Serum vitamin D levels in patients with lung metastases

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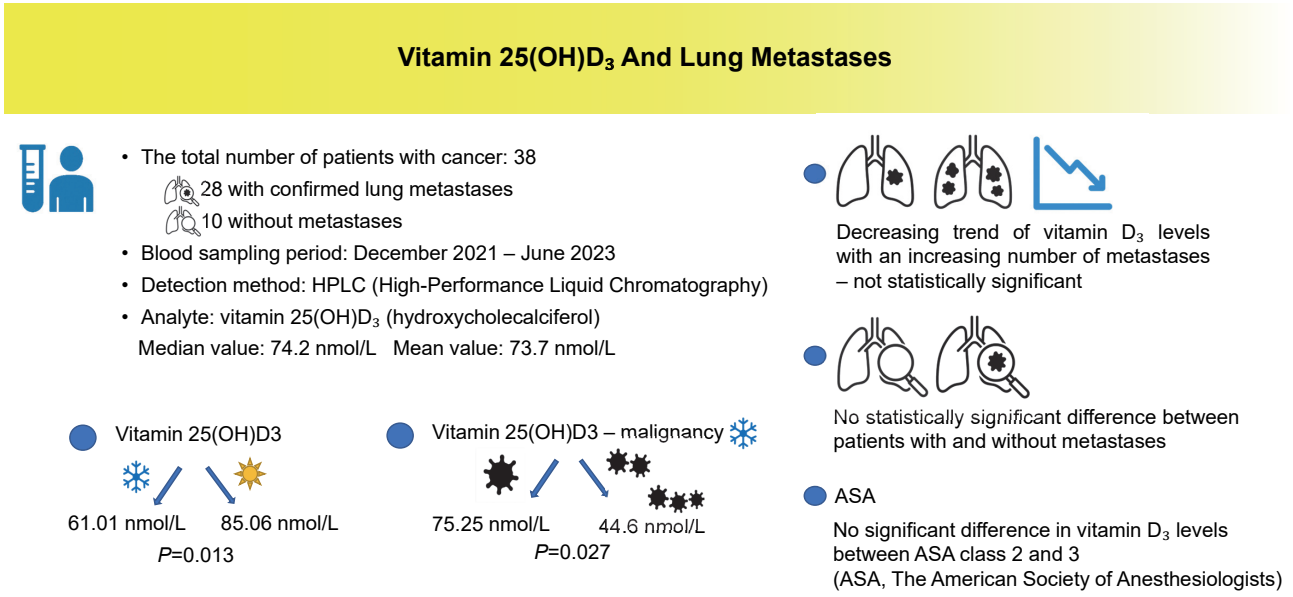
Background and Aim. Vitamin D deficiency is linked to increased cancer risk and death but the effect of vitamin D substitution on the prognosis of patients with malignant disease is debatable. We aimed to investigate the value of serum vitamin D3 levels in patients with a history of malignancy and confirmed lung metastases.
Materials and Method. Serum Vitamin D2 (25-hydroxyergokalciferol) and D3 (25-hydroxycholekalciferol) levels were measured in 38 patients (28 with and 10 without lung metastases) using high performance liquid chromatography (HPLC). Serum Vitamin D2 + D3 levels of patients with lung metastases were analysed with respect to season, number of metastases, number of malignancies in the history, value according to the ASA (The American Society of Anaesthesiologists) physical status classification, and compared to patients with a history of malignancy without confirmed lung metastasis.
Results. Overall, mean serum vitamin D3 levels were significantly higher in summer (summer vs. winter; 85.06 nmol/L vs. 61.01 nmol/L; *P*=0.013). There was no significant difference in vitamin D3 levels in summer or winter between patients with or without lung metastases. There was also no significant difference in vitamin D3 levels in the summer months between patients with a history of one malignancy versus those with two or more. In winter however, patients with a history of one malignancy had significantly higher vitamin D3 levels (mean, 75.25 nmol/L) than those with two or more malignancies (mean 44.6 nmol/L) (*P*=0.027). The differences between vitamin D3 levels in patients with ASA 2 and 3 were not statistically significant.
Conclusion. Vitamin D supplementation may be advisable for patients with a history of multiple malignancies, particularly during the winter months. However, confirmation in a clinical trial with a larger patient cohort is warranted before firm recommendations can be made.



Vitamin D supplementation may be advisable for patients with a history of multiple malignancies, particularly during the winter months. However, confirmation in a clinical trial with a larger patient cohort is warranted before firm recommendations can be made.
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INTRODUCTION

Vitamin D plays important extra skeletal roles, including immunomodulatory and antitumor effects. At supraphysiological doses, it exhibits antiproliferative and differentiating properties, inhibits angiogenesis, induces apoptosis, arrests the cell cycle, and enhances cell adhesion and intercellular communication[1-3]. The metabolism of vitamin D is quite complex with many different active molecules. Detailed description however is beyond the scope of this article. 25-hydroxyvitamin D is used as a biomarker of vitamin D (ref.[1,4]).

Active vitamin D ($1,25(OH)_2D$) exerts its effects via nuclear receptors, primarily in the intestine, bones, and kidneys. Its genomic action involves binding of the $1,25(OH)_2D$ and the activated vitamin D receptor/retinoid X receptor (VDR/RXR) complex to specific DNA sequences[5]. These receptors contain hormone- and DNA-binding domains and, upon forming a complex with RXR, regulate transcription of ~200 genes by binding to DNA (ref.[6-8]). The vitamin D receptor (VDR) has over 470 polymorphisms, with Bsm I (BB, Bb, bb) being most relevant to cancer[9]. The bb genotype, present in ~35% of the U.S. population, is linked to lower $1,25(OH)_2D$ levels and higher risks of prostate cancer in men and breast cancer metastases in women[2]. Furthermore, the Taq I TT genotype of the VDR is associated with a fivefold increased risk of advanced prostate cancer in men and a twofold higher risk of lymph node metastases in women with breast cancer[2,10]. In animal models, vitamin D inhibits metastatic growth of lung cancer cells and may function as an intrinsic metastasis-suppressing factor[11]. Vitamin D deficiency is linked to poor cancer outcomes in humans, while its supplementation or analogs reduce tumor progression and metastasis also in animal models[12]. $1,25(OH)_2D$ exerts anti-invasive, anti-migratory, and anti-proliferative effects on osteosarcoma cells *in vitro* by modulating c-Myc and FOXO1 expression. These findings suggest that effective doses of $1,25(OH)_2D$ may reduce the aggressiveness of osteosarcoma cells[13].

