

Vitamin D and COVID-19: comparative analysis with other respiratory infections and impact of comorbidities

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Abstract

Introduction The COVID-19 pandemic has promoted an intensive investigation into the pathophysiological mechanisms of SARS-CoV-2 infection, risk factors, and its impact on disease severity. Vitamin D has generated significant attention for its potential role in viral prevention and immune defense due to its pleiotropic functions, including immunomodulation and antimicrobial effects. This study aimed to assess serum 25(OH)D3 levels in patients with COVID-19 compared to those with other viral respiratory infections and to evaluate associations of vitamin D levels with symptomatology, clinical characteristics, presence of comorbidities and laboratory investigation.

Methods The study included 78 patients admitted to a hospital with COVID-19 (52 patients) or other viral respiratory infections (26 patients). Routine blood biomarkers, markers of inflammation, markers of endothelial dysfunction, serum 25(OH)D3 were analyzed, and patients were classified according to vitamin D levels and presence of comorbidities.

Results Most patients had vitamin D levels <30 ng/mL and there was no significant difference in 25(OH)D3 levels between patients with and without COVID-19 ($p=0.768$). Aging and comorbidity prevalence were significantly increased in the COVID-19 than in the non-COVID-19 group ($p<0.001$; $p=0.049$). A significant positive correlation was determined between endocan level and serum ferritin concentration in patients with COVID-19 and vitamin D deficiency. A borderline significantly elevated NLR was observed in patients with COVID-19 who were also vitamin D deficient, compared with the similar non-COVID-19 subgroup ($p=0.05$). In patients with COVID-19 and insufficient vitamin D, levels of 25(OH)D negatively correlated with endocan. Interestingly, COVID-19 patients with diabetes exhibited significantly lower 25(OH)D3 levels compared to non-diabetic patients ($p=0.003$), along with higher ferritin levels, suggesting a potential association between vitamin D deficiency and diabetes in COVID-19.

Conclusions These findings contribute to the understanding of the complex interplay between vitamin D status, comorbidities, and COVID-19 outcomes, emphasizing the need for further research to elucidate their underlying mechanisms and clinical implications.

Keywords COVID-19, vitamin D, ferritin, diabetes.

Introduction

The recent COVID-19 pandemic has generated major interest in investigating the

pathophysiological mechanisms of SARS-CoV-2 infection, and in identifying risk factors and strategies to prevent infection or progression to

Received: 15 April 2024; revised: 02 June 2024, 14 June 2024 and 17 July 2024; accepted: 20 July 2024.

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Article downloaded from www.germs.ro

Published September 2024

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ISSN 2248 - 2997

ISSN - L = 2248 - 2997

severe forms. Clinically, many of the cases of COVID-19 requiring hospitalization are characterized by dysregulated hyperactivation of the immune system, inflammation, a pro-thrombotic state and damaged vascular endothelium. The presence of comorbidities further elevates the likelihood of progression to severe disease.¹⁻³

Previous studies indicated vitamin D deficiency as a risk factor for various infections.^{4,5} In the recent COVID-19 pandemic context, the potential significance of vitamin D as a factor involved in viral prevention and immune defense has been the subject of numerous analyses, but the results have so far been inconclusive. Aging and multiple chronic diseases, in particular cardiovascular and metabolic disorders, are risk factors severe COVID-19 and can be linked to vitamin D deficiency.^{6,7}

Vitamin D is a multifaceted hormone involved in phospho-calcium metabolism, also having significant immuno-modulatory properties and antimicrobial effects. Vitamin D is synthesized in the skin through ultraviolet irradiation of 7-dehydrocholesterol,⁸ circulates bound to vitamin D binding protein (DBP) to the liver where is converted to hydroxyvitamin D (25(OH)D) and then hydroxylated by CYP 27B1 in the kidney, macrophages and dendritic cells to its active form, calcitriol (1,25 (OH)₂).^{4,9} The hydroxyderivatives bind to vitamin D (VDR), retinoid X (RXR) or liver X (LXR) expressed by different tissues, indicating the influence of vitamin D on various physiological processes.¹⁰ Beyond the target tissues (bone, kidney, intestines), VDRs are expressed in brain, breast, pancreas, colon and immune cells (macrophages, B and T lymphocytes, neutrophils, dendritic cells).¹¹ VDR activation in immune cells induces production of cathelicidin and defensin, proteins with antiviral activity. Vitamin D reduces inflammation mediated by T cells, enhances the anti-inflammatory functions of Treg cells⁹ alters the expression of genes for cytokine production¹² and reduces oxidative stress.¹³ Serum levels > 30 ng/mL significantly inhibited production of IL-6 and TNF- α induced by lipopolysaccharide.¹⁴ The Endocrine Society recommends the measurement of serum circulating 25-hydroxyvitamin D

[25(OH)D] levels to assess vitamin D status. Vitamin D deficiency is defined as a 25(OH)D below 20 ng/mL (50 nmol/liter) level, vitamin D insufficiency as a 25(OH)D of 21-29 ng/mL (50-75 nmol/liter) level and sufficiency as a 25(OH)D of >30 ng/mL (75 nmol/liter).¹⁵

