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Efficacy and safety of vitamin D supplementation in patients with chronic lymphocytic leukemia

Skuteczność i bezpieczeństwo suplementacji witaminy D u pacjentów z przewlekłą białaczką limfocytową

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A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
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Summary

Background: Vitamin D (VD) deficiency in chronic lymphocytic leukemia (CLL) is associated with inferior prognosis, shorter time to treatment and worse overall survival. VD deficiency is the first potentially modifiable prognostic factor in CLL. Currently, however, there is a lack of studies concerning VD supplementation in CLL patients.

Aim: To evaluate the efficacy and safety of VD supplementation in patients with CLL.

Methods: A 6-month interventional study was conducted in CLL patients with lower serum 25-OH-D_3 concentrations (< 30 ng/ml) than currently recommended. Patients with VD insufficiency (20-30 ng/ml) received 2000 IU of cholecalciferol/day, patients with moderate deficiency (10-19.9 ng/ml) received 4000 IU/day, and patients with severe VD deficiency (<10 ng/ml) received 6000 IU/day.

Results: In the analyzed group of 13 CLL subjects, only 1 patient had a VD level within the optimal range (30-80 ng/ml), 7 had an insufficient concentration, 4 had moderate deficiency, and 1 had severe deficiency. Secondary hyperparathyroidism was diagnosed in 4 subjects. Cholecalciferol supplementation (mean dose of 3384 ± 1211 IU) was followed by a significant increase in 25-OH-D_3 concentration (from 17.3 ± 5.8 to 41.4 ± 17.5 ng/ml; $p<0.05$) and decrease in PTH ($p<0.05$). Five patients did not achieve the recommended 25-OH-D_3 concentration. Calcium level remained unchanged and no patients developed hypercalcemia.

Conclusions: VD replenishment is safe and can be effectively achieved by means of the employed cholecalciferol dosage in the majority of patients. However, some subjects may require higher doses to obtain the optimal level and immune function.

Key words: chronic lymphocytic leukemia • vitamin D

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Introduction

Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in the western hemisphere, with continuously increasing incidence. Accumulation of monoclonal neoplastic B lymphocytes [45] in CLL results from the imbalance between proliferation and apoptosis of leukemic cells [7]. Diverse microenvironmental stimuli confer on these cells a growth advantage and extended survival [18].

Recent findings suggest a role of vitamin D (VD) as a paracrine immunomodulator. The majority of known biological actions of $1,25(OH)_2D_3$ are mediated through the VD receptor (VDR) [24]. VDR expression was found in immune cells, such as antigen-presenting cells (monocytes, macrophages, dendritic cells) and activated CD4+ and CD8+ T lymphocytes [51] [53]. Furthermore, the immune system has the capacity for regulating activity of 1-α-hydroxylase in macrophages and dendritic cells [15]. VD plays a role in maintaining the balance of Th1 and Th2 profiles. $1,25(OH)_2D_3$ appears to inhibit Th1 cells and, under certain conditions, may support a shift to the Th2 phenotype [42].

It is estimated that up to 1 billion people worldwide have VD deficiency or insufficiency [29]. In the general population VD deficiency is associated with cardiovascular [1] and autoimmune diseases [25], various types of cancer, infections and other diseases [21], as well as higher all-cause mortality [17]. A meta-analysis summarizing the findings from five prospective studies suggested an 11% reduction of overall cancer incidence for an increase of 25-OH-D_3 serum level by 20 ng/ml [55]. A different meta-analysis of randomized controlled studies showed that VD supplementation consistently reduced total cancer mortality but not cancer incidence [37]. Moreover, supplementation of intermittent, high dose VD may not be effective in preventing overall mortality [56].

The importance of VD in lymphoid malignancies epidemiology is being steadily revealed. Living in an area with higher ambient ultraviolet radiation, which would be anticipated to increase the VD levels, was found to be related to reduced risk of NHL (non-Hodgkin lymphoma), especially DLBCL (diffuse large B-cell lymphoma) and CLL [6]. High dietary intake of VD lowered the risk of NHL in African American women [14]. In an older population a higher lymphoma risk was observed in subjects with 25-OH-D_3 concentrations <12 ng/ml, with the dose–response relationship showing a tendency towards decreasing lymphoma risk with higher 25-OH-D_3 [46]. Higher concentrations of 25-OH-D_3 were found to be associated with a reduced risk of CLL [40].

