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Patients with COVID-19 pneumonia with 25(OH)D levels lower than 12 ng/ml are at increased risk of death

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ABSTRACT

Objectives: There is no consensus on specific serum 25-hydroxy vitamin D (25(OH) D) levels associated with higher risk of severe outcome in patients with coronavirus disease 2019 (COVID-19). According to the literature patients with serum 25(OH) D levels <12 ng/ml are clearly deficient at all ages. Our aim was to assess COVID-19 mortality in the settings of severe 25(OH) D deficiency. A cohort study of 357 patients with COVID-19 was conducted. Subjects were monitored until discharge or in-hospital death. At admission, severity parameters (C-reactive protein (CRP), IL-6, Charlson comorbidity index, etc.) were assessed. These parameters were compared regarding 25(OH) D levels threshold 12 ng/ml, where values below 12 ng/ml were considered absolute vitamin D deficiency.

Results: 25(OH) D levels at the time of admission were independently associated with mortality ($p < 0.05$). Nonsurvivors (N = 168) had lower 25(OH) D levels, SO2, higher age, CRP, viral load, and Charlson comorbidity index in comparison to survivors. Patients with serum 25(OH) D levels <12 ng/ml had higher mortality (55% vs 45 %), viral load (21.5 vs 23.1), and Charlson comorbidity index (5.3 vs 4.4) than those with serum 25(OH) D levels >12 ng/ml ($p < 0.05$).

Conclusions: Patients with COVID-19 with serum 25(OH) D levels <12 ng/ml have higher mortality. Among other factors, severe vitamin D deficiency likely leads to poor outcome.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) uses the angiotensin-converting enzyme 2 (ACE2) receptor to enter macrophages and monocytes, resulting in macrophage activation (Merad, Martin, 2020). In some patients, this leads to a hyperinflammatory syndrome, acute respiratory distress syndrome (ARDS), and end-organ damage (Webb et al., 2020). It has been hypothesized that vitamin D sufficiency may modulate this excessive inflammatory response in coronavirus disease 2019 (COVID-19) (Grant et al., 2020). Indeed, low vitamin D levels in patients with COVID-19 have been reported to adversely affect disease severity and course (Pereira et al., 2020). However, not all published literature supports this conclusion. Very recently, a meta-analysis showed that vitamin D deficiency (<20 ng/ml) or insufficiency (<30 ng/ml) was not associated with a significantly increased risk of COVID-19 infection or in-hospital death (Chen et al., 2021). In the context of the possible immunomodulatory role of vitamin D in COVID-19, the problem of reverse causality has also been proposed. Thus, it is possible that 25-hydroxy vitamin D (25(OH) D) is a negative acute phase reactant, with low lev-

Abbreviations: COVID-19, Coronavirus disease 19; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; 25(OH) D, 25-hydroxy vitamin D; ACE2, Angiotensin-converting enzyme 2; ARDS, Acute respiratory distress syndrome; RT-PCR, Real-time reverse transcriptase-polymerase chain reaction; RAAS, Renin-angiotensin-aldosterone-system; Ang II, Angiotensin II; 1,25(OH) D3, 1α, 25-hydroxy vitamin D3; IL-10, Interleukin 10; IL-6, Interleukin 6; TNF-α, Tumor necrosis factor α; INF-γ, Interferon γ.

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els indicating greater inflammation. As such, the relationship between vitamin D status and COVID-19 severity remains controversial (Martineau, Forouhi, 2020).

Although 25(OH) D is widely accepted as the best marker for assessing vitamin D status, the definition and relevance of vitamin D deficiency are still under debate (Amrein et al., 2020). However, there is high level of agreement on 2 points: 25(OH) D levels <12 ng/ml are clearly deficient at all ages and levels above 30 ng/ml are clearly sufficient (Giustina et al., 2020). Severe vitamin D deficiency with a 25(OH) D concentration <12 ng/ml increases the risk of mortality, infections, and other diseases and should be prevented with a public health approach (Amrein et al., 2020; Cashman, 2020; Lips et al., 2019). Specifically, regarding respiratory infections, Martineau et al. (2017) showed that vitamin D supplementation was protective against acute respiratory tract infections, and patients who were severely 25(OH) D deficient experienced the most benefit. More recently, a small study reported that elderly patients with COVID-19 with 25(OH) D levels <12 ng/ml were at higher risk of noninvasive ventilation requirement than those with 25(OH) D levels >12 ng/ml. The effect on mortality was not proven (Baktash et al., 2021). To our knowledge, there is no study defining serum 25(OH) D level of 12 ng/ml as a specific cutoff point associated with excessive mortality in patients with COVID-19 pneumonia.

