VITAMIN D STATUS IN HOSPITALIZED PATIENTS WITH SARS-CoV-2 INFECTION

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ABSTRACT

Background: The role of **v**itamin D status in COVID-19 patients is a matter of debate.

Objectives: To assess serum 25-hydroxyvitamin D (25OHD) levels in hospitalized patients with COVID-19 and to analyze the possible influence of vitamin D status on disease severity.

Methods: Retrospective case-control study of 216 COVID-19 patients and 197 population-based controls. Serum 25OHD levels were measured in both groups. Besides, the association of serum 25OHD levels with COVID-19 severity (admission to the Intensive Care Unit, requirements for mechanical ventilation, or mortality) was also evaluated.

Results: Of the 216 patients, 19 were on vitamin D supplements and were analyzed separately. In COVID-19 patients, mean±SD 25OHD levels were 13.8±7.2 ng/ml, compared to 20.9±7.4 ng/ml in controls ($p<0.0001$). 25OHD values were lower in men than in women. Vitamin D deficiency was found in 82.2% of COVID-19 cases and 47.2% of population-based controls ($p<0.0001$). 25OHD inversely correlate to serum ferritin ($p=0.013$) and D-dimer levels ($p=0.027$). Vitamin D-deficient COVID-19 patients had a greater prevalence of hypertension and cardiovascular diseases, raised serum ferritin and troponin levels, as well as a longer length of hospital stay than those with serum 25OHD levels ≥20 ng/ml. No causal relationship was found between vitamin D deficiency and COVID-19 severity as a combined endpoint or as its separate components.

Conclusions: 25OHD levels are lower in hospitalized COVID-19 patients compared to population-based controls and these patients had a higher prevalence of deficiency. We did not find any relationship between vitamin D concentrations or vitamin deficiency and the severity of the disease.

Keywords: 25OHD; PTH; SARS-CoV-2 infection, COVID-19

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INTRODUCTION

There are several lines of evidence that might support a role for vitamin D status in SARS-CoV-2 infection. Firstly, vitamin D deficiency is a common condition all around the world, and serum 25-hydroxyvitamin D (25OHD) levels follow a well-known seasonal and geographical pattern. Thus, Spain located in temperate zones of the Northern hemisphere, but with a higher prevalence of vitamin D deficiency (**1**) has reached very high rates of SARS-CoV-2 infection and lethality (**2**). Secondly, vitamin D is a steroid hormone involved in the modulation of the innate and acquired immune system and also in the production of antimicrobial peptides, such as cathelicidin and human β-defensin-2, as well as in the expression of genes involved in the intracellular destruction of pathogens (**3-5**). Thirdly, low serum 25OHD levels are frequently found in elderly individuals or in those with chronic conditions, such as hypertension, diabetes, cancer, or cardiovascular diseases, which have also been reported as poor prognostic factors for COVID-19 (**6-11**). Finally, the downregulation of ACE2 by SARS-CoV-2 leads to a dysregulation of the renin-angiotensin system (RAS) that contributes to the "cytokine storm" that precedes the acute respiratory distress syndrome (ARDS) characteristic of the severe form of COVID-19. In this sense, vitamin D can inhibit pro-inflammatory cytokine production in human monocytes/macrophages (**12**), and chronic vitamin D deficiency may induce RAS activation, leading to the production of fibrotic factors and therefore, lung damage (**13**).

Taking into account the above considerations, we aimed to assess the serum 25OHD levels in hospitalized patients with COVID-19 compared to population-based controls. Besides, the possible association between serum 25OHD concentrations and COVID-19 severity and mortality was also analyzed.

SUBJECTS AND METHODS

Study design and participants

The study consists of two parts. Firstly, we have designed a retrospective case-control study including 216 patients aged ≥18 years, with confirmed COVID-19 admitted to the University Hospital Marqués de Valdecilla in Santander, Northern Spain from March 10 to March 31, 2020, and 197 sex-matched population-based controls recruited from the Camargo Cohort (**14,15**) during their last follow-up visit on January-March of the past year. From the present study, we have excluded patients or controls with malabsorption disorders, liver cirrhosis, serum creatinine levels >1.9 mg/dl, or previous treatment with anticonvulsants. Nineteen COVID-19 patients on oral vitamin D supplements for more than 3 months at admission were analyzed as a separate group, and controls who receive these supplements were also excluded from the study. Secondly, we have assessed only the group of COVID-19 patients to evaluate the possible influence of vitamin D deficiency on the outcome of the disease. Participants from the Camargo Cohort gave their informed written consent and the study was approved by the Cantabria Clinical Research Ethics Committee (internal code 2016.003). The present study was approved

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by the Ethics Committee of Cantabria (internal code 2020.55). Serum samples from Covid-19 patients were provided by the IDIVAL Biobank samples collection (internal code 2020-126).

