1 - Descriptive statistics of main prognosis factors and 25(OH)D3 level at baseline in 1171 MelanCohort melanoma patients.

Prognostic factor	Number of cases	Median 25(OH)D3 level at baseline* (in nmol/L)	Interquartile range	
Age				
<50	453	47.91	36.71	62.28
50-69	485	51.35	36.96	68.05
70+	233	46.11	31.92	61.03
Sex				
Men	579	49.42	36.58	64.36
Women	592	48.13	35.42	66.36
AJCC				
IA	425	52.07	39.11	66.79
IB	218	52.27	38.37	66.57
IIA	124	48.69	35.66	70.20
IIB	85	45.59	37.73	69.03
IIC	39	41.15	28.83	59.43
IIIA	61	49.64	35.42	59.90
IIIB	88	44.11	33.77	60.11
IIIC	58	40.74	30.36	60.61
IV	70	35.42	25.63	48.69
Missing	3	42.44	36.07	48.81
Breslow (in mm)				
0-0.5	196	52.20	39.17	66.35
0.5-1	255	51.72	38.52	67.71
1-2	325	49.07	37.45	66.04
2-3	145	46.50	34.69	61.28
3-4	69	47.50	35.89	64.36
4+	136	41.58	30.15	59.43
Missing	45	42.58	29.89	61.00
Ulceration				
No	781	50.63	37.69	66.36
Yes	225	45.14	31.34	61.00
Missing	165	43.57	33.77	58.60

*25(OH)D3 levels were standardized on age, sex, body mass index, and month of blood sampling.

Table 2 – Cox proportional hazards model on the risk of relapse (410 events) in 1171 melanoma patients participating to MelanCohort in France.

Parameter	Number of cases	HR*	95 % CI	
Age (continuous per individual year)	1171	1.02	1.01	1.03
Sex				
Male	579	1	ref	
Female	592	0.83	0.68	1.02
BMI (continuous per unit of BMI)	1171	1.02	0.99	1.04
AJCC stage				
IA	425	1	ref	
IB	218	2.61	1.70	4.03
IIA	124	3.86	2.46	6.05
IIB	85	7.46	4.81	11.58
IIC	39	10.52	6.29	17.58
IIIA	61	7.06	4.33	11.49
IIIB	88	7.69	5.02	11.76
IIIC	58	13.70	8.71	21.55
IV	70	10.30	6.67	15.91
25(OH)D3 baseline† (in nmol/L)				
<37.15	312	0.95	0.71	1.27
37.15;49.77	288	1.04	0.77	1.40
49.77;66.05	273	1	ref	
66.05+	274	0.99	0.72	1.36
Missing	24	2.25	0.98	5.17
Change in 25(OH)D3† level during follow-up (in nmol/L/year)				
<-5.25	214	1.94	1.36	2.76
-5.25;-0.30	214	1.23	0.85	1.78
-0.30;4.60	214	1	ref	
4.60+	214	1.61	1.14	2.28
Only one test	291	2.11	1.50	2.95
Missing	24	‡		

*Hazard ratios (HRs) from a Cox proportional hazards model adjusting on age, sex, body mass index (BMI), American Joint committee on cancer (AJCC) stage, 25(OH)D3 at baseline and change in 25(OH)D3 during follow-up. CI=Confidence interval.

†25(OH)D3 levels were standardized on age, sex, BMI, and month of blood sampling.

‡Same as 25(OH)D3 at baseline.

Figure Legends

Figure 1. First 25(OH)D3 measurements performed within 180 days after diagnosis on melanoma patients participating to MelanCohort. A) before standardization, B) after standardization on age, sex, body mass index, and month of blood sampling.

Figure 2. Kaplan–Meier representation of disease free survival by quarters of 25(OH)D3 yearly trend.