Supplementation with 2000 IU of vitamin D per day in infants is associated with a reduced risk of type I diabetes mellitus[14]. Deficiency through parathyroid hormone increases insulin resistance[15,16]. Vitamin D regulates hematopoiesis and thus immunocompetent cells[17]. Furthermore, deficiency activates the renin-angiotensin-aldosterone pathway and predisposes to hypertension and left ventricular hypertrophy[15,16]. Toll-like receptor activation in macrophages upregulates the vitamin D receptor and 1α-hydroxylase gene, inducing the antimicrobial peptide cathelicidin and promoting killing of intracellular Mycobacterium tuberculosis[18]; vitamin D supplementation also provides protection against respiratory viral infections, including COVID-19, and may improve outcomes in septic and critically ill patients[19]. Vitamin D increases VDR expression, reducing intestinal fibroblast proliferation and fibrosis in Crohn's disease[20]. 1α-hydroxylase and vitamin D receptors (VDR) are present in most tissues, including tumors; thus, vitamin D is believed to affect tumor recurrence by inhibiting proliferation, angiogenesis, metastasis, and by inducing apoptosis and differentiation[4]. That said, the effect of vitamin D substitution on the prognosis of patients with malignant disease is a debatable issue as the results of studies are inconsistent. In this work aimed to investigate the serum vitamin D3 levels in patients with a history of malignancy and confirmed lung metastases.

MATERIALS AND METHODS

The study included patients without Vitamin D substitution with a history of malignant disease admitted for elective lung resection to the First Department of Surgery, St. Anne's University Hospital and the Faculty of Medicine of Masaryk University in Brno for a lesion in the lung parenchyma suspected of metastasis of the primary malignant disease. Patients who agreed to participate in the study were included. Initially, a total of 42 patients were enrolled, three of whom were excluded due to detected vitamin D substitution. One patient with chronic kidney disease on dialysis with secondary hyperparathyroidism was also excluded. The blood draws were performed between December 2021 and June 2023. After admission, patients had peripheral venous blood drawn into a tube for routine biochemical testing and then sent to the Department of Clinical Biochemistry for further processing using high performance liquid chromatography (HPLC). After receipt of the primary blood sample with the request for testing, the sample was processed using a pre-analytical line and divided into aliquots for further determination. For the determination of vitamin 25(OH)D2 and 25(OH)D3, 100 μL of the processed blood sample was required. All samples were processed once a week, otherwise they remained frozen at −20 °C. After the sample was centrifuged, 100 μL was pipetted into the prepared ependorfs and 250 μL of internal standard was added to precipitate interfering organic matter. The mixed preparation was then mixed on a shaker for at least 20 s and stored in a refrigerator at 4 °C for 10 min. This was followed by centrifugation for 5 min at 15 000 rpm. After centrifugation, the prepared eluate (ap-

proximately 250 µL) was pipetted into the described glass vials, which were then placed in a liquid chromatograph to determine the level of vitamin 25(OH)D3 or vitamin 25(OH)D2, if the patient was taking it. Vitamin 25(OH)D2 was not included in the processing of the results, all patients had unmeasurable levels of vitamin 25(OH)D2 because of its plant origin and is measurable in the blood of individuals on vitamin D replacement therapy who were not included in the study, so that the results refer only to 25(OH)D3 (hydroxycholecalciferol). In the following, 'vitamin D' and/or 'vitamin D3' always means 25(OH)cholecalciferol. In our laboratory, the physiological range of vitamin 25(OH)D3 is determined to be between 50–175 nmol/L.

Basic summary statistics as number, means, median, upper and lower quartiles, standard deviation, non-deviating minimum, non-deviating maximum are shown as a "box plot" (see Fig. 2. – Fig. 6).

The TIBCO Statistica 14.0.0 program was used to carry out the statistical tests.

RESULTS

The study included patients without Vitamin D substitution with a history of malignant disease admitted for elective lung resection as mentioned for a lesion in the lung parenchyma suspected of being metastasis of the primary malignant disease. Initially, a total of 42 patients were enrolled, of whom three were excluded due to the detected vitamin D substitution and one with chronic kidney disease on dialysis with secondary hyperparathyroidism. The blood draws were performed between December 2021 and June 2023. The cohort consisted of 21 women and 17 men, aged from 19 to 74 years, mean 58.15 years (Table 1). Of these, 19 patients had one malignancy, 14 had two malignancies and 5 patients had three malignancies either before surgery or subsequently if a primary lung tumor was found in the resection. Regarding the histological outcome of the lung resections, sarcoma was found in seven cases (Ewing's sarcoma, myxoid chondrosarcoma, myxofibrosarcoma, osteosarcoma, conventional chondrosarcoma) and colorectal adenocarcinoma in six, 3 cases of mammary carcinoma, 2 cases of clear cell renal cell carcinoma (ccRCC), one case of metastasis of malignant melanoma and one case of metastasis of adenoid cystic thyroid carcinoma. Two patients were found to have grade 1 neuroendocrine tumor as a primary and five cases were newly diagnosed with non-small cell lung carcinoma (NSCLC). Two patients had lung cancer metastasis in the resection, one of these patients had metastatic NSCLC and new NSCLC of different histological type. Ten cases were without malignancy in resection (2 cases of chondrohamartoma, 3 cases of granulomatous noncaseating inflammation, 2 cases of suspected tuberculosis, 2 cases without malignancy, 1 case of fibrosis, hyalinization, and granulomatous giant cell type reaction around foreign bodies, which could be a focus of nonspecific reparative changes, but also a completely regressed metastatic lesion after adjuvant hormonal therapy and radiotherapy).