After cardiovascular diseases, the second most frequent comorbidity in patients with COVID-19 is diabetes mellitus (DM) and its relationship with SARS-CoV2 infection is complex and bidirectional. Chronic endothelial dysfunction and low-grade chronic inflammation in DM induces metabolic, vascular, immune and hematological abnormalities and increases the risk for severe stages of disease.^{16,17} Alterations of glucose metabolism induce decompensations of preexisting diabetes or may, in extreme cases, create conditions for new onset diabetes.¹⁸ In infection, the hyperglycemic status was associated with a reduced production of interleukins and reduced phagocytic activity of polymorphonuclear leucocytes.^{19,20} Previous analyses indicate that elevated levels of vitamin D are associated with a reduction in the risk of DM.¹⁹

In type 2 diabetes, vitamin D affects the main pathways involved in the pathophysiology of the disease: insulin secretion, insulin sensitivity and systemic inflammation.^{21,22} It contributes to the maintenance of normal pancreatic function by activating VDR and modulating calcium channels in pancreatic beta cells. In the systemic inflammation, vitamin D effects are: deactivation of inflammatory cytokines, increasing calbindin expression and reduction of the glycated end product accumulation.

To improve the understanding of the prognosis of COVID-19 evolution, new inflammatory molecules, along with classical markers such as C-reactive protein (CRP) and fibrinogen, were investigated. In this context, ferritin was used as an inflammation marker and endocan as an indicator of endothelial dysfunction in infectious diseases. High levels of ferritin are products of inflammation and contribute to the cytokine storm evolution. Ferritin protects against oxidative stress by reducing available iron for Fenton reaction, source of the damaging oxygen free radicals at the

inflammation site in any type of inflammatory processes (infection, injury, cancer).²³ Dysfunctions in metabolism of iron and hemoglobin (inhibition of hem metabolism, hemoglobin denaturation) with hyperferritinemia can be a pathological mechanism in COVID-19.²⁴ Iron metabolism is modulated by hepcidin hormone through ferroportin (iron transmembranar transporter) degradation. A structural similarity of distant amino-acid domains exists between hepcidin and SARS-CoV-2 spike glycoprotein and this can explain dysfunction of iron metabolism and hyperferritinemia. Hyperferritinemia can induce coagulopathies, macrophage activation syndrome, hemochromatosis-like liver injury.²⁵ Vitamin D can influence hepcidin antagonist pathways and administration of high doses induces low levels of hepcidin.⁵ The link between vitamin D and COVID-19 inflammation especially in diabetic patients is an important topic to investigate.²⁶

Endothelial dysfunction is an important factor in COVID-19 pathogenesis and is associated with disease severity. Proinflammatory cytokines induce endothelial activation and dysfunction by upregulating endocan synthesis and secretion. Endothelial damage and thrombotic complications increase endocan levels and can be used as markers for endothelial dysfunction.³

This study aimed to evaluate the differences between serum levels of 25(OH)D3 and inflammatory markers in patients with COVID-19 and non-COVID-19 respiratory infections, to investigate the relation between clinical and laboratory characteristics of patients with COVID-19/non-COVID-19 differentiated by the 25(OH)D3 levels and the relation between vitamin D levels and comorbidities in patients with COVID-19.

Methods

The study included 78 consecutive patients admitted at the National Institute for Infectious Diseases “Prof. Dr. Matei Balș”, Bucharest, Romania, between 1 September 2022 and 31 January 2023. The study group was divided into 2 subgroups: the COVID-19 subgroup (52 patients with a positive RT-PCR test) and the non-

COVID-19 subgroup (26 patients with other respiratory viral infections). Conducted according to the principles of the Declaration of Helsinki, the study was approved by the Bioethics Committee of the National Institute for Infectious Diseases “Prof. Dr. Matei Balș” (approval number: C06884/23.06.2022). COVID-19 diagnosis was confirmed by a polymerase chain reaction test (RT-PCR). The inclusion criteria were as follows: 1) positive RT-PCR test for COVID-19 or laboratory diagnosis of respiratory infection for the non-COVID-19 subgroup; 2) age >18 years. Pregnant woman and subjects with malignancies or other viral infections (HIV, viral hepatitis B and C) were excluded. Blood samples were collected after obtaining written consent from all patients enrolled.

Clinical and biological data, including symptomatology and comorbidities, were collected. Routine blood biomarkers (leukocytes, neutrophils, lymphocyte count), inflammation markers (CRP, fibrinogen, interleukin (IL)-6, ferritin), markers of endothelial dysfunction (endocan) were assessed.

Serum levels of glucose (mg/dL), alkaline phosphatase (U/L), lactate dehydrogenase (LDH, U/L), gamma-glutamyl transferase (GGT, U/L) and lipase (U/L) were measured spectrophotometrically using a VITROS FS3 device (QuidelOrtho, USA). Ferritin (ng/mL) levels were determined using a chemiluminescence method on an ADVIA XPT device (Siemens Healthcare, Germany), while IL 6 (pg/mL) and endocan (pg/mL) levels were analyzed using ELISA methods according to manufacturer’s instructions. White blood cell (WBC), lymphocyte and neutrophil counts were determined using a DXH 900 device (Beckman Coulter, USA), with results expressed as cells/ μ L.