A suboptimal level of 25-OH-D_3 is associated with an increased risk of lymphoma. Moreover, recent data also suggest that VD insufficiency is related to inferior prognosis in some hematologic malignancies. In a prospective cohort of 983 newly diagnosed patients with non-Hodgkin's lymphomas, 25-OH-D_3 insufficiency was associated with an inferior event-free survival and overall survival in patients with DLBCL and T-cell lymphoma [12]. Shanafelt et al. revealed inferior prognosis in CLL patients with VD insufficiency. In this prospective study VD status correlated with time to treatment (TTT) and overall survival (OS). 25-OH-D_3 insufficiency was an independent predictor of TTT [52]. The relationship between VD insufficiency and time to first treatment (TFT) was confirmed by Molica et al. in a group of 130 previously untreated Binet stage A CLL patients. More than 80% of the subjects were found to have VD deficiency. In this cohort values of 25-OH-D_3 below 13.5 ng/ml were associated with a shorter TFT [44]. Furthermore, Salah et al. also revealed that serum 25-OH-D_3 levels are an independent prognostic factor associated with survival in CLL patients [2]. These findings suggest that VD insufficiency may not only be a subsequent prognostic factor in CLL but the first potentially modifiable host factor. 25-OH-D_3 level could also affect effectiveness of treatment. The VD deficiency impairs rituximab-mediated cellular cytotoxicity, which is enhanced after the VD substitution [5]. However, there is no prospective study concerning the effect of VD supplementation in patients with CLL. In vitro, VD has been shown to inhibit proliferation and induce differentiation of lymphoma cell lines [28]. Additionally, a VD analog induced apoptosis via a p53-independent mechanism in CLL cells [48]. Moreover, Arlet et al. observed a remission in a VD-deficient patient with CLL, Binet stage A, after supplementation with VD. Apoptosis of cultured lymphocytes, derived from patients, was induced by VD, and the response was associated with VDR expression [3].

The lack of studies assessing VD supplementation in CLL patients encouraged us to evaluate the efficacy and safety of VD supplementation in patients with CLL.

Materials and methods

Patients and samples

Thirteen patients with untreated CLL in the previous year were enrolled between November 2013 and May 2015. The protocol was approved by the Local Bioethics Committee, and written informed consent was obtained from all the participants. Blood samples were collected after an overnight fast (minimum 8 h) during a routine visit. All laboratory tests (25-OH-D_3, total calcium, ionized calcium, phosphate, alkaline phosphatase, creatinine, 24 h urine calcium excretion, PTH, complete blood count, IgG, IgA, IgM, LDH, β2-microglobulin, D-dimer) were repeated after 3 and 6 months of VD supplementation. All analyses were performed in the hospital laboratory. Secondary hyperparathyroidism was defined as a parathormone (PTH) level above the manufacturer's normal reference range (15-68.3 pg/ml) but with a normal concentration of calcium.

The decision concerning treatment with chemotherapy or a "watchful waiting" strategy was made according to the International Workshop on Chronic Lymphocytic Leukemia guidelines [22].

25-OH-D_3 measurements

The serum 25-OH-D_3 levels were measured on a Cobas E422 Roche by electrochemiluminescent immunoassay (ECLIA) with the inter-assay variability below 10.3%.

Intervention

VD deficiency was defined as a serum 25-OH-D_3 level <30 ng/ml. Subjects were stratified into 3 categories according to 25-OH-D_3 levels: mild deficiency (20-30 ng/ml), moderate deficiency (10 - 19.9 ng/ml) and severe deficiency (<10 ng/ml). The study design and cholecalciferol dosage in each category are shown on Figure 1. In group 1 all patients received rituximab, with addition of fludarabine and cyclophosphamide in two cases (R-FC), cladribine and cyclophosphamide in one subject (R-CC), cyclophosphamide, vincristine and prednisone in one patient (R-CVP), and bendamustine in two cases (R-B).

Safety issues

There were two major concerns related to the VD supplementation: hypercalcemia and hypercalciuria were evaluated by quantification of ionized calcium and 24 h urine calcium excretion or urinary calcium-to-creatinine ratio. The measurements were carried out at baseline and then after 3 months or 3 cycles of chemotherapy and at the completion of the intervention. Hypercalcemia was defined as ionized calcium levels more than 1.35 mmol/l and hypercalciuria as urinary calcium excretion over 5 mmol/24 h or urinary calcium-to-creatinine ratio more than 0.4 mg/mg.