Data collection

Demographic, clinical, and outcome data of COVID-19 patients were gathered from the hospital records, stored in a computerized database, and independently reviewed by two researchers. Missing data were not imputed. Smoking status was coded as current or non-smoker. Immunosuppression included prolonged use (≥3 months) of corticoids (>10 mg/day of prednisone or equivalent) or immunomodulatory agents, and bone marrow or organ transplantation. Chest X-ray and/or CT-scans were performed in all COVID-19 patients. Concerning immunomodulatory therapy, patients were selected for tocilizumab according to our institutional protocol. Thus, tocilizumab was indicated if there was clinical worsening with PaO_2/FIO_2 ratio (PaFI) < 300 and high serum acute-phase reactant levels when no contraindication for its use was present. The endpoint variable for COVID-19 severity has been defined as the composite of admission to the intensive care unit (ICU), requirement for mechanical ventilation, or in-hospital mortality. Clinical outcomes were monitored up to May 20, 2020. Overall, the criteria for ICU admission were those of the guidelines by the American Thoracic Society and Infectious Diseases Society of America **(16)** and the critical care ethic recommendations for the SARS-CoV-2 pandemic by the Intensive Medicine Spanish Society **(17)**. ARDS was the main cause of ICU admission and a case-by-case assessment was carried out by the medical COVID team, including intensivists.

Laboratory measurements

Qualitative detection of RNA from the SARS-CoV-2 was performed by using Real-Time PCR. Blood samples from the controls were obtained from an antecubital vein in the morning after a requested 12-hour overnight fast. The serum was divided into 0.5-ml aliquots and stored at -40ºC. Routine biochemical parameters were measured by standard automated methods in a Technicon Dax autoanalyzer (Technicon Instruments, CO. USA). Human IL-6 was measured by enzyme-linked immunosorbent assay (ELISA) (Enzo Life Sciences, Inc. Farmingdale, NY) following the manufacturer instructions. The sensitivity for serum IL-6 levels was 0.057 pg/mL. Intra- and interassay precision was 4.38% and 9.6%, respectively. Serum 25OHD concentrations were determined in controls by a fully automated electrochemiluminescence system (Elecsys 2010, Roche Diagnostics, GmbH, Mannheim, Germany). The detection limit of serum 25OHD was 4 ng/ml. The intra-assay coefficient of variation (CV) was 5% and inter-assay was 7.5%. In COVID-19 patients, serum 25OHD levels were obtained at admission and assessed by automated competitive chemiluminescence assay (Liaison XL, DiaSorin

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Inc, Stillwater MN, USA). Our laboratory is DEQAS (Vitamin D External Quality Assessment Scheme) certified for this parameter. The detection limit of serum 25OHD was 4 ng/ml. The intra-assay and inter-assay CV were 2.58% and 7.83%, respectively. We have previously found a correlation between both techniques of 0.926 ($p<0.0001$) with a random sample of 52 subjects from the Camargo Cohort.

Statistical analysis

Continuous variables were expressed as mean±SD or median and interquartile range (IQR), and compared with the Student's *t*-test or Mann-Whitney U-test according to the distribution of data. Categorical variables were presented as numbers and percentages and compared using the χ^2-test of the Fisher exact test as appropriate. Spearman rho was used to assess the relationships between serum 25OHD levels and several clinical and laboratory parameters. Serum 25OHD levels were stratified into four categories: below 10 ng/ml, between 10 and 20 ng/ml, between 20 and 30 ng/ml, and above 30 ng/ml. Vitamin D deficiency was defined as serum 25OHD levels <20 ng/ml (50 nmol/l) following a recent position paper by the ECTS Working Group (**18**). A multivariable general linear model was set up to compare serum 25OHD levels between COVID-19 patients and controls (Bonferroni test), adjusting for confounding variables. Moreover, in the group of COVID-19 patients, univariable and multivariable binary logistic regression analyses were used to assess the association between vitamin D (as a continuous variable, or expressed as vitamin D deficiency or as quintiles) and the dependent variable of severity of the disease. A two-sided *p*-value of less than 0.05 was considered statistically significant in all the calculations. A post-hoc power analysis with the present sample size and the obtained difference in serum 25OHD levels between cases and controls yields a power of 100% to detect this difference. In fact, a difference of 2.1 ng/ml between groups already yields a potency of 89.8%. Nevertheless, due to the sample size and the lower number of events (especially mortality) in COVID-19 patients with and without vitamin D deficiency, the post-hoc power analysis for the severity endpoints was lower than 40%.

RESULTS

We included 216 adult COVID-19 patients, of whom 19 were on vitamin D supplementation (11 patients were taking cholecalciferol -25,000 IU/monthly in 10 cases and 5,600 IU/weekly in another one- and 8 patients were on calcifediol -0.266 mg/monthly-). The main demographic, epidemiological, and clinical characteristics of the three groups included in the study are summarized in **Table 1**. Noteworthy, COVID-19 patients on vitamin D supplements were mainly women and had a greater prevalence of hypertension and immunosuppression than the other two groups analyzed. Besides, population-based controls were more smokers and had a lower glomerular filtration rate and greater serum parathormone levels than COVID-19 patients.

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Table 2 summarizes the demographic, clinical, and laboratory data of COVID-19 patients (excluding those on vitamin supplements) according to the presence of vitamin D deficiency (serum 25OHD levels <20 ng/ml. Vitamin D-deficient COVID-19 patients had a greater prevalence of hypertension and cardiovascular diseases, raised serum ferritin and troponin levels, as well as a longer length of hospital stay than those with serum 25OHD levels ≥20 ng/ml.