Vitamin D3 levels were measured by quantifying serum levels of 25(OH)D levels using liquid chromatography tandem mass spectrometry (LC-MS/MS). The calibration curve ranges from 2.3 to 118.9 ng/mL. Quality control samples were prepared at 3 concentrations levels (8.6, 59.1 and 105.3 ng/mL). Eighty μ L of d6-25OHD3 deuterate internal standard diluted with Reagent A (deproteinization solution) were

added into each microcentrifuge tube containing 50 µL of calibration standards, quality control or serum samples. The samples were diluted with 100 µL of methanol, vortexed for 4 minutes and centrifuged 4 minutes at 14000 g. Then, 100 µL of supernatant were diluted with 50 µL mobile phase water: methanol (90:10 v/v), transferred into LC insert vials and 10 µL from each was injected into the LC-MS/MS system.

The data presented in this article are part of a more extensive research process regarding COVID-19, the methodology used in this article being also used in our previous published work.³

Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics (IBM Corp, USA). Results for normally distributed parameters were presented as the mean $\pm$ standard deviation, while results for non-normally distributed parameters were presented as the median [interquartile range (IQR): quartile 25; quartile 75]. Normality was assessed using Kolmogorov-Smirnov test. To detect significant differences among study group, a one-way ANOVA (with a Games-Howell post hoc test) or T-test was used for normally distributed data and Kruskal-Wallis or Mann-Whitney test was used for non-normally distributed data, depending on the data distribution and the number of groups assessed. The Spearman rank correlation method was employed to determine significant correlations. The level of significance was set at 0.05.

Results

The study included 52 patients with COVID-19 and 26 patients without COVID-19. In the non-COVID-19 group, patients were diagnosed with different respiratory viral infections (influenza, respiratory syncytial virus, adenovirus, metapneumovirus). Table 1 summarizes the demographic and clinical characteristics of the patients (COVID-19 and non-COVID-19).

In the COVID-19 group, the average age was 72.28 ± 14.84 years, significantly higher than in the non-COVID-19 group, 51.69 ± 19.41 ($p < 0.001$). Body mass index (BMI) did not significantly differ between the two groups ($p = 0.479$). The most common symptoms in the two groups were: cough, fever, headache, myalgia,

dyspnea, pharyngitis and fatigability. Among patients with COVID-19, cough, fever, fatigability were observed in 57.69%, 36.53% and 26.92% of patients, respectively. In the non-COVID-19 group, the incidence of the same symptoms occurred in 50%, 50% and 23.07% of patients, respectively. The rates of headache, fatigability and pharyngitis were higher in the COVID-19 group than in the non-COVID-19 group, but the proportion of patients with dyspnea was higher in those without COVID-19. There were no significant differences between the two groups in the symptom incidence.

The majority of patients with COVID-19 (84.61%) had comorbidities with a significantly increased prevalence compared to the non-COVID-19 group ($p = 0.049$). In both groups, the first comorbidity was hypertension, present in more than a half of patients with COVID-19 (53.84%). In the COVID-19 group, obesity was the second most commonly observed comorbidity, affecting 23.07% of patients, whereas in the non-COVID-19 group it was the fourth comorbidity (15.38%). Moreover, patients with COVID-19 exhibited a higher prevalence of dyslipidemia (21.15%), diabetes (17.3%), coronary artery disease (15.38%) and chronic kidney disease (13.46%) than patients without COVID-19 (diabetes 7.69%; dyslipidemia 3.86%).

A comparative analysis of biochemical and inflammatory profiles of patients from the two groups indicated significantly elevated serum levels of C-reactive protein in the non-COVID-19 group compared to the COVID-19 group ($p = 0.015$). Analysis of the biochemical parameters revealed higher serum lipase levels in the COVID-19 group compared to the non-COVID-19 group, although the difference was not statistically significant ($p = 0.097$).

The median serum levels of 25(OH)D3 did not differ significantly between the two groups ($p = 0.768$). The median vitamin D level was 21.93 ± 10.04 ng/mL for the COVID group (2.25 – 40.8 ng/mL) and 21.23 ± 9.6 ng/mL for the non-COVID-19 group (2.1 – 39.32 ng/mL).

We divided patients from each initial group (COVID-19/non-COVID-19) into three subgroups, according to vitamin D levels, as

Table 1. Demographic, clinical and laboratory characteristics of the study groups

