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Time-course pattern of blood 25-hydroxycholecalciferol is a significant predictor of survival outcome in metastatic colorectal cancer: a clinical practice-based study

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Vitamin D deficiency has been implicated in the epidemiology of common malignancies including colorectal cancer. We studied consecutive blood levels of 25-hydroxycholecalciferol (25-OHD) in relation to other clinical and laboratory variables in metastatic colorectal cancer patients to ascertain whether their variations may be prognostic or predictive parameters of survival outcomes. Eighty four patients treated with first-line oxaliplatin-based chemotherapy with or without bevacizumab were included. The patients were enrolled on the intent-to-treat basis considering their performance status, comorbidities and laboratory parameters to be medically apt for intensive chemotherapy. Overall survival and progression-free survival were selected as the primary outcomes. Progression free survival and overall survival medians were 15.4 months and 41.2 months, respectively. The cut-off levels of 40 nmol/l for 25-OHD and 11 μg/l for first CEA were identified to be clinical decision levels stratifying patients to the respective prognostic groups. We found that the most consistent outcome predictors were i) any patient surgery, ii) CEA and, independently, iii) time-related blood levels of 25-OHD. We confirmed fundamental and consistent vitamin D deficiency in metastatic colorectal cancer. We demonstrated that all patients with at least one blood level above 40 nmol/l versus all below this cut-off showed profound differences in their disease outcomes. The primary disease stage or time to metastatic stage did not influence the predictive power of blood 25-OHD levels, implying that the time-course pattern of 25-OHD but not the first single measurement may be an independent prognostic factor.

Key words: colorectal cancer, vitamin D, outcome predictors

Vitamin D has been implicated in the epidemiology of common malignancies including colorectal cancer [1-5]. Several papers reported substantial deficiency of vitamin D in breast and colorectal cancer patients and therapeutic potential of vitamin D supplementation in cancer has been postulated [6-9] reflecting its pleiotropic biological properties [10, 11]. Blood levels of vitamin D display vast variations depending on the geographical locality, food habits and way of life [12, 13]. Reports on colorectal cancer published to date have usually investigated single-sampled levels of 25-hydroxyvitamin D at diagnosis. These studies were mostly inconclusive in demonstrating associations with disease outcomes, presumably due to wide variability of 25-hydroxyvitamin D blood levels [1, 14].

We studied blood 25-hydroxyvitamin D levels and other clinical and laboratory variables in metastatic colorectal cancer patients treated at our institution hypothesizing that consecutive 25-OHD blood level patterns may be more informative than single sampling-based observation as they may better neutralize variations due to common and objective reasons such as seasonal variations. Our specific aims were to find out whether blood 25-OHD levels demonstrate deficiency of vitamin D and how consistent such deficiency may be in our patient population and second, whether blood 25-OHD level time-course patterns are associated with outcome measure in metastatic colorectal cancer.

Patients and methods

The study was designed as prospective and non-interventional. Our aim was to obtain data from cohort that was homogenous both from the clinical and laboratory viewpoint (Table 1). Inclusion criteria were as follows: i/ metastatic colorectal cancer treated with first-line oxaliplatin-based chemotherapy with or without bevacizumab, and ii/ eligible for intent-to-treat for approach considering their performance status, comorbidities and laboratory parameters so as to be medically apt for intensive chemotherapy with an anti-VEGF component. Exclusion criteria were: i/ PS 3 or ii/ clinical condition not compatible with intensive chemo-biotherapy. We enrolled consecutively 84 patients from May 2010 to November 2013. The cohort was not extracted from any other patient set grouped for other purposes such as another clinical trial. Median follow up was 24.2 months. All clinical and laboratory data were obtained from the hospital

Table 1. Basic description of patient cohort according to primary metastatic cancer