The features of COVID-19 patients according to the active use of vitamin D supplements are shown in **Table 3**. Patients on supplements had a significantly lower PaO_2/FIO_2 ratio<300 prevalence, lower serum ferritin levels, and received less frequently tocilizumab than COVID-19 patients who did not take vitamin D supplements. They also had an overall lower percentage of the combined severity endpoint and ICU admissions, as well as a shorter length of hospital stay, although these data did not reach statistical significance.

Furthermore, when we pooled together patients with 25OHD levels ≥20 ng/ml (both at basal levels and with vitamin D supplements) and compared with patients with vitamin D deficiency, those with higher levels had a slightly better outcome expressed as a lower PaO_2/FIO_2 ratio<300 (12.8% vs. 27.8%; p=0.034), lower requirements for tocilizumab (17% vs. 33.1%; p=0.032), less frequent radiological progression (14.9% vs. 30.2%; p=0.037), lower ICU admissions (12.8% vs. 26.6%; p=0.048), and also a shorter hospital stay (12.0 [8.0-17.0] vs. 8.0 [6.0-14.0] days; p=0.002). No difference was found regarding neither the composite severity endpoint (21.3% vs. 30.8%; p=0.203) nor mortality (12.9% vs. 9.8%; p=0.590).

In COVID-19 patients, mean±SD 25OHD levels were 13.8±7.2 ng/ml, compared to 20.9±7.4 ng/ml in controls (p<0.0001). The distribution of serum 25OHD levels in hospitalized COVID-19 patients with or without vitamin D supplements and controls, grouped by gender was shown in **Figure 1.** Noteworthy, serum 25OHD values were lower in men than in women. **Figure 2** shows the percentage of COVID-19 cases (without vitamin D supplementation) and controls within the different intervals of serum 25OHD levels. Vitamin D deficiency was found in 82.2% of COVID-19 cases and 47.2% of population-based controls (p<0.0001). 25OHD inversely and significantly correlated with serum ferritin and D-dimer, and there was a trend with C-reactive protein levels (**Figure 3**). We did not find any statistical relationship between serum vitamin D and IL-6 levels in patients with COVID-19 (rho -0.032; p=0.67), although levels of this cytokine were lower, albeit non-significant, in patients with serum vitamin D levels ≥ 20 ng/ml and those on vitamin D supplements (**Table 3**).

In the multivariable general linear model, mean serum 25OHD levels persisted significantly lower in COVID-19 patients (excluding those on vitamin D supplements) than in population-based controls after adjusting for age, smoking, hypertension, diabetes mellitus, history of cardiovascular events, immunosuppression, body mass index, serum corrected calcium, glomerular filtration rate and the month of vitamin D determination; 11.9 (95%CI, 9.6-14.3) ng/ml versus 21.2 (95%CI, 19.7-22.7) ng/ml (p<0.0001).

In COVID-19 patients (once excluding those on vitamin D supplements at admission), no relationship was found between serum vitamin D levels (as a continuous variable or expressed as vitamin D deficiency or as quintiles),

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and the composite severity endpoint or its separate components, in crude or adjusted logistic regression models (combine severity endpoint: unadjusted OR 1.55, 95%CI 0.66-3.65; p=0.315; adjusted OR 1.13, 95%CI 0.27-4.77; p=0.865; for vitamin D deficiency).

DISCUSSION

We have found that serum 25OHD levels are significantly lower in hospitalized COVID-19 patients compared to population-based controls of similar age and sex and that these differences remain significant even once adjusting for the main confounding factors. These levels were especially lower in the group of men with COVID-19. Despite the high frequency of vitamin D deficiency in patients hospitalized for COVID-19, we did not find an association between circulating levels of 25OHD and the severity of SARS-CoV-2 infection.

Vitamin D is a hormone with a pleiotropic role and there is compelling evidence for an epidemiological association between low serum 25OHD levels and human infections such as influenza, HIV, and hepatitis C virus infection (**19**). The interplay between vitamin D and viral infection is an area of growing interest, and interaction with host and viral factors, immunomodulatory effects, induction of autophagy and apoptosis, and even genetic and epigenetic factors have been reported as antiviral effects of this hormone (**20**). In this scenario, the SARS-CoV-2 virus pandemic has rapidly spread in the wintertime with extreme virulence through the Southern European countries, like Italy and Spain. Although there was a considerable variation in the prevalence of vitamin D deficiency across countries, mainly dependent on age and the use of vitamin D supplements or food fortification, vitamin D deficiency (25OHD levels <20 ng/ml) is found in 40% of the European citizens irrespective of age group, ethnic mix, and latitude (**18**). Noteworthy, the population with a more severe COVID-19, such as the elderly and patients with comorbidities with the highest case-fatality rates (**21**), are also those with lower serum 25OHD levels according to published data (**18**). Thus, the Seneca study showed a mean 25OHD concentrations of 10.4 ng/ml (26 nmol/l) in elderly subjects aged 70-75 years in Spain **(22).** Recently, Ilie et al. **(23),** found significant crude associations between serum vitamin D levels and the number of COVID-19 cases and mortality when they analyzed, in some European countries, the mean 25OHD levels reported in some population studies.