This study assessed COVID-19 mortality in the settings of absolute 25(OH) D deficiency. We speculate that in patients who are severely vitamin D deficient, the peripheral tissues including lung parenchyma are depleted of 25(OH) D. In this scenario, the immune system is unable to exert proper lung-protective immune response, which ultimately leads to a severe course and higher mortality in patients with COVID-19.

Material and methods

Patient cohort

This study was undertaken as a longitudinal single-center cohort study at the 5th Internal medicine department of Comenius University and University Hospital Bratislava, Slovakia. The study included all consecutive patients who were admitted to our department between November 1, 2020 and April 30, 2021.

Inclusion criteria were as follows:

- COVID-19 pneumonia as the main diagnosis at admission;
- positive real-time reverse transcriptase-polymerase chain reaction (RT-PCR) test for SARS-CoV-2 on nasopharyngeal swab; and
- specimen for serum 25(OH) D level obtained on admission.

The patients were monitored until discharge (survivors) or in-hospital death (nonsurvivors).

Demographic characteristics, comorbidities, hematologic and biochemical laboratory results on admission, information regarding intensity of care during hospitalization, and information regarding pharmacologic treatment before and during hospitalization were collected from electronic medical records and discharge summaries by 2 physicians using a standardized approach.

All patients received pharmacologic and supportive measures according to the interim COVID-19 guidance for treatment approved by the University Hospital Bratislava. These guidelines were based on current Centers for Disease Control and Prevention (US) recommendations for treatment (https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-guidance-management-patients.html).

All patients, regardless of their levels of 25(OH) D, were supplemented by a loading dose of 30 000 IU cholecalciferol for the first 3 days, followed by 7500 IU cholecalciferol per day.

The Charlson comorbidity index was used to provide a single score, reflecting a range of comorbidities in our cohort of patients. An online calculator (www.mdcalc.com/charlson-comorbidity-index-cci.com) was used for its calculation.

Laboratory evaluation

1) COVID-19 severity parameters: complete blood count, serum C-reactive protein (CRP), interleukin 6 (IL-6), procalcitonin (PCT), D-dimer, and fibrinogen. Other standard tests such as liver tests, kidney functions, serum minerals, and fasting plasma glucose tests and basic coagulation tests were performed in each patient. All measures were assessed with commercial standardized tests and blood sampling was done between 7:00 and 8:00 A.M.;
2) viral load: SARS-COV-2 virus load was assessed by RT-PCR using the cycle threshold;
3) blood oxygen saturation was assessed using arterial blood sampling ±2 hours from venous blood sampling;
4) 25(OH)D: Serum 25(OH) D concentrations (in ng/mL) were obtained at admission using an automated electrochemiluminescence system (Eclesys Vitamin D Total II, 2019, Roche Diagnostics GmBH, Mannheim, Germany). The detection limit of serum 25(OH) D was 3 ng/mL. In accordance with existing guidelines, a serum 25(OH) D cutoff level <12 ng/mL was used to determine tissue vitamin D deficiency (Giustina et al., 2020).

Statistical analysis

Results of numerical parameters are displayed as means and standard deviations; proportions are given as numbers and percentages. Comparison of the groups (survivors vs nonsurvivors, or vitamin D <12ng/ml vs >12ng/ml) was performed using the analysis of variance for continuous variables and chi-square test for categoric variables. To assess predictors of mortality, multivariate logistic regression with death as a dependent variable was used. Independent variables, according to previous studies and our expert opinion, were proposed. Backward selection method for identifying independent predictors of death in the final model was used. P values <0.05 were considered statistically significant.

Results

A total of 558 patients with acute COVID-19 were admitted between November 1, 2020 and April 30, 2021. A total of 201 patients were excluded because 25(OH) D was not measured, COVID-19 pneumonia was not the primary diagnosis at admission, or their SARS-CoV-2 RT-PCR test was negative, with a final number of 357 patients (198 male and 159 female) participating in the study (see Figure 1). In total, 168 (47 %) patients from the study population died of COVID-19. Baseline demographic and clinical and laboratory characteristics of survivors and nonsurvivors are summarized in Table 1. Overall, low serum 25(OH) D was common, with 25(OH) D levels in 80% of patients being either insufficient or deficient according to existing guidelines (i.e., 25[OH] D <30 ng/ml).