Figure 3. Hazard ratio (HR) of risk of relapse by yearly trend in 25(OH)D3 during follow–up of melanoma patients participating to MelanCohort adjusting on age, sex, body mass index, AJCC stage, and baseline 25(OH)D3 level. The reference category is -0.5 to 0.5 nmol/L/year. Grey vertical lines correspond to quartile of the distribution of the yearly trend in 25(OH)D3. CI=confidence interval.

Figure 4. Hazard ratio of risk of relapse according to Breslow's thickness (in mm) entered either as a linear (dashed line) or categorical variable (plain line). Hazard ratios are displayed on a log scale. Cox proportional hazards model was used adjusted on age, sex, body mass index, anatomical body site and Breslow's thickness. Vertical grey lines correspond to quartiles of the distribution of Breslow's thickness (0.6, 1.25, 2.3mm).

Figure--final

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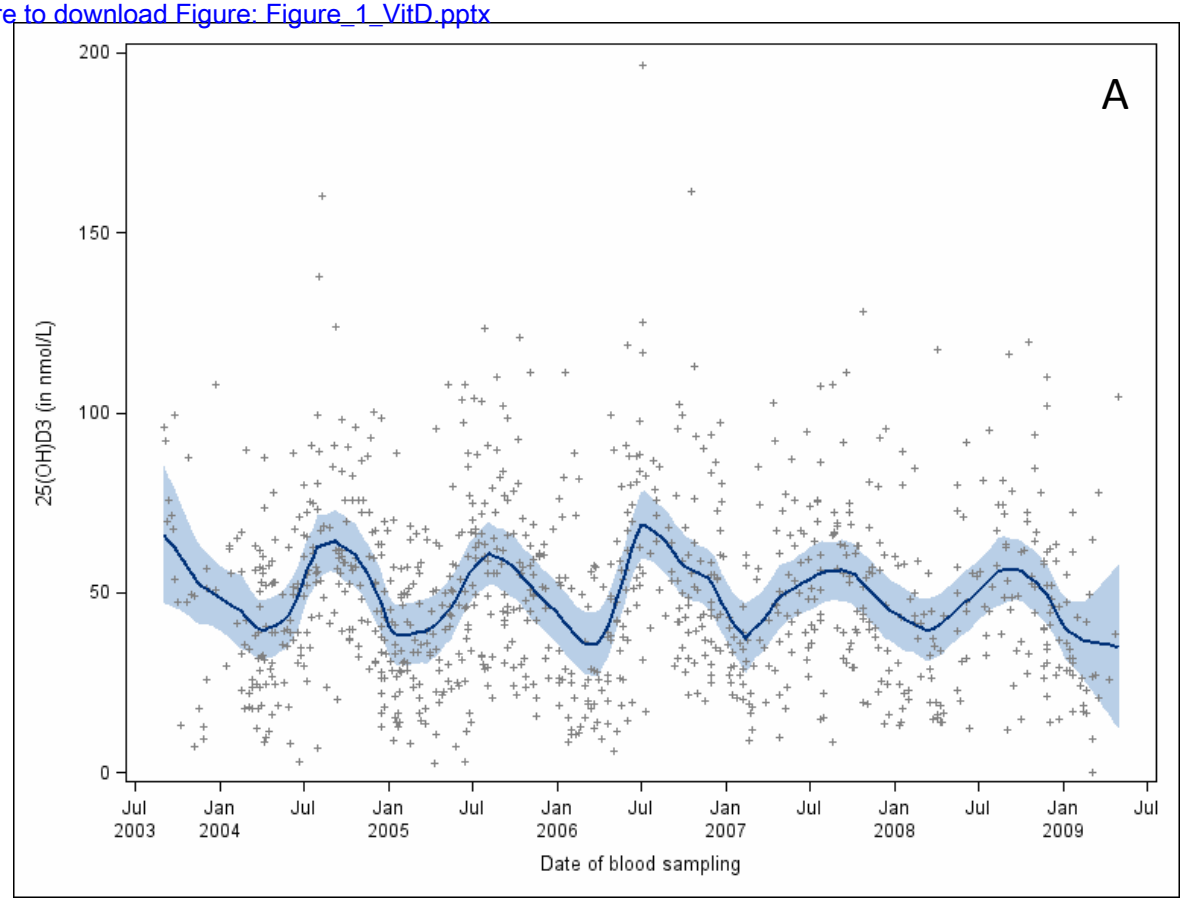