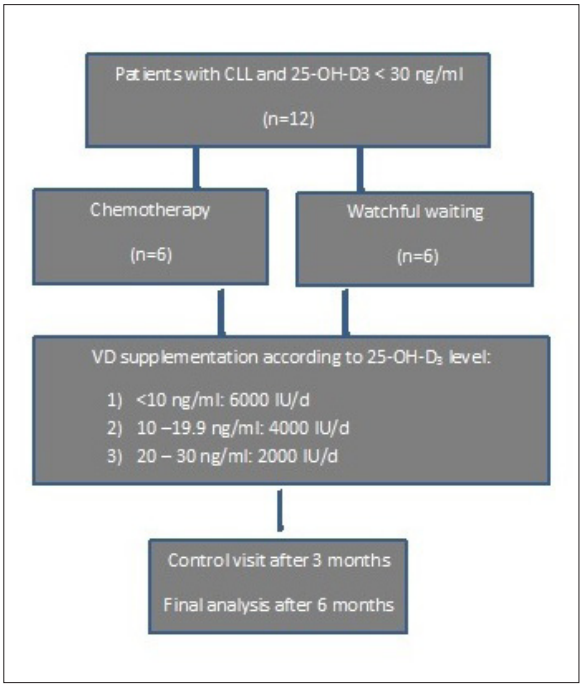

Fig. 1. Flow diagram displaying design of the study.

Statistical analysis

Differences in variables between groups at the beginning of the study, as well as after 6 months of supplementation, were examined by Mann–Whitney U test or Kruskal–Wallis one-way analysis of variance. The changes of values from serum, plasma and urine examinations were compared using the Wilcoxon signed-rank test for paired data. All analyses were performed using the statistical package Statistica, version 10 (StatSoft, Inc.). $P < 0.05$ was considered significant in all analyses.

Results

The characteristics of the study group are shown in Table 1.

In the group of 13 CLL analyzed patients, 1 patient had the 25-OH-D_3 level within the optimal range (30-80 ng/ml), 7 had a mildly reduced (insufficient) level, 4 moderate deficiency and 1 a severely deficient measurement. In total, more than 90% had suboptimal levels of 25-OH-D_3. The mean 25-OH-D_3 level at the baseline for the study subjects was 18.6 ± 7 ng/ml (range 7.6-33.5). The distribution of VD levels by category is shown in Fig. 2.

25-OH-D_3 insufficiency had no association with CLL stage or FISH risk category as classified using the Dohner system [11].

Table 1. Baseline characteristics of the study subjects with chronic lymphocytic leukemia [N=13]

Characteristic	
Age, years (median, range)	67 (57-74)
Male	6 (46%)
BMI (median, range)	28 (21,5-36,8)
Binet stage	
A	4 (31%)
B	4 (31%)
C	5 (38%)
Genetics	
del 11q	4 (31%)
del 13q	9 (69%)
del 13q as a sole mutation	8 (62%)
del 17p	0
trisomy 12	1 (8%)
B symptoms	6 (46%)
Splenomegaly	5 (38%)
Previous treatment	6 (46%)
Time from diagnosis, years (median, range)	2 (0-7)
Concomitant diseases	
Type 2 diabetes	2 (15%)
Coronary artery disease	5 (38%)
Hypertension	8 (62%)

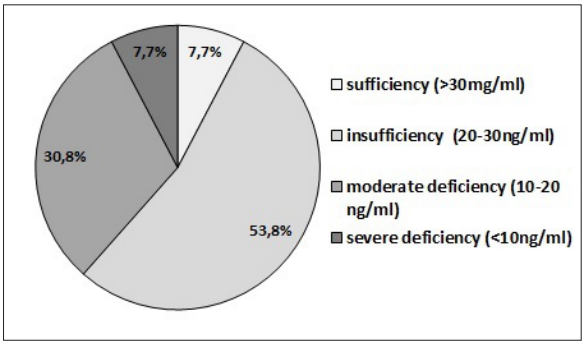

Fig. 2. The distribution of vitamin D levels by category in study subjects.

Twelve patients received cholecalciferol supplementation in a mean dose of 3384 ± 1211 IU. It was followed by a significant increase in 25-OH-D_3 in study subjects ($p < 0.01$), as well as in both subgroups ($p<0.05$). On completion of the study, the mean 25-OH-D_3 level was 41.4 ± 7 ng/ml (range 20.3-70). None of the patients had severe or moderate VD deficiency. Five subjects (42%) had mild VD deficiency and 7 (58%) had optimal VD levels.