Table 1. Baseline characteristics of the study population.

Sex	Age (years)	Result
17 males	Min. 19	Malignant 28
21 females	Max. 74	Benign 10
	Average 58.15	

Subsequent analysis revealed that three patients were included in the study twice, as they underwent repeat pulmonary metastasectomy. The first patient had a serum value of 96.2 nmol/L in December, which decreased to 81.7 nmol/L prior to the second surgery in April. The second patient presented with a value of 89.1 nmol/L in August, declining to 28.6 nmol/L before their second operation in February. The third patient exhibited a serum concentration of 120.2 nmol/L in June, which had decreased to 90.3 nmol/L prior to their second surgery in September. A consistent reduction in serum values was observed before the second surgical intervention.

The median vitamin 25(OH)D3 of all patients in all seasons was 74.15 nmol/L, mean 73.67 nmol/L, maximum value 176.5 nmol/L, minimum value 16.6 nmol/L, and modus 71.2 nmol/L. 27 operations were performed by VATS approach, 2 of them converted to thoracotomy (lateral or vertical muscle sparing thoracotomy), 5 vertical muscle sparing thoracotomies, 3 lateral thoracotomies, 2 rethoracotomies, 1 wedge resection of the lung with part of the chest wall. 14 resections were performed after previous CT-guided marking of the lesion. In 35 cases wedge resection was performed, a total of 55 wedge resections were performed. One resection of part of the chest wall was also performed, one segmentectomy, three lobectomies, one pleural biopsy, and one separate lymph-

Table 2. Overview of metastatic origin, surgical approach, and type of resection.

Metastasis	Primary	Approach	Type of resection
7 sarcomas	2 neuroendocrine tumor	27 VATS[3]	55 wedge resections
6 colorectal	5 NSCLC[2]	2 conversions	1 segmentectomy
3 mammary		5 vertical muscle sparing thoracotomies	3 lobectomies
2 ccRCC[1]		3 lateral thoracotomies	1 pleural biopsy
1 melanoma		2 rethoracotomies	1 lymfadenectomy
1 adenoid cystic thyroid carcinoma		1 chest wall resection	1 chest wall resection
2 metastatic NSCLC[2]			

[1]ccRCC, Clear-cell renal-cell carcinoma; [2]NSCLC, Non-small cell lung cancer; [3]VATS, Video-Assisted Thoracoscopic Surgery.

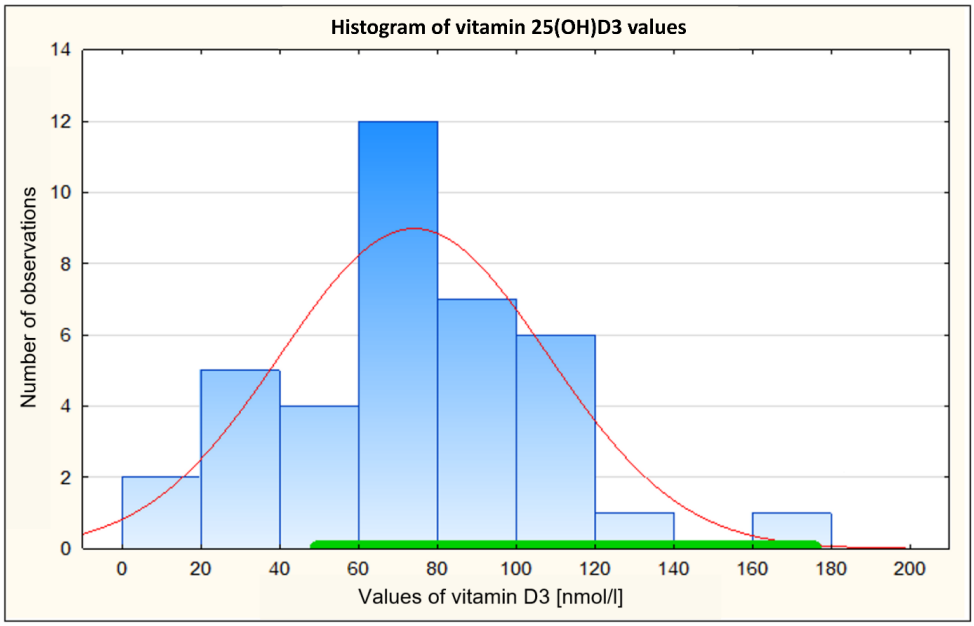

Fig. 1. Histogram of vitamin 25(OH)D3 values. The histogram (y-axis: number of patients; x-axis: vitamin 25(OH)D3 levels in nmol/L) with an overlaid Gaussian curve displays all measured values from patients with a history of malignancy admitted for lung resection due to suspected metastasis. The laboratory reference range (50–175 nmol/L) is marked in green. Among 38 patients, 27 had vitamin D levels within this range; one patient exceeded it (176.5 nmol/L), while the remaining patients fell below the minimum recommended level. The mean and median values were 73.7 nmol/L and 74.2 nmol/L, respectively, close to the lower limit. This indicates that vitamin D levels in this cohort are systematically lower than those recommended for healthy individuals.