Characteristics		Total (N=84)	Primary metastatic disease No (N=29)	Primary metastatic disease Yes (N=55)	p[1]
Season – diagnosis	winter	N=22 (26.2%)	N=10 (34.5%)	N=12 (21.8%)	0.604
	spring	N=23 (27.4%)	N=8 (27.6%)	N=15 (27.3%)	
	summer	N=17 (20.2%)	N=5 (17.2%)	N=12 (21.8%)	
	autumn	N=22 (26.2%)	N=6 (20.7%)	N=16 (29.1%)	
Season – relapse	winter	N=10 (34.5%)	N=10 (34.5%)	-	
	spring	N=7 (24.1%)	N=7 (24.1%)	-	
	summer	N=6 (20.7%)	N=6 (20.7%)	-	
	autumn	N=6 (20.7%)	N=6 (20.7%)	-	
Time to relapse – months		13.0 (2.0; 128.9)	13.0 (2.0; 128.9)	-	
Age		62.0 (39.0; 74.0)	62.0 (29.0; 75.0)	62.0 (39.0; 74.0)	0.598
Age	≤ 65	N=57 (67.9%)	N=17 (58.6%)	N=40 (72.7%)	0.192
	> 65	N=27 (32.1%)	N=12 (41.4%)	N=15 (27.3%)	
Gender	F	N=35 (41.7%)	N=13 (44.8%)	N=22 (40.0%)	0.670
	M	N=49 (58.3%)	N=16 (55.2%)	N=33 (60.0%)	
BMI		26.0 (19.4; 34.8)	27.3 (17.3; 35.8)	25.7 (20.2; 33.8)	0.389
BMI categories	≤20	N=6 (7.1%)	N=4 (13.8%)	N=2 (3.6%)	0.105
	21-25	N=32 (38.1%)	N=7 (24.1%)	N=25 (45.5%)	
	26-30	N=24 (28.6%)	N=8 (27.6%)	N=16 (29.1%)	
	>30	N=22 (26.2%)	N=10 (34.5%)	N=12 (21.8%)	
Stage	1	N=1 (1.2%)	N=1 (3.4%)	N=0 (0.0%)	-
	2	N=6 (7.1%)	N=6 (20.7%)	N=0 (0.0%)	
	3	N=21 (25.0%)	N=21 (72.4%)	N=0 (0.0%)	
	4	N=55 (65.5%)	N=0 (0.0%)	N=55 (100.0%)	
	x	N=1 (1.2%)	N=1 (3.4%)	N=0 (0.0%)	
KRAS	mut	N=30 (35.7%)	N=10 (34.5%)	N=20 (36.4%)	0.947
	wt	N=41 (48.8%)	N=14 (48.3%)	N=27 (49.1%)	
	unknown	N=13 (15.5%)	N=5 (17.2%)	N=8 (14.5%)	
Grade	1	N=11 (15.9%)	N=7 (29.2%)	N=4 (8.9%)	0.142
	2	N=45 (65.2%)	N=14 (58.3%)	N=31 (68.9%)	
	3	N=12 (17.4%)	N=3 (12.5%)	N=9 (20.0%)	
	4	N=1 (1.4%)	N=0 (0.0%)	N=1 (2.2%)	
Diagnosis	C18.0-C18.4	N=14 (16.7%)	N=2 (6.9%)	N=12 (21.8%)	0.108
	C18.5-C18.7	N=21 (25.0%)	N=10 (34.5%)	N=11 (20.0%)	
	C18.8-C20	N=49 (58.3%)	N=17 (58.6%)	N=32 (58.2%)	
PS	0	N=15 (17.9%)	N=5 (17.2%)	N=10 (18.2%)	0.524
	1	N=59 (70.2%)	N=22 (75.9%)	N=37 (67.3%)	
	2	N=8 (9.5%)	N=2 (6.9%)	N=6 (10.9%)	
	3	N=2 (2.4%)	N=0 (0.0%)	N=2 (3.6%)	

Table 1. Basic description of patient cohort according to primary metastatic cancer (continued)