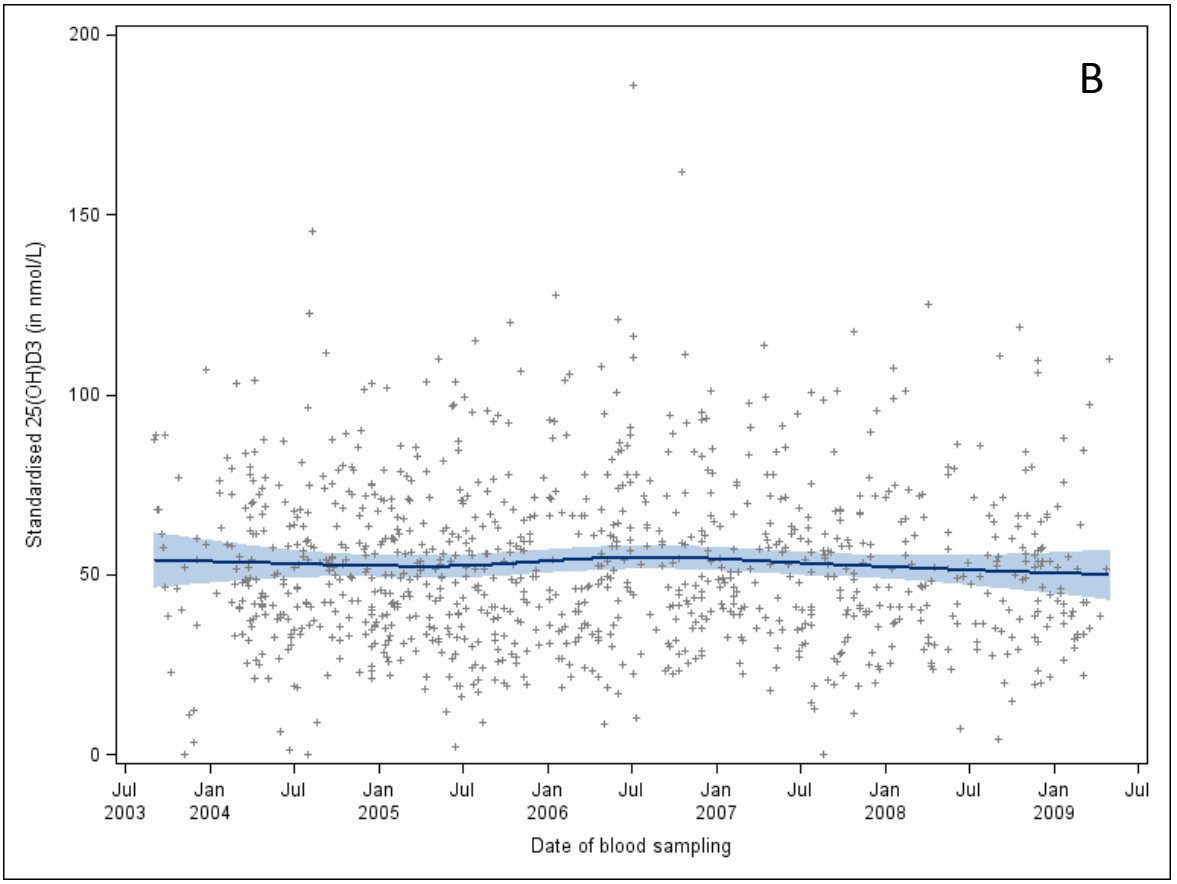

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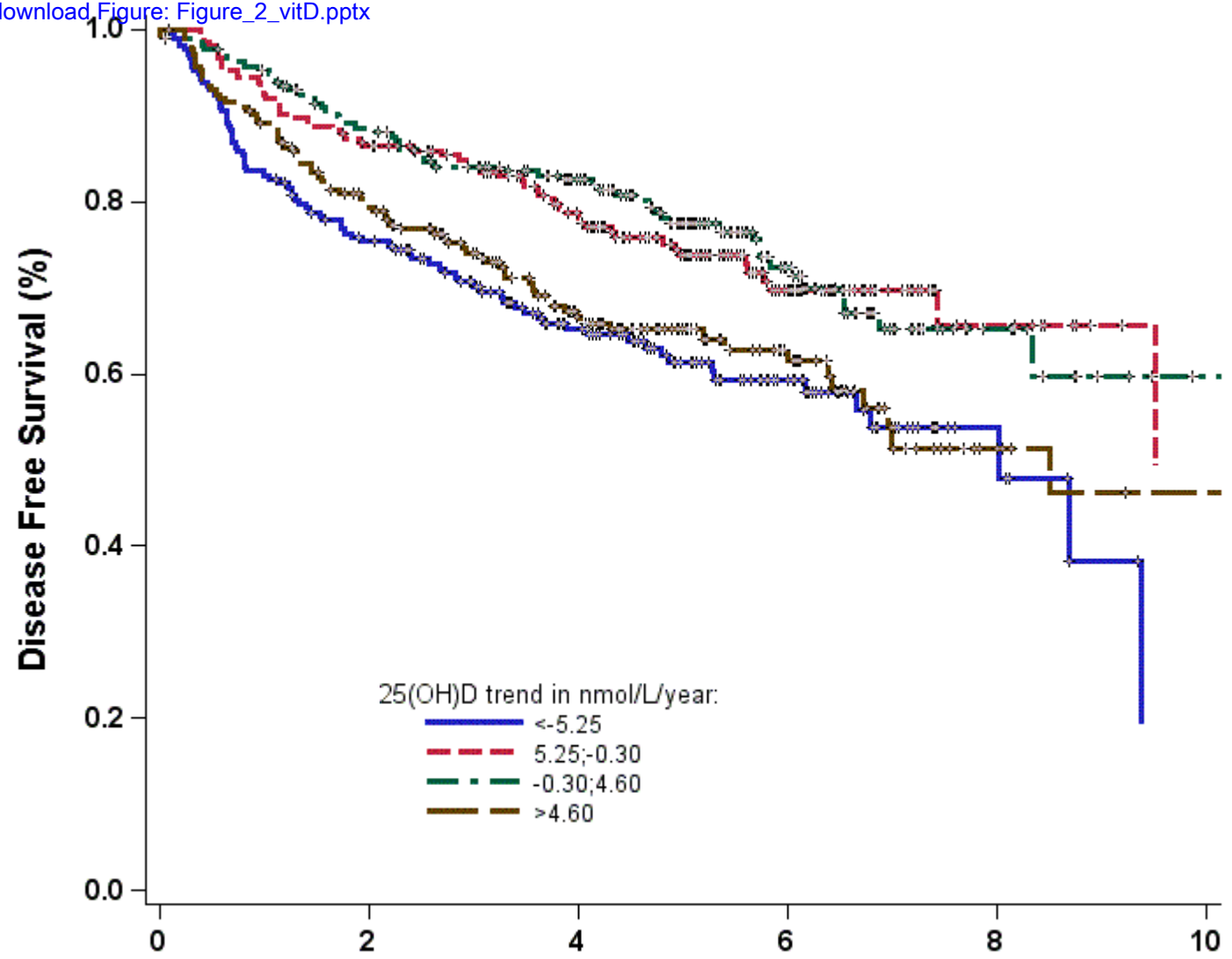

Patients at risk					
<-5.25	214	152	97	46	9
-5.25;-0.30	214	183	142	64	13
-0.30; 4.60	214	182	143	62	18
4.60+	214	157	98	44	13