Moreover, SARS-CoV-2 downregulates ACE2 expression, the main receptor to enter the virus in the human cells, and thereby induces high angiotensin II production leading to myocardial and mainly lung inflammation and ARDS (**24**). In experimental models, vitamin D deficiency induces chronic RAS activation leading to impaired lung function and overexpression of profibrotic factors (**13**). The key pathogenic mechanism for SARS-CoV-2 to develop severe complications and lethality is the hyperinflammatory state (“cytokine storm”) that occurs over the first week of the onset of the symptoms. This cytokine storm may lead to the severe COVID-19 complications, such as ARDS, myocarditis, acute heart and renal failure, causing increased mortality, especially in the elderly or in patients with previous cardiovascular comorbidity (**25**). The intrinsic mechanism of the anti-inflammatory effect of vitamin D remains uncertain, although its role on both, innate and adaptive immunity, has been suggested

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(**26**). In this regard, experimental evidence indicates that vitamin D may inhibit IL-6 and TNF-α by attenuating p38 MAP kinase activation in human monocytes/macrophages, Moreover, 1,25OH2D_3 promotes the induction of the T regulatory cells, thereby inhibiting the production of proinflammatory cytokines, including IL-17, IL-21, and γ-interferon (**27**).

In this scenario, a recent study using 350,000 UK Biobank samples obtained between 2006 and 2010 did not find an association between serum 25OHD concentrations (or vitamin D deficiency –defined as <25 mmol/L- or insufficiency -<50 mmol/L-) and COVID-19 risk (assessed in 449 COVID-19 patients with complete data), after adjusting for potential confounders. Besides, their results did not support that vitamin D might play a role in the reported ethnic variations in COVID-19 incidence (**28**). Nevertheless, baseline 25OHD levels were obtained a decade ago, and also information on the severity of COVID-19 was lacking and the study included all positive tests regardless of its clinical outcome. D'Avolio et al. **(29)**, retrospectively analyzed 107 patients who underwent SARS-CoV-2 PCR testing (80 with a negative and only 27 with a positive result) and simultaneous 25OHD measurement, in a Swiss hospital, from March 1 to April 14, 2020. A control cohort that included 1377 patients with serum 25OHD levels obtained in the same period of the year 2019 was also analyzed. The authors found that SARS-CoV-2 infected patients had median 25OHD levels of 11.1 ng/ml, compared to 24.6 ng/ml in SARS-CoV-2 negative subjects and controls.

Our study was carried out in a hospitalized population, and in this sense, it is worth mentioning that serum 25OHD has been considered as a negative acute-phase reactant and its values have been reported to be decreased during acute inflammatory diseases (**30**). Thus, our COVID-19 patients had a high prevalence of vitamin D deficiency, and serum 25OHD levels significantly and negatively correlated with ferritin and D-dimer values, indicating that vitamin D might have a beneficial role on the systemic inflammatory state of this viral disease. Interestingly, 25OHD concentrations in COVID-19 patients on previous hormone supplements were lower than expected, supporting its behavior as a negative acute-phase reactant. Therefore, 25OHD levels should be interpreted with caution in this scenario, although the population at risk for a more severe SARS-CoV-2 infection is probably the same that is at risk for vitamin D deficiency, especially elderly individuals with comorbidities.

We did not find any relationship between serum 25OHD levels and the parameters of COVID-19 severity, such as ICU admission, the need for mechanical ventilation or mortality, assessed as a combined endpoint, or separately. In contrast to other studies (**31,32**), we did not find an association between serum 25OHD levels and the severity of the disease. However, it cannot be completely ruled out due to the small number of events and the statistical power of the present study. Nevertheless, we had the opportunity to assess a group of 19 COVID-19 patients who were on oral vitamin D supplements at hospital admission. We observed that they had a slightly minor unfavorable outcome than COVID-19 patients who did not take vitamin D supplements, with a significantly more favorable PaO_2/FIO_2 ratio, lower ferritin levels, and decreased requirements for tocilizumab, and even a trend for lower ICU admissions.

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Interestingly enough, 6 out of 19 COVID-19 patients on vitamin D supplements also received chronic corticosteroids or immunosuppressant agents at least since the previous 3 months because of immune-mediated inflammatory diseases (IMIDs) or suprarenal insufficiency. This is an interesting matter of debate since it has been recently suggested that the use of anti-cytokine and other immunosuppressive therapies is not associated with worse COVID-19 outcomes **(33)**. In this sense, IMID patients on chronic corticosteroids usually received vitamin D supplements as prophylaxis or treatment of bone disease. Furthermore, they are under tight control of their comorbidities, and vitamin D deficiency is more frequently checked and treated than in the general population. Whether the outcome of COVID-19 patients on previous vitamin D might have been influenced by vitamin D status itself or by the presence of an important number of patients with IMIDs on corticosteroids and/or immunosuppressant agents is difficult to determine due to the size of the sample.