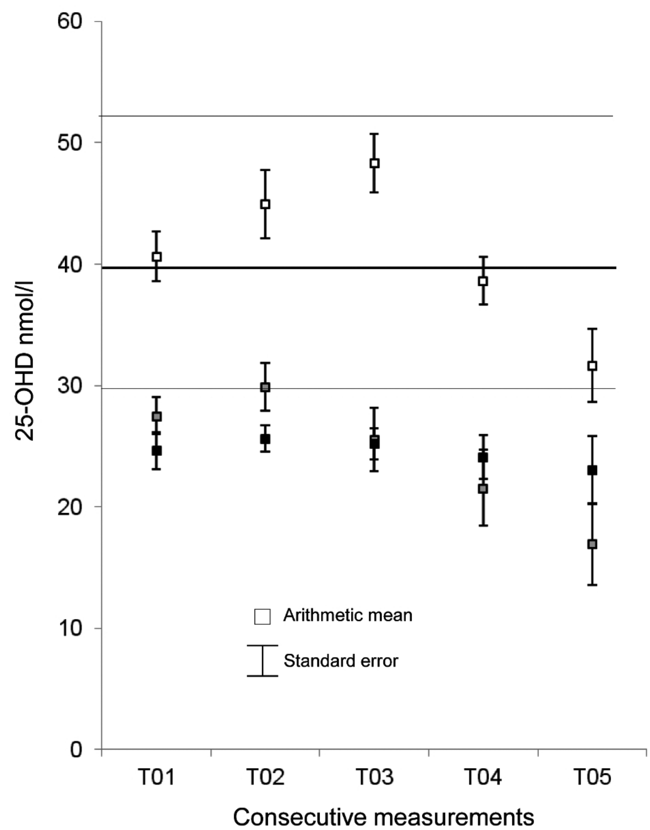
Characteristics		Total (N=84)	Primary metastatic disease No (N=29)	Yes (N=55)	p[1]
MT – number	1	N=43 (51.2%)	N=17 (58.6%)	N=26 (47.3%)	0.792
	2	N=25 (29.8%)	N=7 (24.1%)	N=18 (32.7%)	
	3	N=13 (15.5%)	N=4 (13.8%)	N=9 (16.4%)	
	4	N=3 (3.6%)	N=1 (3.4%)	N=2 (3.6%)	
Loc. – liver	no	N=25 (29.8%)	N=18 (62.1%)	N=7 (12.7%)	**<0.001**
	yes	N=59 (70.2%)	N=11 (37.9%)	N=48 (87.3%)	
Loc. – peritoneum	no	N=50 (59.5%)	N=14 (48.3%)	N=36 (65.5%)	0.129
	yes	N=34 (40.5%)	N=15 (51.7%)	N=19 (34.5%)	
Loc. – lungs	no	N=72 (85.7%)	N=25 (86.2%)	N=47 (85.5%)	0.925
	yes	N=12 (14.3%)	N=4 (13.8%)	N=8 (14.5%)	
Loc. – other	no	N=52 (61.9%)	N=15 (51.7%)	N=37 (67.3%)	0.165
	yes	N=32 (38.1%)	N=14 (48.3%)	N=18 (32.7%)	
Any surgical procedure	no	N=43 (51.2%)	N=16 (55.2%)	N=27 (49.1%)	0.596
	yes	N=41 (48.8%)	N=13 (44.8%)	N=28 (50.9%)	
Radical resection	no	N=68 (81.0%)	N=22 (75.9%)	N=46 (83.6%)	0.394
	yes	N=16 (19.0%)	N=7 (24.1%)	N=9 (16.4%)	
CT type	Length of CT[2]				0.647
bev+FOLFOX	10.0 (9.3; 10.9)	N=7 (8.3%)	N=2 (6.9%)	N=5 (9.1%)	
bev+XELOX	9.0 (5.5; 16.3)	N=49 (58.3%)	N=15 (51.7%)	N=34 (61.8%)	
bFOL	8.2 (2.5; 9.1)	N=3 (3.6%)	N=1 (3.4%)	N=2 (3.6%)	
FOLFOX	7.9 (5.5; 10.7)	N=8 (9.5%)	N=2 (6.9%)	N=6 (10.9%)	
XELOX	6.7 (5.9; 10.4)	N=12 (14.3%)	N=6 (20.7%)	N=6 (10.9%)	
other	5.9 (3.1; 7.8)	N=5 (6.0%)	N=3 (10.3%)	N=2 (3.6%)	
Time to therapy cessation		8.5 (3.1; 23.9)	7.8 (3.0; 16.3)	8.8 (3.1; 23.9)	0.255
Reason for therapy cessation	remission	N=9 (12.2%)	N=0 (0.0%)	N=9 (18.8%)	**0.001**
	patient decision	N=8 (10.8%)	N=5 (19.2%)	N=3 (6.3%)	
	progression	N=46 (62.2%)	N=13 (50.0%)	N=33 (68.8%)	
	other	N=11 (14.9%)	N=8 (30.8%)	N=3 (6.3%)	
Best response	CR	N=16 (19.0%)	N=3 (10.3%)	N=13 (23.6%)	0.091
	PD	N=5 (6.0%)	N=4 (13.8%)	N=1 (1.8%)	
	PR	N=25 (29.8%)	N=8 (27.6%)	N=17 (30.9%)	
	SD	N=38 (45.2%)	N=14 (48.3%)	N=24 (43.6%)	
First clinically accessible levels[3]:	25-OHD	30.4 (17.7; 57.3)	25.9 (10.9; 57.3)	31.5 (18.6; 58.1)	0.102
	Ca	2.3 (2.1; 2.6)	2.3 (2.1; 2.6)	2.3 (2.2; 2.6)	0.399
	CEA	6.8 (0.9; 892.7)	4.9 (0.9; 701.6)	9.2 (0.6; 1 242.0)	0.556
	Hb	126 (98; 150)	125 (98; 150)	130 (97; 149)	0.501
	Albumin	41.0 (35.0; 46.0)	43.0 (38.0; 49.0)	41.0 (34.0; 46.0)	0.056
	eGFR	1.5 (0.9; 1.9)	1.5 (0.7; 2.0)	1.6 (1.0; 1.9)	0.332
	Cholesterol total	5.0 (2.7; 7.7)	4.7 (2.3; 6.8)	5.1 (3.6; 8.6)	0.086
	LDL cholesterol	3.0 (1.3; 4.6)	2.8 (0.8; 4.6)	3.0 (1.7; 5.6)	0.393
	HDL cholesterol	1.1 (0.5; 1.7)	1.1 (0.4; 1.9)	1.2 (0.6; 1.7)	0.724
	Triacylglycerols	1.6 (0.8; 3.9)	1.6 (0.6; 3.2)	1.7 (0.8; 4.2)	0.335
25-OHD time-course patterns	NORMAL:	N=40 (47.6%)	N=14 (48.3%)	N=26 (47.3%)	0.984
	LOW-fluctuating	N=14 (16.7%)	N=5 (17.2%)	N=9 (16.4%)	
	LOW-stable	N=30 (35.7%)	N=10 (34.5%)	N=20 (36.4%)	