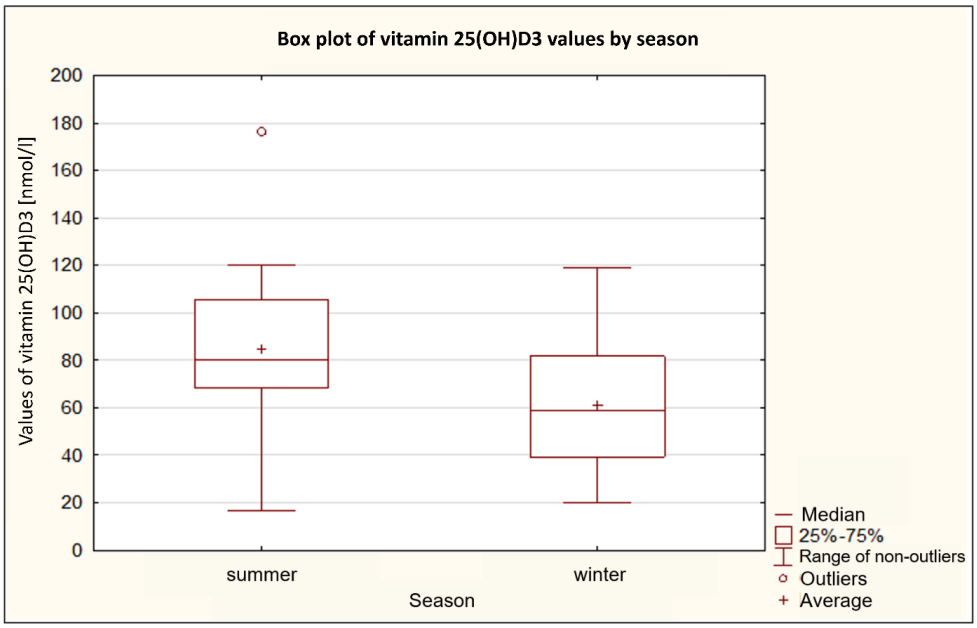

Fig. 2. Box blot of vitamin 25(OH)D3 values by season (*P*=0.013).

adenectomy without resection of the lung parenchyma. Malignancy was found in 46 specimens (Table 2).

Vitamin D3 levels according to season

The season was divided into winter (November–April) and summer (May–October), with data presented in Table 3 and as a box plot (Fig. 2). While the overall range of values was similar between seasons, the central tendency differed: summer median values ranged from 68.1 to 105.5 nmol/L (mean 85.06 nmol/L), whereas winter values ranged from 39.4 to 81.7 nmol/L (mean 61.01 nmol/L). The average winter value was less than 25% of the summer quartile, indicating higher vitamin D levels in summer. Statistical testing of the null hypothesis (equal means) versus the alternative (summer mean higher) yielded a *P*-value of 0.013, leading to rejection of the

Table 3. Vitamin 25(OH)D3 values by season in nmol/L.

	Patient count	Mean	Median	Lower quartile	Upper quartile	Standard deviation	Non-outlier minimum	Non-outlier maximum
Summer	20	85.06000	80.45000	68.10000	105.50000	34.60141	16.600000	120.20000
Winter	18	61.01111	58.85000	39.40000	81.70000	28.61030	19.900000	118.80000

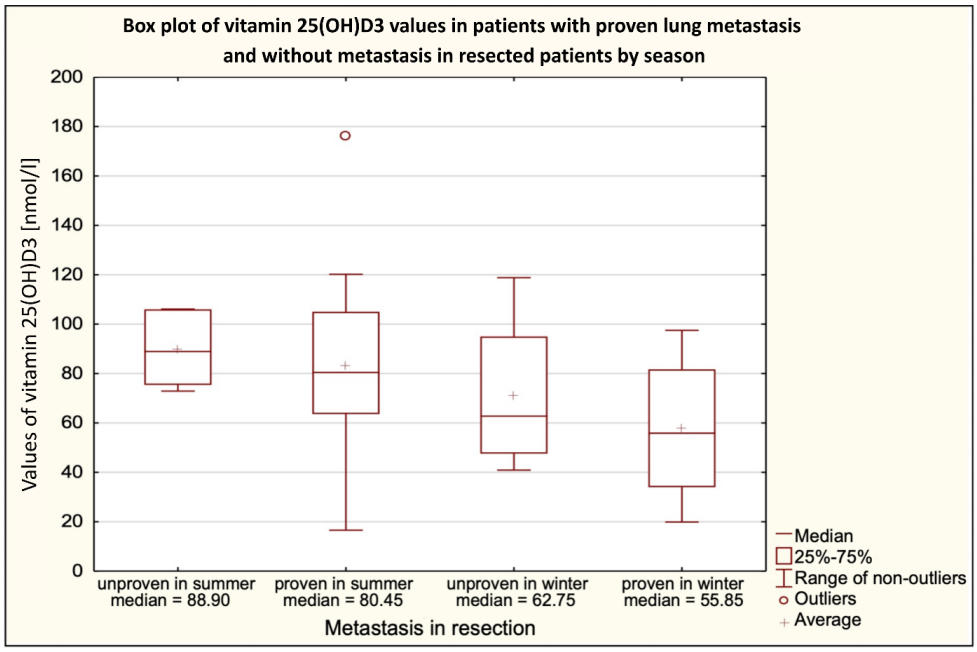

Fig. 3. Box plot of vitamin 25(OH)D3 values in patients with proven lung metastasis and without metastasis in resected patients by season.

null hypothesis at the 5% significance level. Thus, vitamin 25(OH)D3 levels were significantly higher in summer, consistent with vitamin D physiology.

Vitamin D3 levels according to the presence of pulmonary metastases:

Patients admitted for pulmonary lesions suspected of metastasis included 10 histologically confirmed metastasis-free cases (chondrohamartoma or inflammatory changes), considered as controls. We compared vitamin 25(OH)D3 levels between patients with and without confirmed metastatic lung disease. Although levels were higher in non-metastatic patients, the difference was not statistically significant. The null hypothesis (equal means) was tested against the alternative (higher means in non-metastatic patients), yielding a *P*-value of 0.151. Thus, at the 5% significance level, we do not reject the null hypothesis and conclude that vitamin 25(OH)D3 levels do not differ significantly between groups.

Data separated according to the presence of metastasis in the resection and season:

To account for seasonal influence, vitamin 25(OH)D3 levels were compared between patients with and without metastasis within summer and winter periods (Fig. 3). Although means and medians were consistently lower in metastatic patients across both seasons, differences were not statistically significant (*P*=0.216 for both summer and winter). A Wilcoxon test for medians in the summer group, which borderline violated normality, also showed no significant difference (*P*=0.328). Thus, at the 5% significance level, vitamin D3 levels appear similar between groups within each season.