Noteworthy, COVID-19 hospitalized patients had lower serum corrected calcium levels than the control population. In this regard, Di Filippo et al. **(34)**, conducted a hospital-based retrospective study on 531 COVID-19 patients in Italy. Hypocalcemia, defined as serum ionized calcium level < 1.18 mmol/L, was observed in 82% of patients, mainly elderly males. They also found that hypocalcemia was an independent predictor for hospitalization. However, and despite lower calcium and vitamin D levels in our COVID-19 patients, serum PTH was higher in controls. This could be related to a lower GFR in the control population, since there is no reason to suspect relative hypoparathyroidism.

Finally, it is worth mentioning that SARS-CoV-2 pandemic represent a challenging scenario in the management of osteoporosis and fragility fractures. Thus, COVID-19 hospitalized patients are mainly frailty older individuals with comorbidities, who are in many cases exposed to systemic corticosteroids as part of the treatment of the disease and may require prolonged immobilization periods for a complete recovery. Besides, as we observed, they have a high percentage of vitamin D deficiency that may also contribute to a loss of muscle strength and to an increase of the risk of falls. All these factors put these individuals on an increased risk for fragility fractures **(35,36)**. Under these circumstances, prevention strategies should be implemented. According to our results, vitamin D treatment should be recommended in COVID-19 patients with serum 25OHD deficiency since this approach might have beneficial effects in both the musculoskeletal and the immune system **(36)**.

Our study has several limitations. First of all, those inherent to an observational study that does not permit to establish whether vitamin D is simply a biomarker of exposure or a biomarker of effect on the disease. Other vitamin D-related parameters such as the free fraction of 25OHD, 1,25 dihydroxyvitamin D, and vitamin D-binding protein were not measured. The number of COVID-19 patients who were on oral vitamin D supplements is too small and on different dosages to draw solid conclusions on its role in the clinical outcomes of the disease, although we think that it represents a unique opportunity to preliminary explore the differences between both groups of COVID-19 patients. Furthermore, the study has been conducted in a single Spanish tertiary-care hospital, and data may not be generalized to other settings, ethnicities, or countries, especially those with specific policies for vitamin D supplementation or food fortification. The methods to assess serum 25OHD levels

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in cases and controls were different, although, as stated, we have found a very good correlation between both techniques. Finally, no dietary assessment has been carried out, and therefore information on dietary habits is lacking.

In summary, serum 25OHD levels of hospitalized COVID-19 patients are lower than sex-matched population-based controls of similar age. Men with this viral disease represent the group with lower serum vitamin D levels compared to women. Serum vitamin D levels below 20 ng/ml were detected in 82% of COVID-19 patients, indicating that they represent a population with a higher risk for vitamin D deficiency. In our COVID-19 patients, 25OHD was inversely associated with some inflammatory parameters, such as ferritin and D-dimer. We did not found any relationship between vitamin D concentrations or vitamin deficiency and the severity of the disease, including mortality, although further studies including a large sample size should be done to determine the real impact of vitamin D deficiency on the severity of COVID-19. Probably, the best approach should be to identify and treat vitamin D deficiency, especially in high-risk individuals such as the elderly, patients with comorbidities, and nursing home residents, to maintain serum 25OHD levels above 20 ng/ml, and probably with a target between 30 ng/ml and 50 ng/ml. Whether the treatment of vitamin D deficiency will play some role in the prevention of the viral disease or improve the prognosis of patients with COVID-19 remains to be elucidated in large randomized controlled trials which will be certainly necessary to precisely define the role of vitamin D supplementation in futures waves of SARS-CoV-2 infection.

DATA AVAILABILITY

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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AUTHOR CONTRIBUTION

JLH, PMC, and VMT designed the research and analyzed the data. DN, MFA, and MAH collected the data; MLH and MGU performed the laboratory analyses. JLH and VMT wrote the manuscript; PMC, MLH, JMO, JRB, MGC, MAH, and JC critically revised the manuscript. All authors discussed the results and contributed to the final paper.

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FIGURE LEGENDS

Figure 1. Serum vitamin D levels in hospitalized COVID-19 patients with and without active oral vitamin D supplements and population-based controls, according to gender.

Footnote: Grey bars represent men and white bars represent women.

Figure 2. Percentage of COVID-19 cases (excluding those on vitamin D at admission) and controls according to different intervals of serum 25OHD levels.

Figure 3. Correlation between serum 25OHD and inflammatory markers (ferritin, –panel A-; D-dimer, –panel B- and C-reactive protein, –panel C-).

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Table 1. Main baseline features of COVID-19 patients and controls.