	COVID-19	Non-COVID-19	p
General characteristics			
Age (years)	72.28 ± 14.84	51.69 ± 19.41	p<0.001
Male/female	26/26	13/13	
Body mass index (BMI), kg/m ²	23.75 [22.36; 25]	24.6 [23.12; 26.06]	0.479
Comorbidities, n (%)			
Hypertension, n (%)	44 (84.61%)	17 (65.38%)	0.049
Obesity, n (%)	28 (53.84%)	8 (30.76%)	0.190
Dyslipidemia, n (%)	12 (23.07%)	4 (15.38%)	0.457
Dyslipidemia, n (%)	11 (21.15%)	1 (3.86%)	0.937
Heart failure, n (%)	10 (19.23%)	6 (23.07%)	0.385
Rhythm disturbances, n (%)	10 (19.23%)	5 (19.23%)	0.427
Diabetes, n (%)	9 (17.3%)	2 (7.69%)	0.706
Coronary artery disease, n (%)	8 (15.38%)	0	N/A
Chronic kidney disease, n (%)	7 (13.46%)	0	N/A
Chronic obstructive pulmonary disease, n (%)	3 (5.76%)	4 (15.38%)	0.762
Symptomatology			
Cough, n (%)	30 (57.69%)	13 (50%)	0.798
Fever, n (%)	19 (36.53%)	13 (50%)	0.298
Fatigability, n (%)	14 (26.92%)	6 (23.07%)	0.844
Headache, n (%)	13 (25%)	4 (15.38%)	0.451
Pharyngitis, n (%)	12 (23.07%)	2 (7.69%)	0.154
Dyspnea, n (%)	9 (17.3%)	7 (26.92%)	0.316
Myalgia, n (%)	9 (17.3%)	5 (19.23%)	0.769
Biochemical and inflammatory parameters			
Glucose (mg/dL)	106.5 [98; 126.25]	113.27 ± 35.23	0.699
LDH (U/L)	234 [187; 305]	241 [214; 285]	0.819
GGT (U/L)	33 [25; 51]	57.96 ± 35.07	0.058
Alkaline phosphatase (U/L)	78 [55; 94]	91.13 ± 28.42	0.929
Lipase (U/L)	117.5 [55; 193.75]	88.11 ± 75.16	0.097
C-reactive protein (mg/L)	27.6 [7.52; 85.3]	98.55 ± 76.78	0.015
Fibrinogen (mg/dL)	367.17 [307.5; 575.7]	507.76 ± 221.33	0.126
White blood cell count × 1000/μL	7.4 [4.56; 9.28]	8.53 ± 3.72	0.651
Neutrophils × 1000/μL	5.5 ± 2.59	6.18 ± 3.2	0.409
Lymphocytes × 1000/μL	0.85 [0.69; 1.28]	1.47 ± 0.96	0.901
Neutrophil/lymphocyte ratio	7.51 ± 6.59	6.18 ± 4.08	0.345
25(OH)D3 (ng/mL)	21.93 ± 10.04	21.23 ± 9.6	0.768

defined in the methods section. Among the COVID-19 group, vitamin D deficiency, insufficiency and sufficiency were observed in 42.3%, 32.69% and 25% of patients, respectively. In the non-COVID-19 group, the same vitamin D distribution occurred in 50%, 26.92% and 23.07% of patients, respectively. In the COVID-19 subgroups symptoms incidence did not significantly differ between patients with different

serum levels of vitamin D. In the non-COVID-19 group, patients with deficient vitamin D had a significantly higher incidence of fever (p=0.033). The prevalence of comorbidities was similar between subgroups of each group.

Patient with COVID-19 with deficient vitamin D had elevated levels of LDH, CRP, ferritin, IL-6, neutrophils and neutrophil/lymphocyte ratio (NLR). Elevated IL-

6 levels, above the normal limits, were found in all patients. Large variability among them was documented and no correlation was detectable between vitamin D levels and IL-6 values. Moreover, in patients with deficient vitamin D, the IL-6 values were more elevated in comparison with patients with insufficient or sufficient vitamin D levels, but not significantly. Alkaline phosphatase activity was significantly elevated in the COVID-19 group with sufficient vitamin D ($p=0.033$) than in patients from the other two subgroups. In the COVID-19 and deficient vitamin D subgroup, ferritin correlated with endocan ($r=0.607$, $p=0.048$) and NLR ($r=0.692$; $p=0.018$). In the insufficient subgroup vitamin D correlated negatively with endocan ($r=-0.611$, $p=0.009$) and endocan positively correlated with GGT ($r=0.563$, $p=0.023$) and alkaline phosphatase ($r=0.541$, $p=0.031$). Patients with COVID-19 and sufficient vitamin D presented negative correlation between vitamin D and lipase ($r=-0.786$, $p=0.048$) and GGT ($r=-0.636$, $p=0.040$); endocan positively correlated with lymphocytes ($r=0.602$; $p=0.029$).

In the non-COVID-19 group, biomarkers of inflammation (CRP, fibrinogen) were significantly elevated in group with deficient vitamin D than in the insufficient or sufficient subgroups ($p=0.005$; $p=0.036$). Patients from the non-COVID-19 group with insufficient vitamin D had a significantly elevated NLR compared to patients with deficient or sufficient vitamin D ($p=0.012$). A correspondence between vitamin D and inflammatory markers has been documented, although not statistically significant (Table 2).

To complete the study, we include a comparative analysis of levels of biochemical and inflammatory parameters, presence and type of comorbidities between the COVID-19 and non-COVID-19 groups with the same vitamin D status. A borderline statistically significant increase in NLR was detected in COVID-19 vitamin D deficient compared to non-COVID-19 ($p=0.05$). The non-COVID-19 vitamin D deficient group had statistically elevated levels of GGT and alkaline phosphatase than the COVID-19 subgroup ($p=0.007$; $p=0.012$). The comparison between the COVID-19/non-COVID-19 groups with insufficient vitamin D

revealed significantly higher levels of lactate dehydrogenase ($p=0.044$), CRP ($p<0.001$), fibrinogen ($p=0.018$) and neutrophil/lymphocyte ratio ($p=0.04$) in non-COVID-19 (Table 3).

Analysis of the vitamin D status in patients with COVID-19 and different comorbidities revealed no significant difference between patients with and without cardiovascular diseases, dyslipidemia or obesity. Significant differences were observed in the COVID-19 group between subjects with or without diabetes. Patients with diabetes and COVID-19 had lower serum levels of 25(OH)D3 compared to patients without diabetes ($p=0.003$) – Table 4. In patients without cardiovascular diseases, 25(OH)D3 negatively correlated with ferritin ($r=-0.829$, $p=0.042$) and IL6 ($r=-0.786$; $p=0.021$).