Secondary hyperparathyroidism was observed in 4 subjects at the beginning of the study. Serum PTH concentration significantly decreased ($p<0.01$) after 6 months of VD supplementation and all patients achieved normal PTH level.

Total serum calcium or ionized calcium remained unchanged after 6 months of supplementation. The urinary

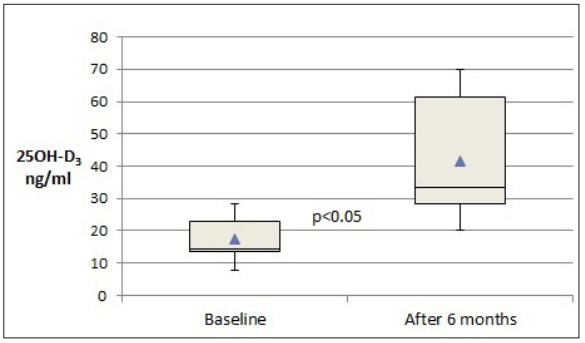

Fig. 3. Levels of 25-OH-D_3 at baseline and after 6 months of cholecalciferol supplementation in 12 patients (median, quartiles, range, mean)

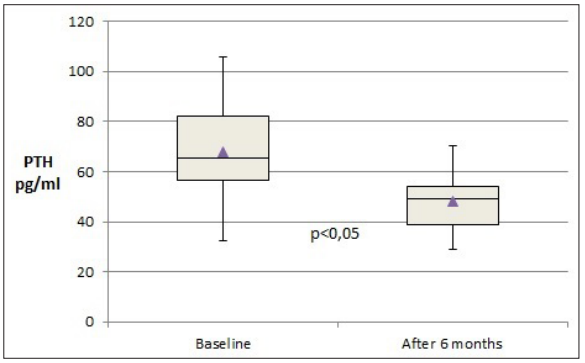

Fig. 4. Levels of parathormone (PTH) at baseline and after 6 months of cholecalciferol supplementation in 12 patients (median, quartiles, range, mean)

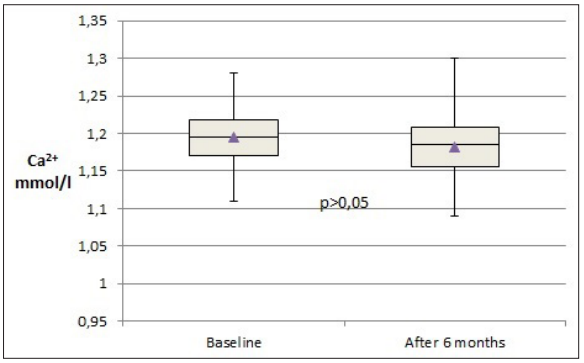

Fig. 5. Levels of ionized calcium (Ca^{2+}) at baseline and after 6 months of cholecalciferol supplementation in 12 patients (median, quartiles, range, mean)

calcium excretion increased, significantly only in group 2 (Table 2), but none of the patients developed hypercalciuria or hypercalcemia during the study. Patients did not complain of constipation.

Changes in the 25-OH-D_3, PTH and serum calcium in 12 patients are shown in Figure 3, 4 and 5.

A significant decrease in LDH ($p<0.05$), B2-microglobulin ($p<0.05$) and lymphocyte count was observed in group 1. A control bone marrow biopsy was performed only in the

group receiving immunochemotherapy, in which a significant decrease in bone marrow infiltration by leukemic cells was found (p=0.03). Additionally, a significant decrease in D-dimer level was found only in group 2 (Table 2).

Two patients from the "watchful waiting" group reported alleviation of bone and muscle pain.

Discussion

The role of VD in the regulation of calcium homeostasis is well known, though it has become clear that the functions of the vitamin extend far beyond. The identification of the presence of common VD deficiency is increasing [31], especially in countries located at high geographical latitudes [43]. The outcomes of this pandemic VD deficiency are still not well understood. In our study more than 90% of subjects had suboptimal levels of VD, and one third of them had secondary hyperparathyroidism. This suggests that our patients had insufficient sun exposure. It was shown that at least 12 h/weekend sun exposure is associated with VD levels above 24 ng/ml, but many subjects did not achieve optimal levels without supplementation [8]. The approach to recommend sun exposure remains controversial, since benefits of sun exposure in terms of its effects on VD status must be weighed against an increased risk of melanoma [19].