Legend: [1] Mann-Whitney U test for continuous variables; maximum likelihood chi-square test for categorical variables; Units of "first clinically accessible" blood levels of analytes: plasma 25-OHD (25-hydroxyvitamin D) = nmol/l, serum CEA = ug/l, blood Hb = g/l, plasma albumin = g/l, plasma creatinine-based eGFR = ml/s, plasma calcium, cholesterols and triacylglycerols = mmol/l; note – Li-heparin plasma and additive-free serum were used for biochemical analyses. [2] The chemotherapy regimen were as follows: FOLFOX (oxaliplatin 85 mg/m^2 IV day1; leucovorin 200 mg/m^2 IV days 1 and 2; 5-FU bolus 400 mg/m^2 IV days 1 and 2; 5-FU 600 mg/m^2 IV 22-hour continuous infusion days 1 and 2 every 2 weeks) or XELOX (oxaliplatin 130 mg/m^2 IV day 1; capecitabine 1000 mg/m^2 twice daily PO for 14 days every 3 weeks). bFOL (oxaliplatin 85 mg/m^2 IV days1,15; leucovorin 20 mg/m^2 IV and 5-FU bolus 500 mg/m^2 IV days 1,8,15 every 4 weeks. Bevacizumab was administered at a dosage of 5 mg/kg IV every 2 weeks or 7.5 mg/kg IV every 3 weeks depending on the chemotherapy regimen. [3] Median (inter-quartile range). 25-OHD time-course patterns contains two types NORMAL and LOW, the LOW being presented here with its subtypes showing subtle but insignificant variations.

information system besides blood levels of 25-OHD, calcium, albumin, total cholesterol, LDL-measured and HDL cholesterol that were additionally determined in tumour marker serum specimen biobanked aliquots [15] using standard clinical laboratory methods (25-OHD – Abbott Architect, all other – Roche Cobas Integra). Estimated glomerular filtration rates were calculated using CKDepi equations [16] based on Li-heparin plasma creatinine enzyme determination. The reference ranges of 25-OHD were derived in-house from a set of healthy individuals from South Moravian region. Complete blood cell count was determined using Sysmex XE5000 instrument. Patients were monitored using common clinical, laboratory and imaging methods and were not provided with any special procedure such as a clinical trial, special counselling services and/or dietary or vitamin supplements.