Variable	COVID-19[1] N=197	COVID-19_D[2] N=19	Controls[3] N=197	p[1-2]	p[1-3]	p[2-3]
Age (years), *median (IQR)*	61.0 (47.5-70.0)	60.0 (59.0-75.0)	61.0 (56.0-66.0)	0.082	0.153	0.182
Sex (male), *n (%)*	123 (62.4)	7 (36.8)	123 (62.4)	**0.030**	0.999	**0.030**
BMI (kg/m²), *mean±SD*	29.2±4.7	30.9±6.3	28.9±4.0	0.134	0.557	**0.035**
Current smoker, *n (%)*	14 (7.1)	2 (10.5)	34 (17.3)	0.638	**0.002**	0.747
Hypertension, *n (%)*	76 (38.6)	12 (63.2)	87 (44.2)	**0.037**	0.260	0.113
Diabetes, *n (%)*	34 (17.3)	0 (0.0)	31 (15.7)	**0.049**	0.684	0.083
Cardiovascular disease, *n (%)*	21 (10.7)	3 (15.8)	22 (11.2)	0.451	0.872	0.468
COPD, *n (%)*	15 (7.6)	2 (10.5)	9 (4.6)	0.650	0.206	0.250
Active cancer, *n (%)*	7 (3.6)	0 (0.0)	8 (4.1)	0.999	0.792	0.999
Immunosuppression, *n (%)*	16 (8.1)	6 (31.6)	2 (1.0)	**0.006**	**0.001**	**<0.0001**
ACEI / ARA2 agents, *n (%)*	58 (29.4)	7 (36.8)	47 (23.9)	0.502	0.210	0.265
GFR-MDRD-4 (ml/min/1.73m²), *median (IQR)*	92.2 (73.9-113.4)	85.9 (69.9-104.9)	71.9 (63.3-91.5)	0.213	**<0.0001**	0.053
C-reactive protein (mg/dl), *median (IQR)*	5.60 (2.63-11.85)	7.30 (2.90-15.10)	0.25 (0.10-0.50)	0.756	**<0.0001**	**<0.0001**
Corrected calcium (mg/dl), *median (IQR)*	8.5 (8.3-9.0)	8.7 (8.4-9.0)	9.1 (8.9-9.3)	0.175	**<0.0001**	**<0.0001**
25OHD (ng/ml), *mean±SD*	13.8±7.2	21.1±5.9	20.9 ± 7.4	**<0.0001**	**<0.0001**	0.914
PTH (pg/ml), *median (IQR)*	42.6 (32.3-62.6)	53.7 (28.8-67.4)	51.6 (42.5-65.2)	0.389	**<0.0001**	0.719

BMI: body mass index; COPD: chronic obstructive pulmonary disease; ACEI: angiotensin-converting enzyme inhibitors; ARA2: angiotensin-receptor 2 antagonists; GFR: glomerular filtration rate. MDRD: modification of diet in renal disease; 25OHD: 25-hydroxyvitamin D; PTH: parathormone.

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Table 2. Main characteristics of COVID-19 patients according to the presence of vitamin D deficiency.

Variable	25OHD <20 ng/ml N=162	25OHD ≥20 ng/ml N=35	p
Baseline characteristics			
• **Age (years),** *median (IQR)*	62.0 (48.0-70.3)	58.0 (45.0-69.0)	0.292
• **Sex (male),** *n (%)*	106 (65.4)	17 (48.6)	0.062
• **BMI (kg/m²),** *mean±SD*	29.0±4.9	29.8±4.1	0.428
• **Current smoker,** *n (%)*	13 (8.0)	1 (2.9)	0.471
• **Hypertension,** *n (%)*	68 (42.0)	8 (22.9)	**0.035**
• **Diabetes,** *n (%)*	28 (17.3)	6 (17.1)	0.984
• **Cardiovascular disease,** *n (%)*	21 (13.0)	0 (0.0)	**0.029**
• **COPD,** *n (%)*	13 (8.0)	2 (5.7)	0.999
• **Active cancer,** *n (%)*	7 (4.3)	0 (0.0)	0.357
• **Immunosuppression,** *n (%)*	11 (6.8)	5 (14.3)	0.169
• **ACEI / ARA2 agents,** *n (%)*	52 (32.1)	6 (17.1)	0.078
Clinical and laboratory data			
• **Pneumonia,** *n (%)*	155 (95.7)	33 (94.3)	0.662
• **Respiratory rate>22,** *n (%)*	36 (22.2)	4 (11.4)	0.150
• **CURB-65 score,** *median (IQR)*	1 (1-2)	1 (1-1)	0.229
• **SBP<100 mmHg,** *n (%)*	4 (2.5)	1 (2.9)	0.999
• **PaO_2/FIO_2 ratio (PaFI),** *median (IQR)*	444 (424-452)	444 (436-452)	0.168