Cholecalciferol dosage in our study was adjusted to the degree of the VD deficiency. According to the guidelines for the supplementation of VD and the treatment of deficits in Central Europe [50], adults and the elderly (65 years and above) with 25-OH-D3 concentration lower than 20 ng/ml should receive 7000–10 000 IU/day for a period of 1-3 months. However, special caution is required with respect to conditions with excessive extrarenal 1-a-hydroxylase. Since such a condition was reported in CLL [27] and our intervention lasted longer, we used lower cholecalciferol doses. The Endocrine Society recommended intake of 6000 IU/day in adults with 25-OH-D3 concentration below 20 ng/ml [30]. The VD supplementation necessary to achieve a 25-OH-D3 increment of 10 ng/ml may be at least 1500 IU/day [20]. Thus, in our study, patients with a 25-OH-D3 level between 20 and 30 ng/ml received 2000 IU/d.

The optimal VD serum level remains uncertain. The current evidence indicates a serum status of 25-OH-D3 of at least 20 ng/ml as needed for optimal bone and muscle function. However, estimates of optimal levels focused on 30 ng/ml as

Table 2. Comparison between baseline and after 6 months of supplementation characteristic in each group (mean±SD, P1, P2 differences in changes between baseline and 6 months, P3 differences between two groups at baseline, P4 differences between two groups at 6 months)

Characteristics	Group 1 (n=6) Baseline	At 6 months	P1	Group 2 (n=6) Baseline	At 6 months	P2	P3	P4
Lymphocytes (10^3/µl)	58.62±68.4	3.19±1.4	0.02	22.4±9.4	26.8±13.7	0.2	0.32	0.001
Hemoglobin (g/dl)	11.3±0.82	11.4±1,6	0.17	13.5±1.3	13.2±1.2	0.5	0.006	0.001
Platelets (10^3/µl)	171±69	202±81	0.2	187±56	163±71	0.1	0.7	0.4
Lymphocytes % in bone marrow	63±23	14±2.6	0.04	43.8±13.8	-	-	0.08	-
LDH (IU/l)	189±30	143±21	0.02	140±11	134±17	0.3	0.04	0.4
B2-microglobulin (µg/ml)	5.0±1.4	3.2±0.8	0.02	2.8±1.0	3.4±1.0	0.1	0.09	0.8
Immunoglobulins								
IgG (g/l)	13.5±6.2	7.4±3.7	0.12	9.1±2.8	9.1±3.3	0.7	0.4	0.4
IgA (g/l)	1.1±0.5	0.84±0.6	0.3	2.3±2.0	2.1±1.7	0.7	0.1	0.1
IgM (g/l)	0.29±0.1	0.28±0.1	0.7	1.1±2.0	1.1±1.2	0.7	0.1	0.1
Creatinine (µmol/l)	81±10	77±8	0.4	75±20	76±18	0.5	0.2	0.2
25-OH-D3 (ng/ml)	18.1±6.1	38.7±18.7	0.03	16.5±5.8	44.1±17.4	0.03	0.4	0.6
Total calcium (mmol/l)	2.3±0.1	2.2±0.1	0.1	2.3±0.1	2.3±0.1	0.14	0.5	0.1
Ionized calcium (mmol/l)	1.19±0.05	1.18±0.07	0.2	1.20±0.03	1.19±0.02	0.3	0.7	0.8
24 h urine calcium excretion (mmol/24h)	2.04±1.35	1.8±1.01	1.0	1.57±1.69	2.04±1.65	0.04	0.8	0.7
Phosphate (mmol/l)	1.11±0.14	1.16±0.12	1.0	1.09±0.17	1.03±0.22	0.6	0.4	0.2
Alkaline phosphatase (IU/l)	71±15	64±14	0.7	57±17	54±16	0.3	0.2	0.2
Intact PTH (pg/ml)	62.4±18.8	45.3±9.1	0.02	73.6±19.7	51.1±12.9	0.03	0.3	0.4
D-dimer (µg/ml)	1.17±1.48	0.36±0.13	0.34	0.42±0.17	0.33±0.13	0.04	0.3	0.4

the threshold for peak bone health [9]. Levels around 30-40 ng/ml are required for optimal immune function [33]. We observed a decrease in PTH level and its normalization in subjects with secondary hyperparathyroidism after 6 months of VD supplementation. Several studies have reported that PTH levels are inversely associated with 25-OH-D3 and reached a plateau in subjects with serum levels of 25-OH-D3 between 30 and 40 ng/ml [32].