Lower 25(OH) D levels, platelet counts and oxygen saturation, and higher age, CRP, viral load, white blood cells, neutrophils, and Charlson comorbidity index in nonsurvivors than in survivors were observed (all p <0.005). Arterial hypertension, chronic kidney disease, diabetes mellitus with complications, chronic heart failure, and chronic kidney disease were more prevalent in nonsurvivors compared with survivors (p <0.05) (see Table 1). Nonsurvivors ended more frequently on high-flow nasal cannula and invasive mechanical ventilation (p <0.0001). There was no difference in

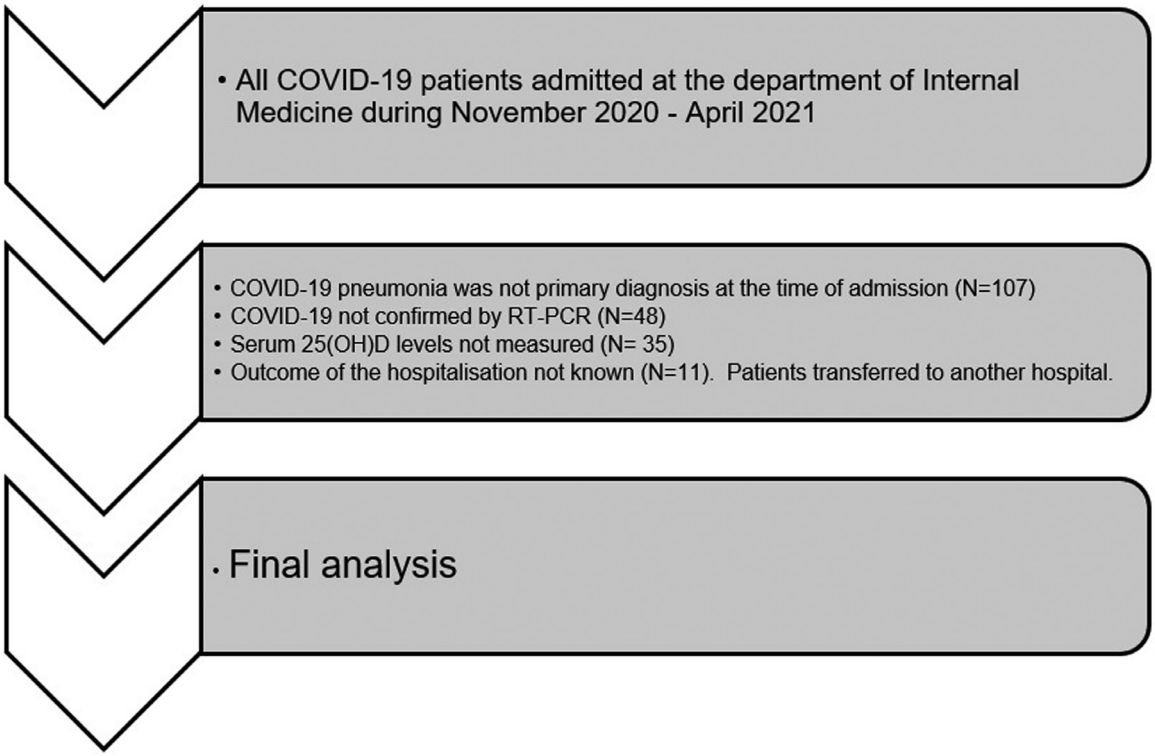

Figure 1. The study flowchart.

Table 1
Baseline demographic, clinical and laboratory characteristics of survivors and nonsurvivors.