Statistical methodology

Standard descriptive statistics were applied in the analysis; absolute and relative frequencies for categorical variables and median estimates supplied with 5th-95th percentile range or mean and standard error for continuous variables. Statistical significance of differences between groups of patients was tested using maximum likelihood chi-square test for categorical variables and Mann-Whitney U test for continuous variables. Correlations between continuous variables were quantified and tested by means of Spearman rank correlation (robust non-parametric method) and Pearson correlation coefficients (parametric product-limit correlation). Progression-free survival and overall survival were taken as principal outcome endpoints of the study. Both the time-to-event endpoints were calculated since the onset of therapy of metastatic CRC. Time-to-event data sets were visualized using standard Kaplan-Meier methodology. Comprehensive set of statistics was derived from time series formed by biochemical parameters consecutively measured during therapy. We defined the "first clinically accessible levels" as those measured in the specimen for first CEA determination and denoted T01 in the subsequent time series as described in the Fig 1. Subsequent time series then provided estimates of min-max values, their ranges, relative median and maximum changes in time (related to the first clinically accessible level), integrated area under time-related concentration curve, and absolute changes per given time interval. All time-related statistics were subsequently examined as potential predictors of outcome endpoints. Quantitative biochemical indicators (both first accessible values and time-series statistics) as potential predictors of survival endpoints were separately examined to define informative cut-off points. Effective cut-off values were optimized on the basis of Receiver Operating Characteristics curves [17]. The computation was based on binormal assumption. Standard measures of sensitivity and specificity were estimated and supported by 95% confidence intervals for each analyzed indicator and associated cut-off points [18]. The final set of potential prognostic factors and interaction terms coded as binary variables according to the cut-off points was subjected to Cox proportional hazards regression model and the prognostic power was quantified by hazard ratios and corresponding 95% confidence intervals. Both univariate and multivariate regression models were applied, the latter using forward stepwise selection algorithm driven by maximum likelihood ratio test. An "alfa" value =



Number of samples in given time	T01	T02	T03	T04	T05
□ At least 1 25-OHD value 40 or above	40	40	32	24	8
▩ All 25-OHD < 40 - fluctuating	14	14	12	9	6
■ All 25-OHD < 40 - stable	30	30	15	11	7

Time between biochemical sample and start of chemotherapy

Time between start of the therapy[1] and „first clinically accessible value T01"	N (%)	Cumulative N (%)
0 months	39 (46.5%)	39 (46.4%)
0 - 2 month	29 (34.5%)	68 (80.9%)
> 2 months	16 (19.0%)	84 (100.0%)

Figure 1. Time-course typology patterns of 25-OHD blood levels during therapy of metastatic CRC
Legend: [1] absolute difference between date of biochemical sample and start of chemotherapy

0.05 was used as limit of statistical significance in all performed analyses. Analyses were computed using SPSS 22 [19].