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• **PaFi<300,** *n (%)*	46 (28.4)	6 (17.1)	0.171
• **Lymphocytes (mm^3),** *median (IQR)*	900 (600-1200)	1100 (700-1250)	0.255
• **Neutrophils (mm^3),** *median (IQR)*	3900 (2875-6125)	3700 (2900-4600)	0.207
• **Neutrophil/lymphocyte ratio,** *median (IQR)*	4.85 (3.00-7.52)	3.63 (3.00-6.67)	0.422
• **Platelet count (x10^9/L)**, *median (IQR)*	167 (138-217)	169 (143-211)	0.833
• **D-dimer (ng/ml),** *median (IQR)*	710.5 (469.0-1021.0)	575.0 (434.0-693.0)	0.057
• **Ferritin (ng/ml),** *median (IQR)*	833.0 (330.8-1488.3)	310.0 (137.3-764.0)	**<0.0001**
• **hs-Troponin I (ng/L),** *median (IQR)*	6.0 (3.0-12.0)	3.0 (3.0-6.0)	**0.015**
• **C-reactive protein (mg/dl),** *median (IQR)*	6.10 (3.10-13.60)	3.20 (2.30-8.70)	0.064
• **IL-6 (pg/ml),** *median (IQR)*	58.9 (19.1-124.0)	45.6 (20.5-119.0)	0.63
• **GFR-MDRD-4 (ml/min/1.72m^2),** *median (IQR)*	91.4 (73.5-114.7)	98.0 (82.4-113.1)	0.323
• **Corrected calcium (mg/dl),** *median (IQR)*	8.5 (8.3-9.0)	8.7 (8.4-9.0)	0.289
• **25OHD (ng/ml),** *mean±SD*	11.2±4.3	25.8±5.6	**<0.0001**
• **PTH (pg/ml),** *median (IQR)*	44.2 (32.3-64.8)	35.6 (30.9-46.4)	0.092
Therapeutic scheme			
• **Hydroxychloroquine,** *n (%)*	156 (96.3)	35 (100)	0.593
• **Lopinavir/ritonavir,** *n (%)*	122 (75.3)	31 (88.6)	0.088
• **Azithromycin,** *n (%)*	117 (72.2)	30 (85.7)	0.096
• **Corticosteroids,** *n (%)*	40 (24.7)	7 (20.0)	0.555
• **β-interferon,** *n (%)*	37 (22.8)	7 (20.0)	0.715
• **Tocilizumab,** *n (%)*	55 (34.0)	8 (22.9)	0.202
• **Anakinra,** *n (%)*	12 (7.4)	1 (2.9)	0.471

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• **Non-invasive ventilation,** *n (%)*	12 (7.4)	1 (2.9)	0.471
Outcome			
• **ICU admission,** *n (%)*	44 (27.2)	6 (17.1)	0.217
• **Mechanical ventilation*,** *n (%)*	37 (84.1)	6 (100)	0.576
• **Radiological worsening,** *n (%)*	50 (30.9)	6 (17.1)	0.103
• **Secondary infection,** *n (%)*	38 (23.5)	6 (17.1)	0.416
• **Thrombotic events**,** *n (%)*	10 (6.2)	0 (0.0)	0.214
• **Death,** *n (%)*	16 (10.2)	4 (11.4)	0.765
• **Composite severity endpoint,** *n (%)*	111 (68.5)	27 (77.1)	0.312
• **Length of stay (days),** *median (IQR)*	12.0 (8.0-17.0)	8.0 (6.0-14.0)	**0.013**

*Refers only to the number of patients admitted to ICU. ** Included pulmonary embolism, deep venous thrombosis, acute coronary syndrome, and cerebrovascular disease. BMI: body mass index; COPD: chronic obstructive pulmonary disease; ACEI: angiotensin-converting enzyme inhibitors; ARA2: angiotensin-receptor 2 antagonists; hs: high-sensitivity. GFR: glomerular filtration rate. MDRD: modification of diet in renal disease; 25OHD: 25-hydroxyvitamin D; PTH: parathormone.

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Table 3. Main features in COVID-19 patients with or without oral vitamin D supplements at admission.

Variable	COVID-19 N=197	COVID-19_D N=19	p
Clinical and laboratory data			
• **Pneumonia,** *n (%)*	188 (95.4)	18 (94.7)	0.999
• **Respiratory rate>22,** *n (%)*	40 (20.3)	3 (15.8)	0.772
• **CURB-65 score,** *median (IQR)*	1 (1-2)	1 (1-2)	0.353
• **SBP<100 mmHg,** *n (%)*	5 (2.5)	0 (0.0)	0.999
• **PaO_2/FIO_2 ratio (PaFI),** *median (IQR)*	444 (428-452)	444 (432-452)	0.524
• **PaFi<300,** *n (%)*	52 (26.4)	1 (5.3)	**0.049**
• **Lymphocytes (mm^3),** *median (IQR)*	900 (700-1200)	900 (500-1400)	0.890
• **Neutrophils (mm^3),** *median (IQR)*	3900 (2900-5600)	4000 (2200-5100)	0.624
• **Neutrophil/lymphocyte ratio,** *median (IQR)*	4.75 (3.00-7.38)	4.58 (2.81-7.82)	0.891
• **Platelet count ($x10^9/L$),** *median (IQR)*	167 (138-214)	168 (142-236)	0.478
• **D-dimer (ng/ml),** *median (IQR)*	735.5 (254.3-1367.3)	599 (431-1336)	0.731
• **Ferritin (ng/ml),** *median (IQR)*	861 (330-1418)	315 (147.0-743.0)	**0.012**
• **hs-Troponin I (ng/L),** *median (IQR)*	6.0 (3.5-125.0)	7.0 (3.5-17.0)	0.979
• **C-reactive protein (mg/dl),** *median (IQR)*	5.55 (2.60-11.85)	7.30 (2.90-15.10)	0.73
• **IL-6 (pg/ml),** *median (IQR)*	57.6 (21.6-125.0)	48.8 (13.0-129.8)	0.80
Therapeutic scheme			
• **Hydroxychloroquine,** *n (%)*	191 (97.0)	19 (100)	0.999
• **Lopinavir/ritonavir,** *n (%)*	153 (77.7)	12 (63.2)	0.164