Variable	Survivors (n=189)	Nonsurvivors (n=168)	p-value
Age (years)	63.5±13.9	73.5 ±10.5	**<0.0001**
BMI (kg/m2)	29.8±7.6	30.4 ±7.1	0.45
Males/Females, n (%)	104 (55)/85 (45)	94 (56) /74 (44)	0.86
Arterial hypertension, n (%)	130 (69)	132 (78.5)	**0.05**
Chronic heart failure, n (%)	17 (9)	35(21)	**0.002**
Diabetes mellitus w/o complications, n (%)	48 (26)	27(16)	**0.03**
Diabetes mellitus w/complications, n (%)	23(12)	40(24)	**0.003**
Chronic kidney disease, n (%)	34 (17)	65(39)	**<0.0001**
Cirrhosis, n (%)	2 (1)	1(0.5)	0.63
Charlson comorbidity index	3.6 ±2.7	5.7±2.6	**<0.0001**
Use of vitamin D supplements prior hospitalization, n (%)	31(16)	32 (19)	0.51
High-flow nasal cannula, n (%)	44 (24)	132 (79)	**<0.0001**
Invasive mechanical ventilation, n (%)	2 (1)	22 (13)	**<0.0001**
White blood cells (10 × 9/L)	7.4 ±3.3	9.0±5.1	**0.0006**
Neutrophils (10 × 9/L)	6.0±3.1	7.7±4.6	**0.0001**
Lymphocytes (10 × 9/L)	1.0 ±0.8	0.8 ±0.9	0.08
Platelets (10 × 9/L)	263.2 ±110.5	231.2 ±97.0	**0.0042**
CRP (mg/L)	107.3 ±88.1	153.6 ±90.9	**<0.0001**
IL-6 (ng/L)	122.7±435.5	215.5±476.5	0.07
D-dimer (mg/L)	2.7±4.6	3.6±5.1	0.09
Procalcitonin (ug/L)	1.5±10.4	2.6±9.7	0.30
25(OH) D (ng/ml)	24.6±14.6	20.8±11.1	**0.007**
Cycle threshold	24.2±5.0	21.0±4.6	**<0.0001**
Oxygen saturation (%)	89.6±7.47	86.1±10.3	**0.0003**
continuous variables are expressed as mean±SD			

Abbreviations: BMI – Body Mass Index; CRP – C-reactive protein; 25(OH) D – 25-hydroxy vitamin D, IL-6 - interleukin 6.

sex, body mass index (BMI), liver cirrhosis prevalence, lymphocyte count, PCT, D-Dimer, vitamin D use before hospitalization, and IL-6 in survivors compared with nonsurvivors.

In multivariate linear regression analysis (see Table 2), serum level of 25(OH) D remained independently associated with in-hospital mortality ($p = 0.0398$). Among other independent variables, age, CRP, blood oxygen level saturation, platelet count, Charlson comorbidity index, and BMI were significantly and independently associated with mortality (all $p < 0.05$).

Patients with 25(OH) D <12 ng/ml ($N = 74$) had more prevalent chronic kidney disease, higher Charlson comorbidity index, and higher viral load than those with vitamin D >12 ng/ml ($N = 283$). Mortality was 11% higher in the group with vitamin D <12 ng/ml (~55% vs ~44%; $p < 0.05$) (see Table 3 and Figure 2). There was no difference in age, BMI, other comorbidities, blood count parameters, CRP, D-dimer, PCT, IL-6, oxygen saturation, use of vitamin D supplements prior hospitalization, or ventilation outcome between the groups according to the 25(OH) D threshold.

Table 2
Results of multivariate linear regression analysis. Only findings with p <0.05 are displayed.

Independent variables	Coefficient	Std. Error	t	P	$r_{partial}$	$r_{semipartial}$	VIF
(Constant)	0.2628						
Age (years)	0.01049	0.002462	4.258	**<0.0001**	0.2365	0.2075	1.815
BMI (kg/m2)	0.007869	0.003524	2.233	**0.0263**	0.1266	0.1088	1.063
CRP (mg/L)	0,001071	0.000275	3,899	**0.0001**	0.2176	0.19	1.086
Charlson Comorbidity Index	0.02327	0.01136	2.048	**0.0414**	0.1163	0.09976	1.797
Platelets (10 × 9/L)	−0.0007356	0.000239	−3.077	**0.0023**	−0.1732	0.1499	1.031
Oxygen saturation (%)	−0.008144	0.002905	−2.803	**0.0054**	−0.1582	0.1366	1.09
25(OH) D (ng/mL)	−0.00395	0.001913	−2.065	**0.0398**	−0.1172	0.1006	1.028

Abbreviations: BMI – Body Mass Index; CRP – C-reactive protein; 25(OH) D – 25-hydroxy vitamin D.

Table 3
Comparison of clinical parameters according to vitamin D levels.