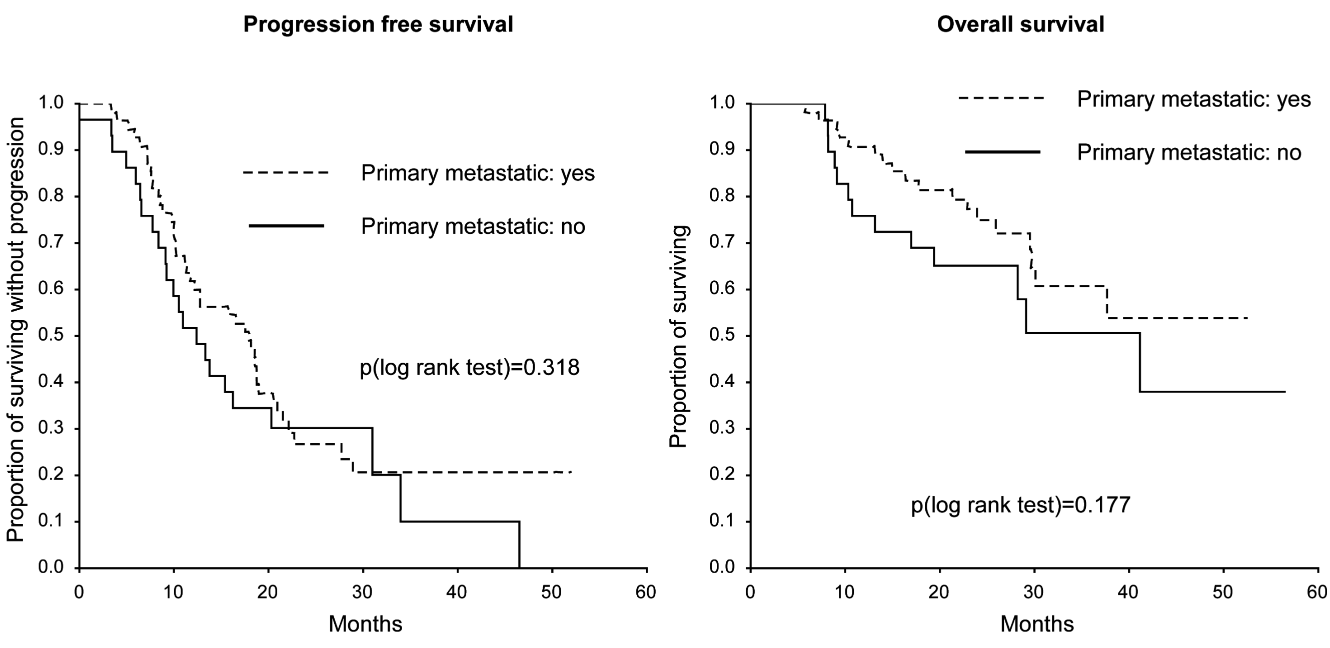
Results

Baseline overall characteristics of patients are presented in the Table 1. The median age was 62 years (range 39-74) with slight prevalence of males (58.3 %). The majority of patients were of performance status ECOG 0-1 (88.1 %). Synchronous primary and metastatic disease was seen in 65.4 % of patients. We found no statistically significant differences in survival outcomes between primary metastatic and nonmetastatic disease (Fig. 2). Medians of progression free survival (PFS) and overall survival (OS) were 15.4 months for PFS and 41.2 months for OS, respectively. Predictors of PFS and OS not statistically significant are listed in the Legend to the Table 2. The cut-off levels of 40 nmol/l for 25-OHD and 11 ug/l for first CEA derived from the ROC analyses proved to be clinical decision levels for stratifying patients to the respective prognostic groups. Time-related blood levels of 25-OHD segregated in two typology patterns denoted in relation to the reference ranges as being NORMAL or LOW (Fig. 3) and along with "first CEA" and "patient surgery" consisting of either any surgical procedure or radical resection of metastases showed significant associations with disease outcomes (Table 2).

Discussion

In this study we confirmed fundamental vitamin D deficiency [20] in our set of metastatic colorectal cancer patients. As expected, our results showed the critical effect of surgery on OS and the strong predictive and prognostic power of CEA.