Since the synthesis of 1,25(OH)2D3 is tightly regulated and the therapeutic index for 25-OH-D3 is wide, VD toxicity is extremely rare and usually occurs at excessively high doses [38]. The main clinical manifestation of VD hypervitaminosis is hypercalcemia and its associated symptoms [13]. VD intoxication usually occurs at levels of 25-OH-D3 >100 ng/ml [47], as a result of consecutive intramuscular injections of 600 000 IU of cholecalciferol [26]. Patients developed VD toxicity with exclusively oral intake of VD extremely rarely, when very high doses, such as 60 000 IU daily, were given over a period of 1-3 months [35]. An oral daily dose of 10 000 IU of cholecalciferol was unlikely to cause any adverse effects in the healthy population and is accepted as the tolerable upper intake level (UL) [23]. The postulated risk factors for VD toxicity comprise older age, impaired renal function, use of thiazide diuretics, intramuscular injections of VD, concomitant disorders such as sarcoidosis and tuberculosis. Additionally, lymphomas were found to be related to a higher prevalence of hypercalcemia, most likely mediated by 1,25(OH)2D3, which is secreted from lymphoma-adjacent macrophages [27]. Parathyroid hormone-related protein released by neoplastic cells might be the other cause of hypercalcemia in hematologic malignancies [16]. In our study the highest observed 25-OH-D value was 70 ng/ml. The serum total calcium and ionized calcium level remained unchanged, and not a single patient developed hypercalcemia or hypercalciuria. However, in five study subjects, the applied doses of cholecalciferol were insufficient to achieve a 25-OH-D3 level above 30 ng/ml. A prospective study with higher VD doses is needed to reevaluate the safety of such supplementation.

Older age groups in studies from all over the world have been reported to have lower serum concentrations of VD [43]. It is of great importance for patients with CLL, since the median age for diagnosis of the disease is 70 years for men and 74 years for women [10]. The median age of the study subjects in our study was 67 years. The elderly (65 years and above) are recommended to take VD throughout the whole year, because of the reduced efficacy of VD skin synthesis [50].

Both leukemic cells and lymph nodes in CLL could contribute to lower concentrations of VD in blood through receptor-ligand binding [49]. Whether tumor burden can impact the level of VD remains a question. We found no association between serum level of VD and parameters reflecting tumor burden such as Binet or Rai substages, β2-microglobulin, or total leukocyte count, which is in agreement with other studies [52]. A significant decrease in LDH and B2-microglobulin and total leukocyte count was observed only in the group receiving immuno-chemotherapy.

The decrease in the D-dimer level after supplementation in the "watchful waiting" group was an unexpected finding, although an inverse correlation between VD level and D-dimer level has been found before [36]. VD exerts anticoagulant effects by upregulating thrombomodulin and downregulating tissue factor expression [39]. Gene expression studies suggest that VD analogs may suppress thrombogenicity [54]. The VD status was associated with tPA concentrations, fibrinogen and D-dimer, which suggests that VD intake may be important for maintaining antithrombotic homeostasis [34]. In contrast to our data, Marckmann et al. [41] found no impact of cholecalciferol supplementation on D-dimer level. Although there is a shortage of data related to the state of the coagulation system in patients with CLL, there are some studies which suggest ongoing abnormal activation of coagulation and altered endothelial cell function, especially in relapsed patients with extensive disease [4].

VD deficiency was suggested to be the first potentially modifiable prognostic marker in CLL. Because measurement of VD concentrations is easily done and VD supplements are readily available, safe and inexpensive, prospective trials are necessary to determine whether this represents a causal relationship and whether VD supplementation can reduce the associated increased risk in CLL and improve the outcome.

We are aware that our study has several limitations such as a small sample size and a short follow-up to analyze the patients' outcomes. However, to our knowledge, our results represent the first attempt to apply VD replacement therapy in CLL patients, and show that higher VD doses should be used in future trials to obtain optimal levels in all patients.

In conclusion: VD replenishment is safe and can be effectively achieved with the employed cholecalciferol dosage in the majority of patients. However, some subjects may require higher doses to obtain the optimal level and immune function.

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