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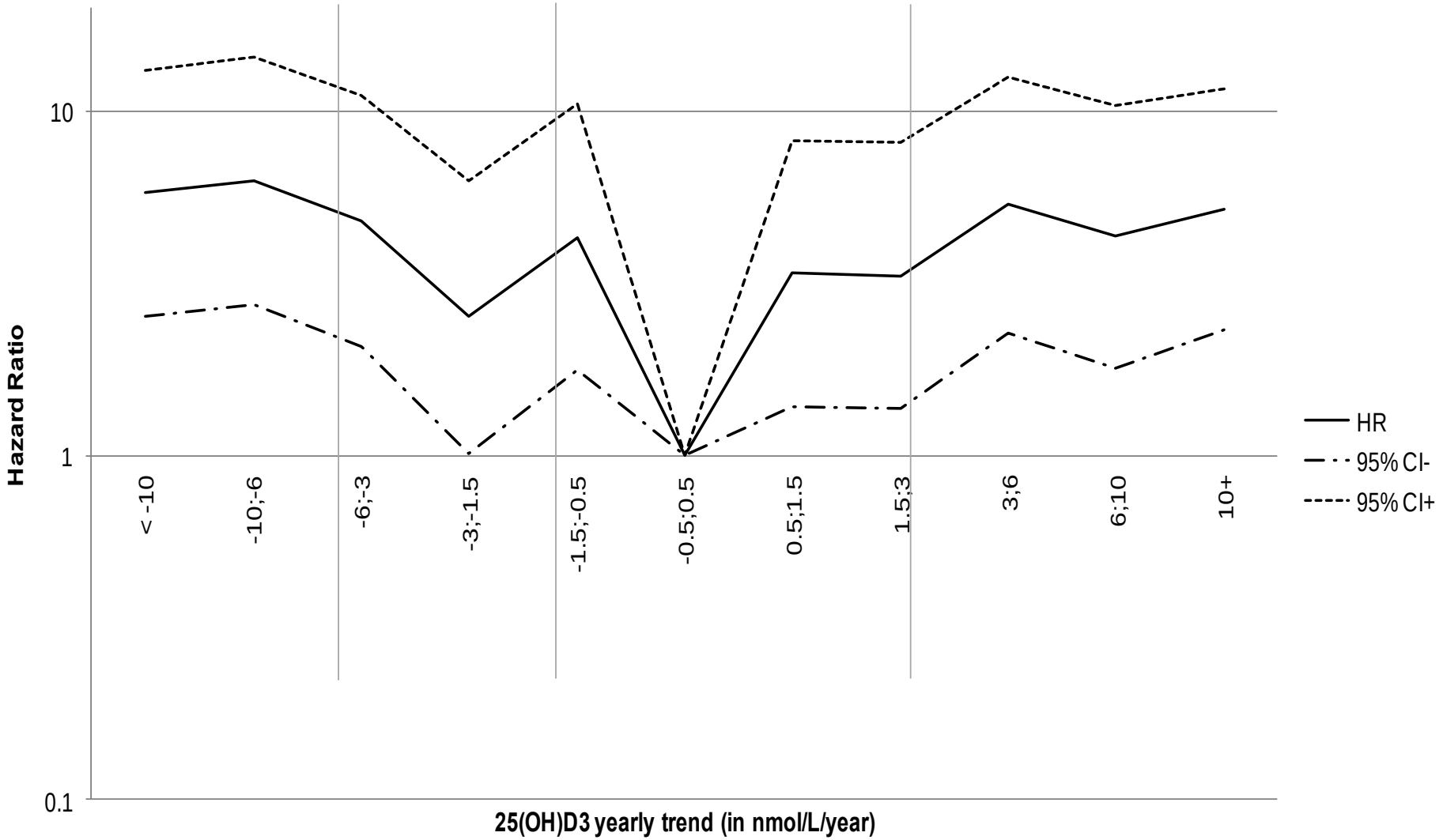