However, the box plot suggests a downward trend, with the lowest vitamin D3 levels in metastatic patients during winter compared to non-metastatic patients in summer (mean difference 31.63 nmol/L). A larger sample size might reveal statistically significant differences.

Data broken down by ASA of patients:

Patients' preoperative physical status, assessed by the ASA (The American Society of Anaesthesiologists) classification, was compared with vitamin 25(OH)D3 levels. With a *P*-value of 0.849, the null hypothesis was not rejected, indicating no significant difference in 25(OH)D3 levels between ASA 2 and ASA 3 groups at the 5% significance level. Due to seasonal variation in sampling, further analysis was stratified by season.

Data broken down by patient ASA and season:

Box plots (Fig. 4) reveal an unexpected trend, with higher mean vitamin 25(OH)D3 levels in ASA 3 patients compared to ASA 2 in both summer and winter. Although the summer median is lower in ASA 3 than ASA 2, the difference is not significant; in winter, the ASA 3 median is higher. Thus, seasonal stratification likely does not affect the results. At the 5% significance level, vitamin 25(OH)D3 levels are assumed identical between ASA 2

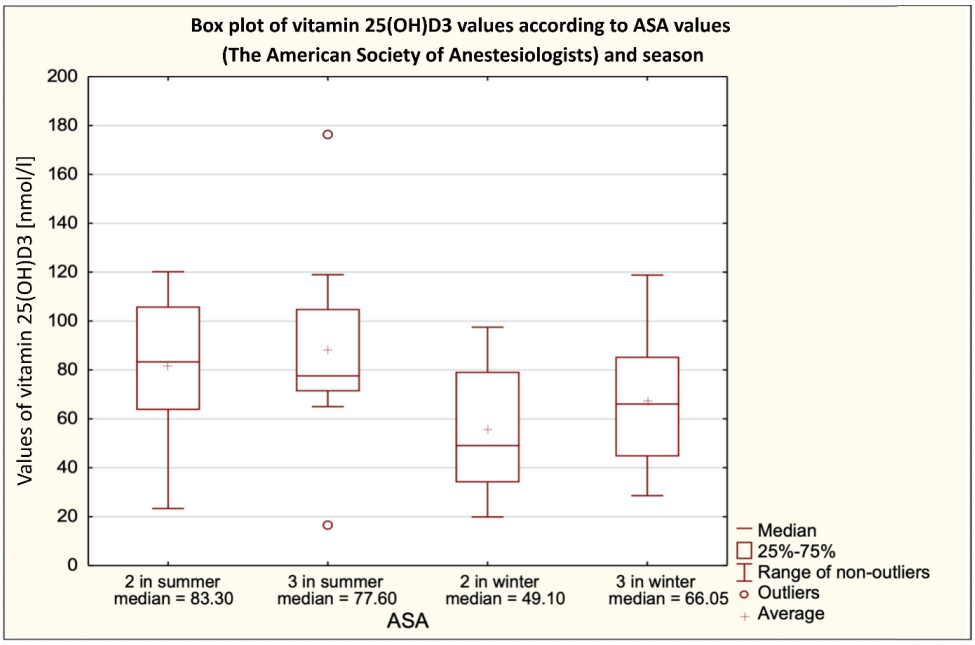

Fig. 4. Box plot of vitamin 25(OH)D3 values according to ASA values and season.

and ASA 3 groups. However, small sample sizes may limit the findings, and larger cohorts could yield statistically significant differences.

Data broken down by number of malignancies in history and season:

Patients were grouped by number of malignancies: Group 1 included patients with a single malignancy (11 in summer, 8 in winter), while Group 2 included those with multiple malignancies (19 total; 9 in summer, 10 in winter), including newly diagnosed NSCLC cases without lung metastasis. The analysis was stratified by season, as presented in Table 4.

In summer (Fig. 5), Group 2 showed higher mean and median vitamin 25(OH)D3 levels than Group 1, contrary to expectations, but differences were not significant. Quartile ranges were nearly identical. In winter, results aligned with expectations, with Group 2 having lower mean, median, and quartile ranges.

Testing the null hypothesis of equal means yielded a *P*-value of 0.585 for summer (no significant difference) and 0.027 for winter, where the null was rejected. Thus, at the 5% significance level, Group 1 had significantly higher vitamin 25(OH)D3 levels than Group 2 during winter.

Data broken down by number of metastases:

Patients were grouped by number of metastases: 10 patients with benign findings, 18 patients with up to 2 metastases (Group 1 and 2), and 10 patients with 3 or more metastases, including disseminated disease. Box plots (Fig. 6) show a gradual decrease in mean vitamin 25(OH)D3 levels with increasing metastases, while medians remain relatively stable. Quartile ranges and spread of values also decrease with metastasis number.

The null hypothesis of equal means across groups was tested against the alternative that at least one group differs. With a *P*-value of 0.280, the null hypothesis was not rejected at the 5% significance level. Thus, the observed downward trend was not statistically significant. Increasing sample sizes could potentially reveal significant differences in an ANOVA test.

DISCUSSION

In relation to pleiotropic effects, a concentration above 75 nmol/L is recommended[21]. Vitamin D deficiency is a global world problem[21] and affects 30–50% of the population[15] and nearly 72% of cancer patients[22]. In our study, we found that 50% of the most typical values ranged from 68.1 to 105.5 nmol/L with a mean of 85.06 nmol/L

Table 4. Vitamin 25(OH)D3 values according to the number of malignancies in the anamnesis and season in mmol/L.