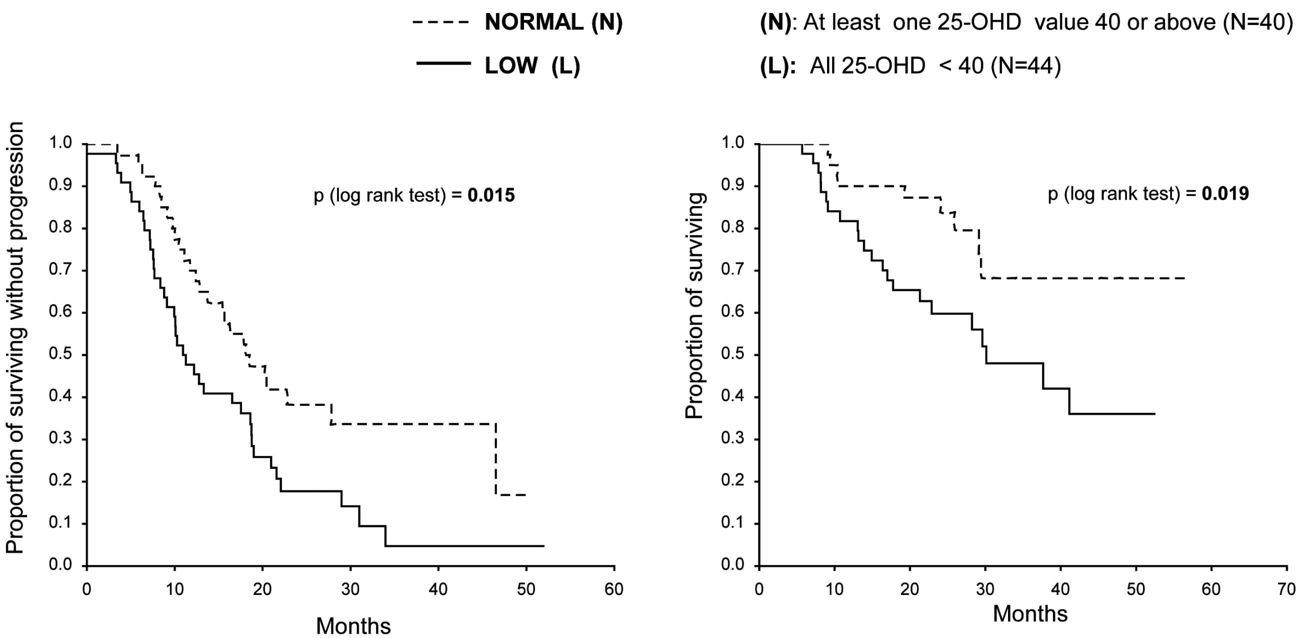
The overall host/disease responses to vitamin D and related compounds appear to be very complex involving host immunity modulation and VDR-dependent differentiating properties at the disease level as well. Brenner et al published a meta-analysis of prospective cohort studies in breast and CRC patients, concluding that 25-OHD levels higher than 75 nmol/l were associated with reduced mortality [21]. Very recently, Ng and coworkers presented data indicating possible associations of "postdiagnostic" blood levels of vitamin D with disease outcomes in a set of North American patients [22]. The published reports analyzed mostly single-sampled "prediagnostic or postdiagnostic" levels of 25-OHD and were



Time	Primary metastatic: no No at risk	Surviving (95% CI)	Primary metastatic: yes No at risk	Surviving (95% CI)
0	29	1.000	55	1.000
12	15	0.517 (0.335; 0.699)	34	0.618 (0.490; 0.747)
24	5	0.302 (0.131; 0.472)	11	0.267 (0.144; 0.389)
36	1	0.101 (0.007; 0.271)	2	0.207 (0.087; 0.327)
48	0	0.000	2	0.207 (0.087; 0.327)

Time	Primary metastatic: no No at risk	Surviving (95% CI)	Primary metastatic: yes No at risk	Surviving (95% CI)
0	29	1.000	55	1.000
12	22	0.759 (0.603; 0.914)	49	0.909 (0.832; 0.985)
24	11	0.651 (0.476; 0.826)	31	0.749 (0.629; 0.868)
36	4	0.507 (0.283; 0.730)	9	0.606 (0.445; 0.766)
48	1	0.380 (0.107; 0.652)	4	0.539 (0.349; 0.728)

Figure 2. Survival of patients stratified according to primary diagnosis of CRC
Legend: No statistically significant difference in survival outcomes between primary metastatic and nonmetastatic disease was observed.



Time No at risk	0	12	24	36	48
N	40	28	11	2	1
L	44	21	5	1	1

Time No at risk	0	12	24	36	48
N	40	36	24	5	3
L	44	35	18	8	2

Figure 3. Survival of patients according to time-course typology patterns of 25-OHD blood levels (nmol/l)
Legend: Blood levels of 25-OHD showing typology patterns classified against the local winter (i.e. lower) reference ranges as being NORMAL or LOW; the LOW group showed subtle variations as "All 25-OHD < 40 – fluctuating" (meaning any blood level elevation relative to the previous sampling i.e. due to improved conditions because of response to treatment), and "All 25-OHD < 40 – stable" (meaning profound, consistently low levels)

Table 2. Potential predictors of PFS and OS in Cox proportional hazard regression model