In patients with COVID-19 and diabetes, values of blood glucose, WBC, and neutrophils were significantly different compared to patients with COVID-19 without diabetes ($p=0.002$; 0.015 ; 0.011). Ferritin positively correlated with CRP ($r=0.464$; $p=0.022$), fibrinogen ($r=0.526$; $p=0.008$) and IL6 ($r=0.62$; $p=0.008$). Ferritin levels were three times the upper limit in the patients with COVID-19 and diabetes and were significantly higher than in the patients with COVID-19 without diabetes ($p=0.017$). In the patients with diabetes, 25(OH)D3 levels positively correlated with alkaline phosphatase ($r=0.714$, $p=0.047$) and ferritin negatively correlated with endocan ($r=-0.900$; $p=0.037$) – Table 5.

Discussion

The immune-modulatory effects of vitamin D can influence the risk and evolution of infectious diseases and deficient serum vitamin D is related to infections in the respiratory tract and acute lung injury.²⁷

Few published reports have compared COVID-19 and non-COVID-19 respiratory infections. The present study analyses symptomatology, comorbidities, clinical characteristics and laboratory investigations between patients with COVID-19 and without COVID-19. Presence of comorbidity is a risk factor for COVID-19 susceptibility and severity. In our study, aging and comorbidity incidence

Table 2. Comparative analysis of biochemical and inflammatory parameters, presence and type of comorbidities between patients with different vitamin D levels in the COVID-19/non-COVID-19 groups

	COVID-19				Non-COVID-19			
	Deficient (1) N=22 (42.3%)	Insufficient (2) N=17 (32.69%)	Sufficient (3) N=13 (25%)	p	Deficient (1) N=13 (50%)	Insufficient (2) N=7 (26.92%)	Sufficient (3) N=6 (23.07%)	p
Symptoms								
Cough, n (%)	14 (63.63%)	8 (47.05%)	8 (61.53%)	0.553	7 (53.84%)	2 (28.57%)	5 (83.33%)	0.142
Fever, n (%)	9 (40.90%)	6 (35.29%)	4 (30.76%)	0.827	8 (61.53%)	1 (14.28%)	5 (83.33%)	0.033
Headache, n (%)	5 (22.72%)	3 (17.64%)	3 (23.07%)	0.910	1 (7.69%)	0	3 (50%)	N/A
Myalgia, n (%)	5 (22.72%)	3 (17.64%)	1 (7.69%)	0.523	1 (7.69%)	2 (28.57%)	2 (33.33%)	0.320
Dyspnea, n (%)	4 (18.18%)	3 (17.64%)	2 (15.38%)	0.976	2 (15.38%)	2 (28.57%)	3 (50%)	0.284
Pharyngitis, n (%)	4 (18.18%)	4 (23.52%)	4 (30.76%)	0.693	1 (7.69%)	1 (14.28%)	0	N/A
Fatigability, n (%)	4 (18.18%)	8 (47.05%)	2 (15.38%)	0.072	3 (23.07%)	1 (14.28%)	2 (33.33%)	0.585
Comorbidities, n (%)								
Diabetes, n (%)	19 (86.36%)	12 (70.58%)	12 (92.3%)	0.248	7 (41.17%)	4 (57.14%)	3 (50%)	0.967
Cardiovascular, n (%)	6 (27.27%)	3 (17.64%)	0	N/A	1 (7.69%)	1 (14.28%)	1 (16.66%)	0.820
Obesity, n (%)	13 (59.09%)	11 (64.70%)	9 (69.23%)	0.827	5 (38.46%)	4 (57.14%)	4 (66.67%)	0.471
Dyslipidemia, n (%)	7 (31.81%)	2 (11.76%)	3 (23.07%)	0.905	1 (7.69%)	0	3 (50%)	N/A
	6 (27.27%)	1 (5.88%)	4 (30.76%)	0.165	0	1 (14.28%)	0	N/A
Biochemical and inflammatory parameters								
LDH (U/L)	304.89 ± 217.43	211.76 ± 46.17	271.18 ± 71.9	0.169	308.9 ± 161.51	266.71 ± 80.11	214.66 ± 36.74	0.322
GGT (U/L)	31.5 [25; 38]	52.12 ± 32.91	48.45 ± 28.02	0.238	70.15 ± 41.34	59.28 ± 21.49	30 ± 12.94	0.061
Alkaline phosphatase (U/L)	69.45 ± 26.96	84 [72; 108.5]	99.9 ± 80.56	0.033	98.88 ± 31.6	84.85 ± 14.17	86.83 ± 37	0.586
Lipase (U/L)	125.54 ± 85.55	153.33 ± 115.08	111.71 ± 55.67	0.642	91.22 ± 71.03	98.4 ± 109.12	63.4 ± 39.74	0.743
C-reactive protein (mg/L)	70.88 ± 84.25	33.06 ± 41.57	54.06 ± 51.15	0.209	103.75 ± 76.96	168.8 ± 45.21	31.36 ± 29.67	0.005 2: 3= 0.001
Fibrinogen (mg/dL)	428.22 ± 153.7	447.88 ± 154.42	447.55 ± 169.2	0.787	500.08 ± 210.80	656.42 ± 229.66	349.66 ± 114.03	0.036 2: 3= 0.016
Ferritin (ng/mL)	479.07 ± 352.3	508 ± 419.79	247.68 ± 146.3	0.276	-	-	-	-