	Patient count	Mean	Median	Lower quartile	Upper quartile	Standard deviation	Non-outlier minimum	Non-outlier maximum
1 in summer	11	83.50000	77.20000	71.20000	106.10000	28.10512	23.30000	120.20000
2 in summer	9	86.96667	83.30000	65.00000	105.00000	42.99625	16.60000	106.00000
1 in winter	8	75.31250	75.25000	56.30000	93.30000	27.62904	34.00000	118.80000
2 in winter	10	49.57000	44.60000	28.60000	63.40000	25.00347	19.90000	96.20000

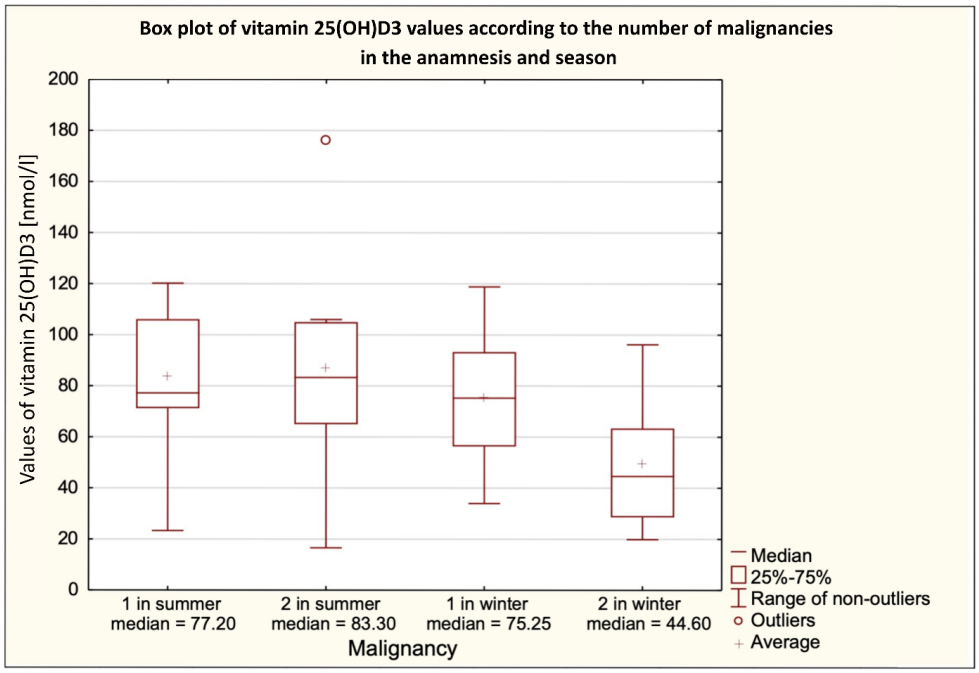

Fig. 5. Box plot of vitamin 25(OH)D3 values according to the number of malignancies in the anamnesis and season (P=0.585 for summer, P=0.027 for winter).

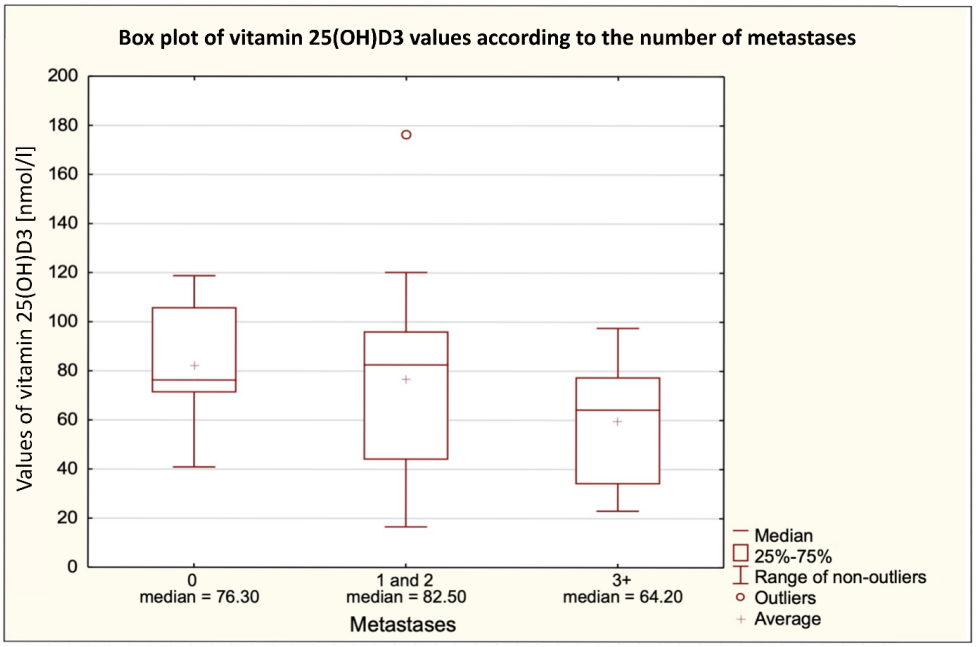

Fig. 6. Box plot of vitamin 25(OH)D3 values according to the number of metastases.

in summer and from 39.4 to 81.7 nmol/L with a mean of 61.01 nmol/L in winter. This shows that the average value in winter does not even reach 25% of the quartile of the summer period. Of the 38 patients, 19 had deficits below 75 nmol/L and of these, 10 patients had severe deficits below 50 nmol/L.

According to the literature, vitamin D3 statistically significantly reduced cancer mortality in one study[23], and its use is associated with a 17% reduction in the risk of advanced cancer[22]. Another study reported that vitamin D supplementation was associated with reduced overall mortality and mortality from differentiated thyroid cancer[24]. Increasing calcium and vitamin D3 intake to 1100 IU per day substantially reduces the risk of cancer in postmenopausal women[25]. Other authors have reported that supplementation of 1100 IU per day was insufficient to correct vitamin D deficiency in patients with nonspecific intestinal inflammation; also, patient compliance was low[26]. Akiba et al. conducted a randomized, double-blind, placebo-controlled trial of vitamin D supplementation (1200 IU/day for 12 months) in patients with non-small cell lung cancer (NSCLC). No survival difference was observed in the overall cohort, but in early-stage NSCLC patients with 25(OH)D levels <50 nmol/L, supplementation

significantly improved 5-year survival. Polymorphisms of the vitamin D-binding protein, affecting affinity for calcidiol and calcitriol, also influenced survival outcomes[4].