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• **Azithromycin,** *n (%)*	147 (74.6)	14 (73.7)	0.999
• **Corticosteroids,** *n (%)*	47 (23.9)	5 (26.3)	0.783
• **β-interferon,** *n (%)*	44 (22.3)	2 (10.5)	0.378
• **Tocilizumab,** *n (%)*	63 (32.0)	1 (5.3)	**0.015**
• **Anakinra,** *n (%)*	13 (6.6)	1 (5.3)	0.999
• **Non-invasive ventilation,** *n (%)*	13 (6.6)	2 (10.5)	0.627
Outcome			
• **ICU admission,** *n (%)*	50 (25.4)	1 (5.3)	**0.05**
• **Mechanical ventilation*,** *n (%)*	43 (86.0)	1 (100)	0.999
• **Radiological worsening,** *n (%)*	56 (28.4)	2 (10.5)	0.093
• **Secondary infection,** *n (%)*	44 (22.3)	2 (10.5)	0.378
• **Thrombotic events**,** *n (%)*	10 (5.1)	1 (5.3)	0.999
• **Death,** *n (%)*	20 (10.4)	2 (10.5)	0.999
• **Composite severity endpoint,** *n (%)*	59 (29.9)	3 (15.8)	0.193
• **Length of stay (days),** *median (IQR)*	12.0 (8.0-16.0)	8.0 (6.0-14.0)	0.107

*Refers only to the number of patients admitted to ICU. ** Included pulmonary embolism, deep venous thrombosis, acute coronary syndrome, and cerebrovascular disease. BMI: body mass index; COPD: chronic obstructive pulmonary disease; ACEI: angiotensin-converting enzyme inhibitors; ARA2: angiotensin-receptor 2 antagonists; hs: high-sensitivity. GFR: glomerular filtration rate. MDRD: modification of diet in renal disease; 25OHD: 25-hydroxyvitamin D; PTH: parathormone.

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Figure 1

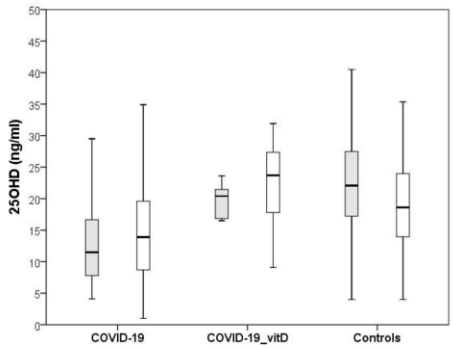

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Figure 2

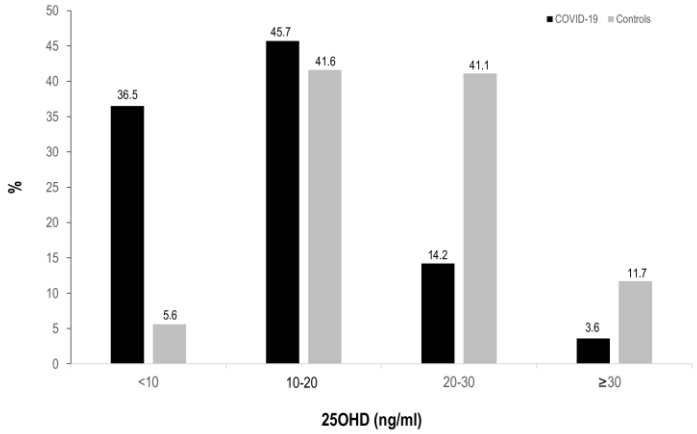

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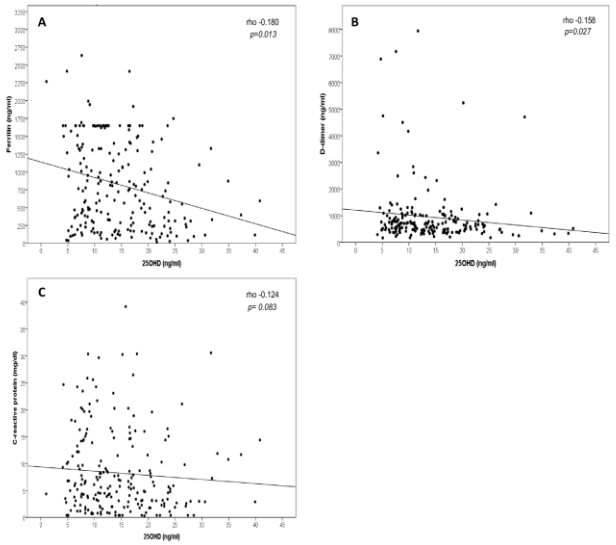
Figure 3



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