	COVID-19				Non-COVID-19			
	Deficient (1) N=22 (42.3%)	Insufficient (2) N=17 (32.69%)	Sufficient (3) N=13 (25%)	p	Deficient (1) N=13 (50%)	Insufficient (2) N=7 (26.92%)	Sufficient (3) N=6 (23.07%)	p
Interleukin-6 (pg/mL)	268.69 ± 379.54	166.28 ± 243.13	47.5 ± 209.28	0.640	-	-	-	-
White blood cells × 1000/ μ L	9.33 ± 7.47	6.73 ± 2.43	7.41 ± 2.79	0.298	9.46 ± 4.57	8.78 ± 2.54	6.21 ± 1.54	0.209
Neutrophils × 1000/ μ L	6.17 ± 2.75	4.98 ± 32.16	5.55 ± 2.74	0.359	6.64 ± 4.05	6.85 ± 2.07	4.43 ± 1.34	0.320
Lymphocyte × 1000/ μ L	0.78 [0.5; 1.1]	1.06 ± 0.54	1.11 ± 0.43	0.238	1.9 ± 0.96	1.03 ± 0.99	1.05 ± 0.51	0.068
Neutrophil/ lymphocyte ratio	9.53 ± 8.39	5.98 ± 3.82	3.76 ± 4.86	0.183	4.63 ± 3.29	9.86 ± 4.32	5.23 ± 2.96	0.012 1: 2=0.020 2: 3=0.042
Endocan (pg/mL)	65.48 ± 29.57	85.08 ± 30.55	84.19 ± 36.4	0.107	-	-	-	

Data are expressed as the mean and standard deviation for values with a normal distribution and the median (quartile 25, quartile 75) for those without a normal distribution.

Table 3. Comparative analysis of biochemical and inflammatory parameters, presence and type of comorbidities between the COVID-19 and non-COVID-19 groups divided according to vitamin D levels

Parameters	Deficient Vitamin D		p	Insufficient Vitamin D		p	Sufficient Vitamin D		p
	COVID-19	Non- COVID-19		COVID-19	Non-COVID-19		COVID-19	Non-COVID-19	
LDH (U/L)	304.89 ± 217.43	308.9 ± 161.51	0.893	211.76 ± 46.17	266.71 ± 80.11	0.044	271.18 ± 71.99	214.66 ± 36.74	0.095
GGT (U/L)	31.5 [25; 38]	70.15 ± 41.34	0.007	52.12 ± 32.91	59.28 ± 21.49	0.605	48.45 ± 28.02	30 ± 12.94	0.151
Alkaline phosphatase	69.45 ± 26.96	98.88 ± 31.6	0.012	84 [72; 108.5]	84.85 ± 14.17	0.400	86.83 ± 37	99.9 ± 80.56	0.714
Lipase (U/L)	125.54 ± 85.55	91.22 ± 71.03	0.371	153.33 ± 115.08	98.4 ± 109.12	0.401	111.71 ± 55.67	63.4 ± 39.74	0.129
C-reactive protein (mg/L)	70.88 ± 84.25	103.75 ± 76.96	0.256	33.06 ± 41.57	168.8 ± 45.21	<0.001	54.06 ± 51.15	31.36 ± 29.67	0.330
Fibrinogen (mg/dL)	428.22 ± 153.79	500.08 ± 210.80	0.227	447.88 ± 154.42	656.42 ± 229.66	0.018	447.55 ± 169.2	349.66 ± 114.03	0.222
White blood cells × 1000/ μ L	9.33 ± 7.47	9.46 ± 4.57	0.955	6.73 ± 2.43	8.78 ± 2.54	0.077	7.41 ± 2.79	6.21 ± 1.54	0.344
Neutrophils × 1000/ μ L	6.17 ± 2.75	6.64 ± 4.05	0.690	4.98 ± 32.16	6.85 ± 2.07	0.065	5.55 ± 2.74	4.43 ± 1.34	0.362
Lymphocytes × 1000/ μ L	0.78 [0.5; 1.1]	1.9 ± 0.96	0.851	1.06 ± 0.54	1.03 ± 0.99	0.909	1.11 ± 0.43	1.05 ± 0.51	
Neutrophil/lymphocyte ratio	9.53 ± 8.39	4.63 ± 3.29	0.050	5.98 ± 3.82	9.86 ± 4.32	0.04	3.76 ± 4.86	5.23 ± 2.96	0.626
Comorbidities									
Diabetes	6 (27.27%)	1 (7.69%)	0.161	3 (17.64%)	1 (14.28%)	0.840	0	1 (16.66%)	N/A
Cardiovascular diseases	13 (59.09%)	5 (38.46%)	0.238	11 (84.61%)	4 (66.66%)	0.727	9 (69.23%)	4 (66.66%)	0.911
Obesity	7 (31.81%)	1 (7.69%)	0.100	2 (15.38%)	0	N/A	3 (23.07%)	3 (50%)	0.240

Data are expressed as the mean and standard deviation for values with a normal and the median (quartile 25, quartile 75) for those without a normal distribution.