Parameter	25(OH) D ≥12 ng/ml (n=283)	25(OH) D <12 ng/ml (n=74)	p-value
Age (years)	67.9±13	69.4±14.7	0.39
BMI (kg/m2)	30.5±7.4	28.7±7.2	0.08
Males/Females, n (%)	164(58) / 119 (42)	34 (46) /40 (54)	0.07
Arterial hypertension, n (%)	210 (74)	52 (70)	0.60
Chronic heart failure, n (%)	38 (13)	14 (19)	0.41
Diabetes mellitus w/o complications, n (%)	62 (22)	13 (17)	0.41
Diabetes mellitus w/complications, n (%)	50 (18)	13(18)	0.8
Chronic kidney disease, n (%)	70 (25)	29 (40)	**0.007**
Cirrhosis, n (%)	3 (1)	0	0.38
Charlson comorbidity index	4.4±2.8	5.3±3.09	**0.022**
Use of vitamin D supplements prior hospitalization, n (%)	50(18)	13 (17)	0.81
High-flow nasal cannula, n (%)	141 (49)	35(46)	0.56
Invasive mechanical ventilation, n (%)	18 (6)	4 (5)	0.61
White blood cells (10 × 9/L)	8.4±4.6	8.1±3.6	0.65
Neutrophils (10 × 9/L)	7±4.2	6.8±3.5	0.73
Lymphocytes (10 × 9/L)	0.97±0.8	1.07±1.1	0.43
Platelets (10 × 9/L)	251±108	246±100	0.75
CRP (mg/l)	131.6±93	125.15±91	0.59
IL-6 (ng/l)	171.1±475	143.6±330	0.65
D-dimer (mg/L)	3.4±5.2	3.2±4.8	0.79
PCT	1.7 ±9.3	2.8±12.1	0.41
25(OH) D (ng/ml)	26.7±12	8.2±2.6	**<0.0001**
Oxygen saturation (%)	87.7±9.3	87.6±10.5	0.9
Cycle threshold	23.2±5.2	21.5±4.9	**0.04**
Nonsurvivors/Survivors, n (%)	127(44)/166(54)	41(55)/31(46)	**0.05**
continuous variables are expressed as mean ± SD			

Abbreviations: BMI – Body Mass Index; CRP – C-reactive protein; 25(OH) D – 25-hydroxy vitamin D, IL-6 - interleukin 6.

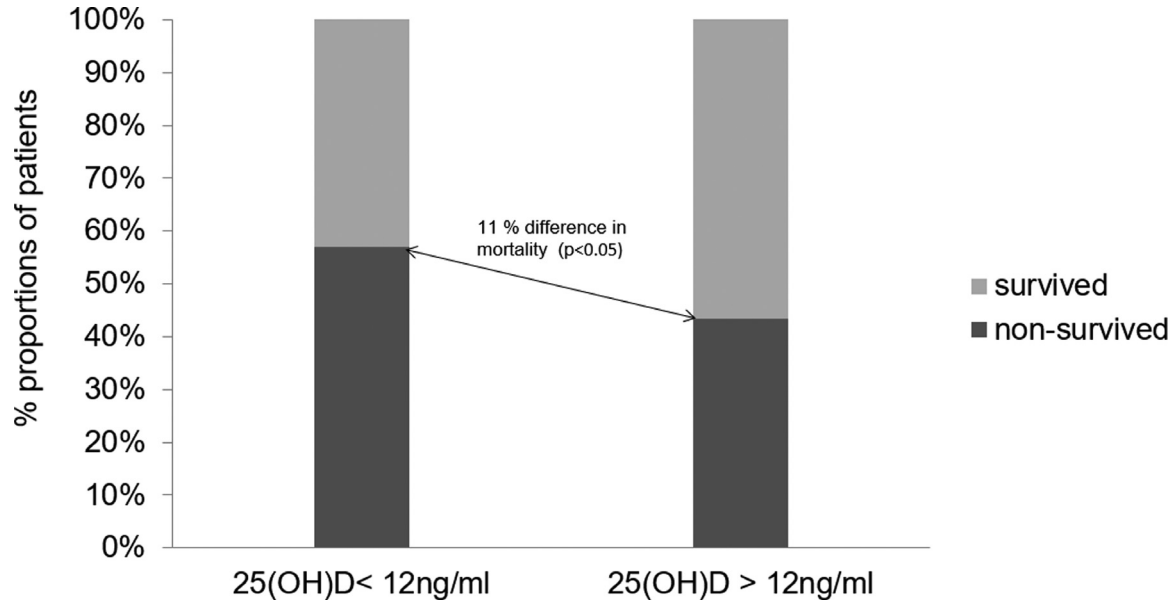

Figure 2. The difference in mortality between groups according to the specific 12 ng/ml cut-off level of 25(OH)D, representing servere 25(OH)D deficiency.