Low vitamin D levels corresponded with a higher prevalence of polymorbidity, especially in patients with vitamin D levels < 25 nmol/L, where each 1 standard deviation decrease in vitamin D levels was associated with an 8% increase in morbidity score[27]. Vitamin D deficiency prolongs intensive care unit stay, increases morbidity and mortality, and is associated with chronic diseases[8]. We were unable to demonstrate this in our study because patients with ASA 3 had the same vitamin D levels as those with ASA 2. However, it can be assumed that the results are to some extent influenced by the relatively small frequencies in each cohort and that there may not be a significant difference in the comorbidities of patients classified into these two grades. Also, this sorting may be partly subjective, or patients without internal comorbidities may have been sorted into ASA 3 due to active cancer disease.

The ability of vitamin D to regulate immune processes depends on whether immune cells express VDR (VDR positive cell line) or not (VDR negative cell line). The ratio between these cell lines is determined by factors, including vitamin D levels, which regulate this ratio. Understanding these processes would be crucial for the design of treatments targeting vitamin D and thus the immune system[17]. As mentioned above, it has been reported that up to 50% of the population is vitamin D deficient, due to poor food fortification and the misconception that a healthy diet contains enough of this vitamin[28]. There is a study that looked at 25-hydroxyvitamin D levels in anaesthetic department staff in hospitals in Iceland and Wisconsin in late winter. The authors of the study found no difference in mean serum vitamin D levels between Iceland (70.53 nmol/L, standard deviation 30.87 nmol/L) and Wisconsin (70.0 nmol/L, standard deviation 30.0 nmol/L). In Iceland and Wisconsin, vitamin D levels below 25 nmol/L were observed in 4.7% and 4.0%, below 50 nmol/L in 34.9% and 25.0%, and below 75 nmol/L in 56.6% and 61.3% of participants[29].

The action of vitamin D in the body is broad and its positive effect is demonstrated by a number of studies. No uniform recommendations on optimal dosage have yet been established[30]. Most studies report better outcomes in chronic, communicable and non-communicable diseases with higher serum 25-hydroxyvitamin D concentrations. Therefore, a number of organizations have established recommendations regarding supplementation and serum concentrations. When targeting bone, a serum concentration of 50 nmol/L and supplementation depending on age of 400–800 IU per day is recommended. For pleiotropic effects, a concentration of 75 nmol/L is recommended[31] with vitamin D intake of at least 1000 IU per day[32], optimal serum concentration 90-100 nmol/L (ref.[33]) or 75–125 nmol/L (ref.[21]) and supplementation with 400–2000 IU(ref.[31]), or 1500 IU(ref.[34]) per day, taking into account age, weight, comorbidities and ethnicity. Other authors recommend a daily dose of vitamin D in the general population of 600 IU/day(ref.[35]). The mean of the values in patients in winter months in our study was 61.01 nmol/L.

Patients with a history of one malignancy had mean values of 83.5 nmol/L in summer, median 77.2 nmol/L, and 75.31 nmol/L and 75.25 nmol/L in winter. Patients with two or more malignancies in the anamnesis had mean values of 86.97 nmol/L in summer, median 83.3 nmol/L, and 49.57 nmol/L and 44.6 nmol/L in winter. Furthermore, patients without metastasis in the lung resection had an average of 89.7 nmol/L in summer, 71.3 nmol/L in winter, a median of 88.9 nmol/L in summer and 62.75 nmol/L in winter. On the contrary, patients with proven metastasis had an average value of 83.07 nmol/L with a median of 80.45 nmol/L in summer, 58.07 nmol/L and 55.85 nmol/L in winter. Patients without metastases in the resection had average vitamin D values of 82.34 nmol/L, median 82.34 nmol/L, with one or two metastases had an average of 76.77 nmol/L, median 82.5 nmol/L. In patients with three or more metastases, the mean values were 59.41 nmol/L and the median was 64.2 nmol/L.

Levels below 75 nmol/L are considered inadequate and conversely, concentrations above 375 nmol/L are potentially toxic[2,8,21]. Another toxic concentration of 25(OH) D is reported to be 750 nmol/L and the recommended upper limit is 250 nmol/L to provide a sufficient safety range[36].

The distribution of vitamin D and its metabolites between the bloodstream and adipose tissue may contribute to lower serum concentrations and may explain, at least in part, the reduced efficacy of supplementation in patients with higher body mass index (BMI) (ref.[37]). Another study evaluated the incidence of advanced malignant disease with possible modification of the effect of supplementation by body mass index (BMI). A significant reduction in the incidence of advanced cancer was found in persons with normal BMI, but not in overweight or obese persons[38]. Adverse effects such as hypercalcaemia and hypercalciuria are rare and associated with prolonged administration of extremely high doses of vitamin D[21]. Vitamin D3 substitution combined with calcium increased the incidence of nephrolithiasis in the study[23]. Because of the risk of hypercalcaemia, vitamin D3 boluses should not be administered for prolonged periods but daily supplementation is recommended[39], however, use of higher doses for 8–12 weeks is not associated with the occurrence of serum 25(OH)D levels potentially at risk for hypercalcaemia[40]. Oral supplementation for vitamin D deficiency is the first choice; if serum vitamin D levels do not rise sufficiently, intramuscular injection with cholecalciferol is indicated[30].

In the VITamin D and OmegA-3 TriaL (VITAL) study, daily supplementation with 2000 IU of vitamin D3 and 1 g of marine omega-3 fatty acids was investigated for the primary prevention of cancer and cardiovascular disease. Vitamin D did not significantly reduce the overall incidence of invasive cancers but showed signs of reducing overall cancer mortality, with other meta-analyses and studies showing similar results[41]. However, it must be taken into account that epigenetic differences are assumed and

each individual responds to supplementation in a different way, with low reactivity in people with higher susceptibility to different types of disease and, conversely, high reactivity may reflect high immune system resistance[39]. However, the daily dose of vitamin D3 should not exceed 4000 IU (ref.[39]) to avoid overdose in persons with a high response to vitamin D. The most appropriate supplementation may be 40 IU/kg body weight with respect to obese individuals[39]. Patients with severe hepatic and renal dysfunction require the use of active metabolites of vitamin D, namely calcidiol for hepatic disease, alfacalcidiol for renal disease or 1,25-hydroxyvitamin D3 (calcitriol) (ref.[21]).