Table 4. Vitamin D serum levels (ng/mL) in patients with COVID-19 with/without comorbidities

Comorbidity	Present	Absent	P value
Cardiovascular	21.93 ± 10.2	21.93 ± 10	0.998
Obesity	19.12 ± 10.06	22.77 ± 10.01	0.274
Dyslipidemia	22.44 ± 12.14	21.79 ± 9.58	0.851
Diabetes	13.29 ± 9.42	23.74 ± 9.29	0.003

Table 5. Serum concentration of inflammatory markers and vitamin D depending on disease severity in the COVID-19 group

	Without diabetes	With diabetes	P value
Age, years	72 [61; 82]	83 [77; 86.5]	0.023
Blood glucose (mg/dL)	104 [96; 121]	141 [107; 178.5]	0.002
CK (U/L)	76 [46.75; 143]	43 [24; 74.5]	0.038
C-reactive protein (mg/L)	25.5 [6.69; 69.4]	32.5 [14.35; 140]	0.233
Ferritin (ng/mL)	234 [163.9; 489]	854 [330.25; 1173.3]	0.017
IL6 (pg/mL)	59.7 [16.4; 185.33]	229.2 [64.8; 0]	0.171
WBC × 1000/μL	6.6 [4.4; 8.85]	9.75 [8.4; 11.55]	0.015
Neutrophils × 1000/μL	5.12 ± 2.47	7.47 ± 2.46	0.011
Lymphocytes × 1000/μL	0.86 [0.69; 1.31]	0.76 [0.6; 1.23]	0.608
Neutrophil/lymphocyte ratio	6.93 ± 6.08	10.25 ± 8	0.213
Fibrinogen (mg/dL)	361.28 [291.72; 556.98]	548 [364.09; 611.01]	0.115
Endocan (pg/mL)	77.2 ± 31.99	77.28 ± 30.31	0.994
Vitamin D (ng/mL)	25.84 ± 11.39	13.3 ± 9.42	0.003

Data are expressed as the mean and standard deviation for values with a normal distribution and the median (quartile 25, quartile 75) for those without a normal distribution.

were significantly increased in the COVID-19 group compared to the non-COVID-19 group ($p < 0.001$, $p = 0.049$) and our data correspond to the study by Goyal et al. (2020).²⁸

The focus of our study was on the relation between vitamin D levels and clinical and inflammation status in COVID-19 vs non-COVID-19. The results of the study support a high prevalence of vitamin D deficiency/insufficiency in patients with SARS-COV-2/other viral respiratory infections. Data addressing the COVID-19-vitamin D relationship have been published, but the results are not conclusive. The present study results indicate that patients from both groups (COVID-19; non-COVID-19) have vitamin D < 30 ng/mL (insufficiency) and it cannot be affirmed that there is a specific association between 25(OH)D insufficiency and greater vulnerability to COVID-19 infection. Our findings are in agreement with the study by Hastie et al. (2020) and do not support a potential link between vitamin D

concentrations and risk of COVID-19. The link between vitamin D deficiency and COVID-19 should be analyzed in correlation with age and presence of comorbidities.

Analyses of inflammatory markers between subgroups according to the vitamin D levels revealed the following results. In the COVID-19 subgroups: significantly higher levels of alkaline phosphatase in sufficient COVID-19 than in the other COVID-19 subgroups, without marked differences of other inflammatory markers, even patients with deficient and insufficient levels had CRP, ferritin and IL6 levels over the normal range and higher than in the sufficient group. In the non-COVID-19 subgroups: the insufficient subgroup had significantly elevated levels of CRP, fibrinogen and NLR compared to the sufficient one.

The non-COVID group had a marked inflammatory response in the insufficient group, whereas, in the COVID-19 subgroups the inflammation status did not differ. Clinical

manifestation, early diagnostic and treatment in COVID-19 and reduced symptoms with extending time to hospital presentation in non-COVID-19 could theoretically influence the differences in inflammatory reactions.

Two new biomarkers were analyzed in the COVID-19 group: ferritin and endocan. Ferritin has a pivotal in SARS-COV-2 infection and inflammation and elevated levels were shown to correlate with disease severity.²⁴ Endocan plays a key role in vascular pathologies, organ-specific inflammation and endothelium-dependent pathological disorders.³ A significant positive correlation was determined between endocan level and serum ferritin concentration in patients with COVID-19 and vitamin D deficiency. Our observation revealed that in the context of vitamin D deficiency, ferritin can have an impact on endothelial function. Few studies analyzed the relation between ferritin and endocan, and found a positive correlation, in patients with hematological or chronic kidney diseases.^{29,30} The influence of iron on endothelial damage attracts attention on the importance of ferritin monitoring.

Inflammation can be predicted by elevated neutrophil count and decreased lymphocytes. COVID-19 induces loss of lymphocyte activity with marked reduction of the immunological responses. Migration and compartmentalization to damaged tissues may be the cause of decreased lymphocyte count.³¹ A combination of these two measures, neutrophil/ lymphocyte ratio is indicative of a dysregulated immune response and considered predictive of an inflammatory status. Elevated NLR could potentially serve as a prognostic biomarker reflecting the intensity of the host's response to the pathogen. In patients with COVID-19, who were deficient, ferritin positively correlated with NLR. The elevation of ferritin, which is produced by hepatocytes and macrophages, may be linked to the activation macrophages.