Discussion

A considerable number of studies, even from the pre-COVID era, have suggested that vitamin D may play an important role in minimizing lung tissue inflammation and improving the antiviral state, thus maintaining local respiratory homeostasis (Hansdottir, Monick, 2011).

In this study, we found that a low serum level of 25(OH) D was highly prevalent in patients with COVID-19 pneumonia, with 80% of patients being 25(OH) D deficient or insufficient. Besides age, BMI, CRP, Charlson Comorbidity Index, platelet count, and oxygen saturation, the concentration of 25(OH) D at the time of admission was also independently associated with mortality. In addition, patients with severe vitamin D deficiency, as defined by 25(OH) D levels $<$12 ng/ml, had higher mortality (55% vs 45 %), viral load (21.5 vs 23.1), and Charlson comorbidity index (5.3 vs 4.4) than those with serum 25(OH) D levels $\geq$12 ng/ml. There was no difference between markers of inflammation in the 2 groups despite a significantly higher mortality rate in the group with 25(OH) D levels below 12 ng/ml. This could support the notion that vitamin D is not simply a bystander reflecting greater inflammation but an independent and potentially modifiable risk factor.

Several plausible pathophysiologic mechanisms of how low 25(OH) D could influence disease severity and progression in patients with COVID-19 have been proposed to date.

The ACE2 receptor is widely expressed in the alveolar compartment, importantly on the lung epithelia type II, vascular endothelial cells, and on the major sentinel immune cells of the lung (i.e., monocytes/macrophages and dendritic cells) (Beyerstedt et al., 2021). SARS-CoV-2 has tropism for these cells and binds to the ACE2 receptor on the cell membrane with subsequent endocytosis (Hussain et al., 2020). ACE2 is an important part of RAAS (renin-angiotensin-aldosterone-system) and consists of 2 pathways. The first pathway (classical) leads to the generation of angiotensin II (Ang II) and is associated with proinflammatory, prothrombotic, and profibrotic effects of RAAS. The result of activation of the second pathway (nonclassical), however, is Ang II degradation and subsequently angiotensin-(1–7) formation, which is associated with anti-inflammatory effects in the lungs (Mascolo et al., 2021). Adequate levels of 25(OH) D favor ACE2/Ang-(1–7) axis, which experimentally was found to be associated with lesser lipopolysaccharide-induced lung injury (Xu et al., 2017). Except serum 25(OH) D level (in)adequacy, the balance between the RAAS axes is also similarly influenced by higher BMI and older age (Getachew, Tizabi, 2021). Interestingly, higher BMI and older age are also associated with poor vitamin D status.

Regarding lung immunity, it has been suggested earlier that vitamin D potentiates innate immune response and inhibits adaptive immune response. The antigen-presenting cells, dendritic cells and macrophages, express 1α-hydroxylase (CYP27B1) which converts 25(OH) D into its active form: 1 α, 25-hydroxy vitamin D3 (1,25(OH) D3) (Bishop et al., 2020). This active form of vitamin D is considered an important immunomodulatory molecule with paracrine/autocrine activity in the pulmonary microenvironment. After the viral stimulus through pattern recognition receptors, vitamin D signaling pathways lead to the synthesis of several antimicrobial peptides like cathelicidin antimicrobial peptides (e.g., CAMP/LL37) and β-defensins responsible for widespread antimicrobial and antiviral activity (Bishop et al., 2020).