Endpoint: progression free survival		**Univariate estimates**		**Multivariate-adjusted estimates**	
		HR (95% CI)	**p**	**HR (95% CI)**	**p**
Grade	≥ 2	2.449 (1.077; 5.567)	0.033		
N	N1+N2	2.159 (1.081; 4.698)	0.045		
25-hydroxyvitamin D (25-OHD), nmol/l	25-OHD (max) < 40	1.881 (1.131; 3.129)	0.014		
	25-OHD (max) < 50	2.055 (1.131; 3.739)	0.018		
All 25-OHD in time series < 50		2.013 (1.124; 3.605)	0.019		
All 25-OHD in time series < 40		1.841 (1.116; 3.038)	0.017		
Typology of 25-OHD time series (binary code)	All values <40 (LOW)	1.637 (1.053; 2.544)	0.031	1.699 (1.016; 2.845)	**0.043**
CEA ug/l	CEA (first) > 11	2.226 (1.355; 3.657)	0.002	2.060 (1.247; 3.402)	**0.004**
	CEA (max) > 11	2.345 (1.141; 3.897)	0.001		
Endpoint: overall survival					
Any surgical procedure		0.239 (0.098; 0.583)	0.002	0.240 (0.097; 0.594)	**0.002**
Radical resection of metastases		0.101 (0.014; 0.747)	0.025		
25-hydroxy D, in nmol/l	25-OHD (max) < 40	2.238 (1.029; 4.868)	0.042		
25-OHD (max) < 50		3.178 (1.107; 9.068)	0.032		
All 25-OHD in time series < 50		3.344 (1.168; 9.576)	0.024		
All 25-OHD in time series < 40		2.466 (1.133; 5.371)	0.023		
Typology of 25-OHD time series (binary code)	All values <40 LOW,	2.710 (1.333; 5.510)	0.006	2.220 (1.074; 4.592)	**0.031**
CEA ug/l	First CEA > 11	4.610 (2.115; 10.051)	<0.001	4.862 (2.196; 10.762)	**<0.001**

Table 2 Potential predictors of PFS and OS in Cox proportional hazard regression model

Predictors of PFS not statistically significant: season – diagnosis and relapse, age over 65 yrs, male gender, BMI, stage 3+4, KRAS, T, M1, diagnosis, type of disease (disseminated relapse or primary metastatic), PS, MT – number, localisation (liver, peritoneum, lungs, other), any surgical procedure, radical metastases resection, chemotherapy type, levels of calcium, haemoglobin, albumin, eGFR, cholesterol total, LDL cholesterol, HDL cholesterol, triacylglycerols.

Predictors of OS not statistically significant: season – diagnosis and relapse, age over 65 yrs, male gender, BMI, stage 3+4, KRAS, grade, T, N, M1, diagnosis, type of disease (disseminated relapse or primary metastatic), MT – number, localisation (liver, peritoneum, lungs, other), chemotherapy type, levels of calcium, haemoglobin, albumin, eGFR, cholesterol total, LDL cholesterol, HDL cholesterol, triacylglycerols.

unable to show consistent associations with PFS and OS [14, 23, 24]. In fact, their results may be well in accordance with our "first clinically accessible levels" that did not show either enough predictive or prognostic power in our statistical models – by definition, our "first clinically accessible value" is either "prediagnostic or early postdiagnostic". Our reason for this design was that repetitive sampling may neutralize seasonal variation, better reflect the patient´s premorbid period and interim clinical status and provide time course of blood concentration data. Furthermore, such a design is an essential component for the planned interventional clinical trial on supplementing vitamin D to personalize vitamin D dosing to the desired target levels.

To make the analysis feasible in this limited size cohort, we had to set forth some initial assumptions. Considering daily clinical practice, one finds it rather unfeasible to obtain consistent "true initial" concentrations. To overcome this, we instead defined "the 25-OHD first clinically accessible value" linked to the first determination of CEA constituting the T_{01} observation that became then consistent among the patients with acceptable variability (Fig. 1). The most informative outcome predictors were patient surgery, CEA and, independently, time-related blood levels of 25-OHD demonstrating that all patients with at least one level above 40 nmol/l and all below behaved differently when associated towards disease outcome. Neither the primary stage of the disease nor the time to metastatic stage interacted with variations and final predictive power of 25-OHD, implying that the time-course pattern of 25-OHD is a true independent prognostic factor of survival outcome. Nevertheless, our data should be interpreted with caution because of the limited cohort size even though our patient set was internally consistent, reflected clinical daily practice well and was free from apparent selection bias.

Our results imply that cholecalciferol may be proposed as an adjuvant component of treatment protocols in colorectal cancer irrespective of mechanisms leading to its deficiency. We suggest that the phenomenon of vitamin D deficiency may carry a therapeutic potential that, when possibly corrected, may enhance vitamin D-dependent biomodulation of host anticancer response.

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