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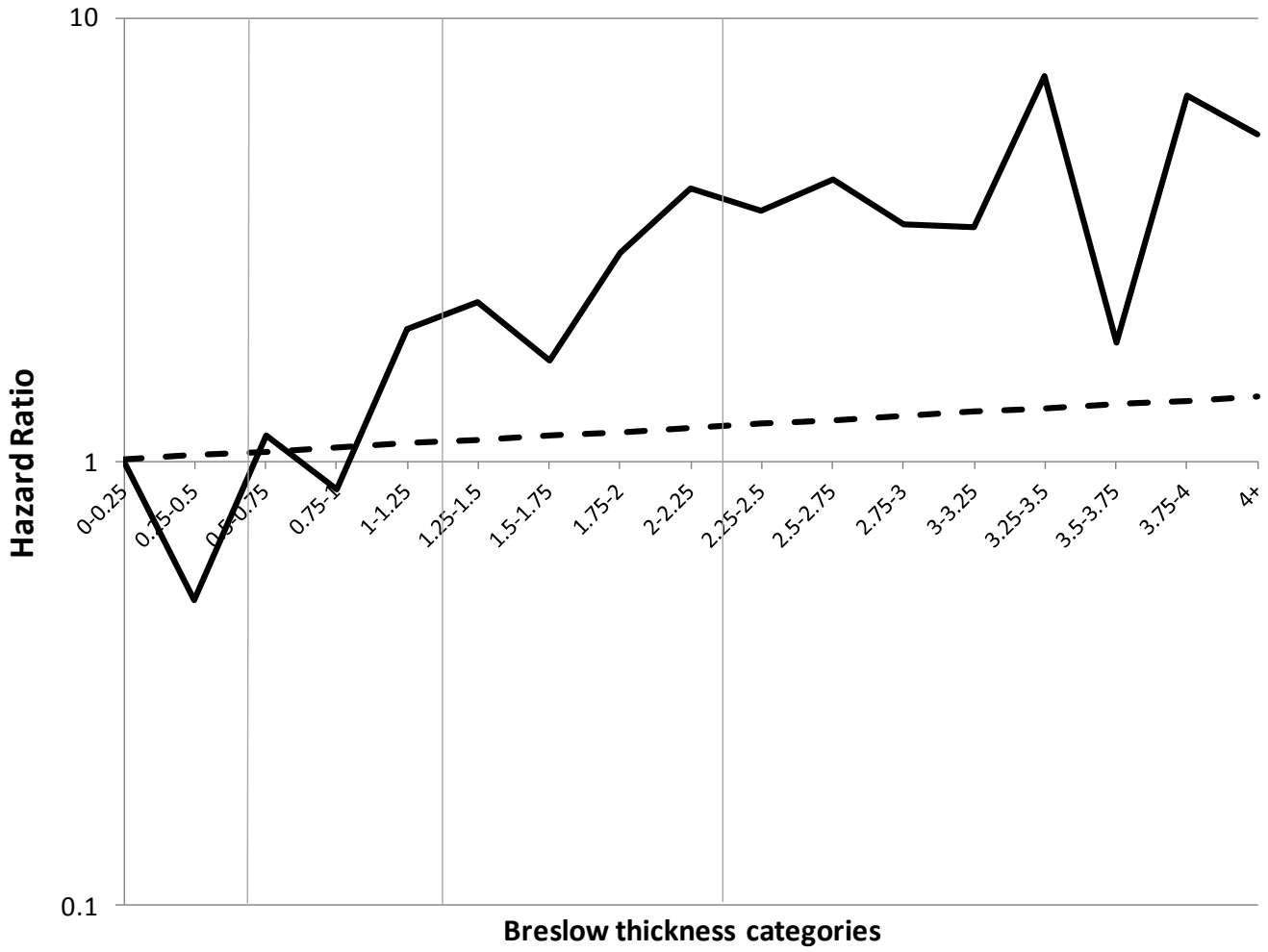

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