Contrary to the stimulatory effect of vitamin D on monocytes, vitamin D signaling pathways in adaptive immune cells are largely inhibitory. Both B and T cells express on their membrane receptor for vitamin D and 1-α hydroxylase and thus can convert 25(OH) D to 1,25(OH)D3 (Hewison, 2012). 1,25(OH)D3 largely potentiates the synthesis of anti-inflammatory cytokine interleukin 10 (IL-10) by regulatory T-lymphocytes and suppresses the production of proinflammatory cytokines like IL-6, tumor necrosis factor α (TNF-α) and interferon γ (INF-γ), thus preventing an overstimulation of the immune response (Hewison, 2012). Overproduction of proinflammatory cytokines may be one of the main factors in the development of viral pneumonia-induced ARDS. Decreased synthesis of inflammatory cytokines with 1,25(OH)D3 was observed in a dose dependent manner and sufficient serum levels of 25(OH) D are needed to increase 1,25(OH)D3 levels to exert a proper immune response to viral respiratory infections (Hetta et al., 2021).

Seeking optimal 25(OH) D cutoff also has great implications for randomized clinical trials (RCTs). Vitamin D is a threshold nutrient, which means that if the threshold value for given physiologic end point is achieved, higher levels of vitamin D do not lead to a greater effect. Thus, if a clinical trial enrolls patients with 25(OH) D levels above that threshold, the possible benefit from vitamin D supplementation is greatly reduced and one would not expect to see the effect of a threshold nutrient if both the control and the supplemented groups are not clearly 25(OH) D deficient at baseline (Giustina et al., 2020). This could be the case why intervention trials have usually not been able to find a benefit of vitamin D supplementation on clinical outcome (Amrein et al., 2020). For example, in one RCT conducted in Brazil, vitamin D supplementation did not significantly reduce the hospital length of stay in patients with moderate to severe COVID-19. However, neither patients in the vitamin D group nor patients in the placebo group were clearly vitamin D deficient, with 25(OH) D levels above 20 ng/ml in each group (Murai et al., 2021).

This study has several limitations. First, the enormous burden of COVID-19 outbreak on healthcare personnel and resources at our center did not allow us to repeatedly assess serum levels of 25(OH) D during the disease, which would have potentially better established the evolving relationship between levels of inflammatory biomarkers and 25(OH) D. Second, we did not know the pre-existing vitamin D status of the patients in our study cohort. Therefore, the possibility of so-called reverse causality to explain the relationship of 25(OH) D with COVID-19, i.e., that a more severe disease produces greater 25(OH) D reduction must still be considered. Very recently, a small but elegant study documented that 25(OH) D declines within 2 hours of onset of inflammatory reaction; thus we can speculate that patients that were ill for a longer time before admission had lower 25(OH) D levels (Smolders et al., 2021). Last, vitamin D supplementation during hospitalization could in fact influence mortality. All our patients were supplemented with vitamin D according to the treatment protocol at our institution, regardless of baseline 25(OH) D serum levels (loading dose: 30 000 IU of cholecalciferol per day for the first 3 days, followed by 7500 IU cholecalciferol per day). We did not have a control group of patients without supplementation. Vitamin D is a threshold nutrient; therefore, patients with severe 25(OH) D deficiency are most likely to benefit from vitamin D supplementation. Given this, we could speculate that some patients who were severely 25(OH) D deficient could improve their nutritional status upon supplementation and thus exhibit a milder course of the disease. Our study also has several strengths. Notably, this is a real-world study that prospectively evaluated a large homogeneous, properly defined group of acutely ill patients with COVID-19 pneumonia. Serum levels of 25(OH) D in all patients were measured exactly at the time of admission together with markers of inflammation. The assessment of 25(OH) D in all patients was performed by the same method and assay. All the patients in our study group had COVID-19 infection confirmed by positive RT-PCR nasopharyngeal smear. Finally, to our knowledge, this is the first study to describe increased mortality specifically among patients with COVID-19 and severe vitamin D deficiency, as defined by 25(OH) D levels less than 12 ng/ml.

In conclusion, this study shows that 25(OH) D insufficiency or deficiency is prevalent among acutely ill patients hospitalized for COVID-19 pneumonia. Importantly, the serum level of 25(OH) D is an independent risk factor of mortality. In addition, a serum 25(OH) D level <12 ng/ml at the time of admission seems to be a good indicator of morbidity and mortality in patients with COVID-19 and is associated with a 11% increased mortality rate than patients with a serum level above 12 ng/ml. According to these results, routine 25(OH) D assessment at admission could be relevant for risk stratification and planning for treatment strategy in patients with COVID-19.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this paper.

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Ethical Approval Statement

The study was conducted in accordance with the Declaration of Helsinki and was approved by The Ethics Committee of the University Hospital Bratislava. Informed and written consent was obtained either from the participants or a first-degree relative.

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