In patients with COVID-19 and insufficient vitamin D, levels of 25(OH)D negatively correlated with endocan and revealed that vitamin D status can influence the endothelial function. This observation supports the idea that vitamin D influences inflammation and

regulation of endothelial dysfunction in COVID-19 patients with 25(OH)D serum levels between 20-30 ng/mL. Atis et al. observed a decrease in the endothelial function marker, endocan, after vitamin D treatment in kidney transplant patients with vitamin D deficiency and negative correlation between the two molecules.³² Our study is the first in which serum vitamin D-endocan levels are evaluated in patients with COVID-19. Experimental studies on animals or in vitro demonstrated the expression of vitamin D receptors in endothelial cells, vascular smooth muscle and direct involvement in vasodilation mediated by endothelium, anticoagulant activity, and modulation of the inflammatory response.³³ Further studies with more participants are now needed.

Regarding the relationship between vitamin D and metabolic markers, a negative correlation exists between 25(OH)D and GGT in the sufficient COVID-19 group. The relation between vitamin D and GGT activity differed across vitamin D statuses (deficiency, insufficiency, sufficiency) in both groups studied, COVID-19/non-COVID-19, being significantly higher in the deficient non-COVID-19 than in the similar COVID-19 group ($p=0.007$). The link between vitamin D and GGT in COVID-19 has not been thoroughly explored; the few data available come from the study of Barchetta et al., which observed that patients with non-alcoholic fatty liver disease have low 25(OH)D concentrations and slightly elevated GGT.³⁴ The association between GGT and vitamin D can be explained by the inflammatory process.³⁵

The final purpose of our study was to evaluate serum levels of vitamin D in patients with COVID-19 and different comorbidities, especially in diabetics. A previous study indicated diabetes as the second common comorbidity found in patients with COVID-19.³⁶ Hyperglycemic status in diabetic patients induces a dysfunctional immune response with a low production of interleukins (IL-1, IL-6). Hyperglycemia can induce a favorable environment for increased virulence of pathogens.³⁴ Diabetes is characterized by a chronic inflammatory status and immune response dysregulation with higher risk of lower

respiratory infection.³⁷⁻³⁹ Glycosylation induces an altered profile of cell membrane receptors and overexpression of ACE-2 molecules in patients with diabetes.⁴⁰ Also, patients with diabetes have overconcentration of ferritin possibly due to up-regulation of hepcidin axis. Data report low levels of 25(OH)D3 in diabetic patients. Possible pathological mechanisms are impaired liver and kidney metabolism of vitamin D and a decreased intestinal absorption caused by diabetic neuropathy.⁴⁰ In agreement with previous studies, our results indicate a significant difference between 25(OH)D3 serum levels in diabetic/non-diabetic COVID-19 groups. Patients with COVID-19 and diabetes are older and have higher levels of blood glucose than patients with COVID-19 without diabetes. Also, ferritin concentration is significantly higher, and patients with diabetes manifest a 25(OH)D3 deficiency. Our results are in accordance with data published in previous studies,^{41,42} which have shown that patients with COVID-19 and diabetes have significantly lower values of vitamin D.

The Di Fillip study (2022) showed an association between low levels of vitamin D, increased blood glucose and COVID-19 severity.⁴³ A pathophysiological and mechanistic link between diabetes and COVID-19 manifests in the presence of vitamin D deficiency. Deficient levels of serum vitamin D are associated with diabetes in COVID-19 patients. The study by Elsheikh et al. (2024) demonstrated that vitamin D status correlates with diabetes prevalence and subject with diabetes have significantly lower level of vitamin D than those without diabetes.⁴⁴

In the COVID-19 group, ferritin levels were higher than in the non-COVID-19 one because its secretion in hepatocytes, macrophages, and Kupffer cells is stimulated by inflammatory cytokines storm.^{45,46} Patients with COVID-19 and diabetes have significantly higher levels of ferritin than those without diabetes, and these data correspond to the results of Varikasuvu et al. (2020) and showed that patients with COVID-19 and diabetes are more susceptible to coagulation dysfunction and inflammation than those with COVID-19 but without diabetes.⁴⁷ Significantly increased ferritin levels indicate the activation of the monocyte-macrophage system in diabetic

patients and indicate susceptibility to inflammatory storm. Hyperferritinemia is prevalent in individuals with COVID-19 and diabetes and ferritin can be considered as a valuable biomarker for diabetic patients.⁴⁵

The most important limitation of our study was the small number of patients with an impact on the statistical significance on the results. To the best of our knowledge, there are limited data about the relation vitamin D-COVID-19 or vitamin D-non-COVID-19 infections in the Romanian population.

Conclusions

The link between serum levels of 25(OH)D3 and COVID-19 is not fully established and the data are contradictory. Insufficient serum vitamin D is related to infections in the respiratory tract and acute lung injury. Vitamin D deficiency can be a risk factor in development of inflammation and endothelial dysfunction and can be associated with an increased risk of infection, mostly in patients with comorbidities, especially in the case of diabetes mellitus. Ferritin can be used a biomarker in infectious diseases, used to improve the evaluation of inflammatory status.

Author contributions statement: LC and DM designed and supervised the study and drafted the manuscript. LC and AU collected and analysed the data and performed the background literature review for the manuscript. LC, AU, IS, EB carried out the laboratory work, conducted the statistical analyses and drafted the manuscript. All authors read and approved the final version of the manuscript.

Conflicts of interest: All authors – none to declare.

Funding: None to declare.

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Please cite this article as:

Constantin L, Ungurianu A, Țârcomnicu I, Bălulescu E, Margină D. Vitamin D and COVID-19: comparative analysis with other respiratory infections and impact of comorbidities. *GERMS*. 2024;14(3):232-245. doi: 10.18683/germes.2024.1435