Different cycles of cholecalciferol administration of 10 000 IU daily for 8 weeks followed by 1000 IU daily for 4 weeks, versus 50 000 IU weekly for 12 weeks or 100 000 IU every other week for 12 weeks, were also investigated. After the first month, 93% had serum 25(OH) D levels ≥75 nmol/L, and after two months 100%, thus all three regimens allow rapid 25(OH)D replacement. Daily administration is more effective than weekly and biweekly administration, and higher 25(OH)D levels are maintained longer with more frequent administration. The bolus regimen results in greater production of inactive $24,25(OH)_2D$ than the daily regimen[40]. Monthly administration of 50 000 IU of vitamin D3 rapidly normalises 25(OH)D3 in deficient subjects, while daily administration of the same cumulative dose is similarly effective but takes two weeks longer to achieve the desired level of 50 nmol/L (ref.[42]). Other work has reported that the increase in serum 25(OH)D concentrations after 28 days was comparable after administration of 100 000 IU intramuscularly and after oral administration with increasing doses of 2000 IU daily for 4 weeks, followed by 4000 IU daily for the next 4 weeks and 8000 IU daily in the last 4 weeks. With a baseline mean serum level of 40 nmol/L, after 4 weeks of oral administration according to the scheme above it was 83.4 nmol/L, after a further month 127.4 nmol/L and at the end it reached 159.7 nmol/L (ref.[30]). There is a tendency to plateau at concentrations of 75–100 nmol/L with a subsequent decrease in the effectiveness of supplementation, which is a protective mechanism against overdose. Higher and more frequent doses are needed to overcome this plateau, with a rapid decrease in serum concentration after the transition from 10 000 IU/day to 1000 IU/day (ref.[40]). Oral supplementation with cholecalciferol at a dose of 2000–4000 IU per day from January to March is sufficient to achieve adequate serum 25(OH)D in healthy adults. Alternatively, an injection of 100 000 IU i.m. can be used without side effects in individuals with, for example, impaired gastrointestinal resorption or limited compliance[30]. Tolerable upper limits of vitamin D doses that do not pose a health risk are given for children up to 1 month up to 1000 IU/day, from 1 month to 10 years of age up to 2000 IU/day, from 11 to 18 years of age up to 4000 IU/day and for adults and elderly up to 4000 IU/day or up to 10 000 IU/day (ref.[21]).

As our study showed, there are statistically significant differences in serum vitamin D concentrations in summer and winter months, which is due to vitamin D physiology. The amount of vitamin D synthesized in the skin is influenced by latitude, with vitamin D not being formed in winter at high latitudes (>40 degrees) due to sunlight, and the levels being 10–100% lower in winter than in summer[34]. In the UK, exposure to midday sunlight three times a week for 13 min in summer is sufficient for the Caucasian race to cover 35% of the skin surface[43]. But 70-year-old person has a 75% reduction in vitamin D3 production in the skin[44]. The minimum erythema dose (MED) is the lowest dose of radiation needed to cause visible erythema. The average MED value for the Czech population in the UVB region ranges from 15–70 mJ/cm^2 (ref.[45]). For example, 1 MED in Oslo represents approximately 30 min of solar radiation (20 mJ/cm^2), and such radiation on the entire skin surface is equivalent to an oral intake of 10 000–20 000 IU of vitamin D, which is equivalent to 200–375 mL of cod liver oil[34]. Going to the solarium also increases vitamin D production. A study was conducted in which volunteers with skin types I and II went to the solarium twice a week, achieving a 0.5–1 MED for four weeks during the winter, and 25(OH)D3 levels increased by an average of 40%. Half subsequently took 200 IU of vitamin D daily. Within eight weeks of the last visit to the solarium, levels had fallen to baseline in all, including those who continued to take supplementation[46]. This suggests that a dose of 200 IU of vitamin D per day in winter is not sufficient to maintain summer levels. With more sun exposure over a lifetime, the likelihood of dying from cancer decreases, so vitamin D deficiency increases this risk. Adults with 25-hydroxyvitamin D levels <50 nmol/L who were followed for 19 years had a 30–50% higher risk of colorectal cancer, breast cancer, prostate cancer, and other cancers[44]. Excessive sun exposure does not cause vitamin D intoxication[47], on the other hand, the increased risk of skin malignancies due to overexposure to sunlight must of course be taken into account.

CONCLUSION

The study investigated 25(OH)D3 levels in cancer patients with metastatic lung disease. Overall, the mean level was 73.7 nmol/L – just above the minimal recommended threshold. A statistically significant seasonal difference emerged (P=0.013): summer levels averaged 85.06 nmol/L (IQR: 68.1–105.5 nmol/L), while winter levels dropped to 61.01 nmol/L (IQR: 39.4–81.7 nmol/L). Furthermore, although 25(OH)D3 levels were similar between patients with and without confirmed pulmonary metastases, winter medians remained below recommended values. In winter patients with multiple malignancies had significantly lower levels (median 44.6 nmol/L, P=0.027) compared to those with a single malignancy (median 75.25 nmol/L). Trends indicating decreasing 25(OH)D3 levels with more metastases did not reach statistical significance, likely due to the small sample sizes. The study is limited by the small cohort size and the multifactorial nature of vitamin D metabolism, which is influenced by seasonal variation, sun exposure, lifestyle, and comorbidities. The single-center design may further reduce the generalizability of the results. Additionally, regional factors such as

climate and lifestyle could have influenced the findings. Given these factors, it remains an open question whether vitamin D supplementation should be recommended for cancer patients, particularly during the winter months or in individuals with multiple malignancies, in whom significantly lower levels have been observed. Although various supplementation regimens have been investigated, further research is required to determine whether vitamin D supplementation has a beneficial impact on the prognosis of malignant diseases.

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