

Clinical Research Article

Calcifediol Treatment and COVID-19–Related Outcomes

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Abbreviations: 1,25(OH)₂D₃, active metabolite of vitamin D; 25(OH)D, 25-hydroxyvitamin D; ARDS, acute respiratory distress syndrome; calcifediol, 25(OH)D₃; CKD, chronic kidney disease; CURB-65, combination of confusion, urea, respiratory rate, blood pressure, and age 65 or older; ICU, intensive care unit; ITT, intention-to-treat; MEWS, Modified Early Warning score; OR, odds ratio; RCT, randomized controlled trial.

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Abstract

Context: COVID-19 is a major health problem because of saturation of intensive care units (ICU) and mortality. Vitamin D has emerged as a potential treatment able to reduce the disease severity.

Objective: This work aims to elucidate the effect of 25(OH)D₃ (calcifediol) treatment on COVID-19–related outcomes.

Methods: This observational cohort study was conducted from March to May 2020, among patients admitted to COVID-19 wards of Hospital del Mar, Barcelona, Spain. A total of 930 patients with COVID-19 were included; 92 were excluded because of

previous calcifediol intake. Of the remaining 838, a total of 447 received calcifediol (532 µg on day 1 plus 266 µg on days 3, 7, 15, and 30), whereas 391 were not treated at the time of hospital admission (intention-to-treat). Of the latter, 53 patients were treated later during ICU admission and were allocated in the treated group in a second analysis. In healthy individuals, calcifediol is about 3.2-fold more potent on a weight basis than cholecalciferol. Main outcome measures were ICU admission and mortality.

Results: ICU assistance was required by 102 (12.2%) participants. Out of 447 patients treated with calcifediol at admission, 20 (4.5%) required the ICU, compared to 82 (21%) out of 391 nontreated ($P < .001$). Logistic regression of calcifediol treatment on ICU admission, adjusted by age, sex, linearized 25-hydroxyvitamin D levels at baseline, and comorbidities showed that treated patients had a reduced risk of requiring the ICU (odds ratio [OR] 0.13; 95% CI 0.07-0.23). Overall mortality was 10%. In the intention-to-treat analysis, 21 (4.7%) out of 447 patients treated with calcifediol at admission died compared to 62 patients (15.9%) out of 391 nontreated ($P = .001$). Adjusted results showed a reduced mortality risk with an OR of 0.21 (95% CI, 0.10-0.43). In the second analysis, the obtained OR was 0.52 (95% CI, 0.27-0.99).

Conclusion: In patients hospitalized with COVID-19, calcifediol treatment significantly reduced ICU admission and mortality.

Key Words: COVID-19, vitamin D, calcifediol, ICU admission, mortality

SARS-CoV-2 is the etiologic agent of COVID-19, a rapidly spreading infection that has caused a global pandemic since its initial identification in China in December 2019. The clinical spectrum of the disease ranges from asymptomatic or mild flu-like symptoms to severe respiratory illness and death (1). Epidemiological studies have estimated that approximately 20% of infected patients sought medical attention, and 10% required hospitalization, thereby tremendously affecting health care services worldwide (2, 3). The burden of COVID-19 has particularly overwhelmed critical care, leading to saturated intensive care units (ICUs) by patients with SARS-CoV-2-mediated acute respiratory distress syndrome (ARDS). Therefore, there is an enormous interest by the medical and scientific community to identify risk and protective factors associated with the development of COVID-19-related severe outcomes. At the beginning of the pandemic no effective treatments were available. As of this writing, only 4 drugs are approved by the US Food and Drug Administration: the antiviral agent remdesivir, effective in reducing recovery time in adults hospitalized with COVID-19 (4), and dexamethasone, or other corticosteroids, the only therapy that has been shown to reduce mortality so far in patients requiring mechanical ventilation or high-flow oxygen (5). More recently, the US Food and Drug Administration has approved by emergency use authorization baricitinib in combination with remdesivir in patients requiring invasive mechanical ventilation or extracorporeal membrane oxygenation (6), as well as the neutralizing antibody bamlanivimab (LY-CoV555) (7).

Adequate vitamin D status has emerged as a potentially preventing factor for COVID-19 progression and mortality (8, 9). Protective effects of vitamin D on the endocrine system are supported by studies that point to a role of the hormone in innate and acquired immunity modulation, autophagy induction, and synthesis of reactive oxygen intermediates (10, 11). In addition, vitamin D is known to be involved in the induction of antimicrobial peptides in response both to viral and bacterial infections (12). A systematic review and meta-analysis from randomized controlled trials (RCTs) including more than 11 000 participants before the onset of COVID-19, showed protective effects of daily or weekly vitamin D administration on the risk of acute respiratory tract infection (13). Many data indicate that vitamin D deficiency, which is common among critically ill patients, is associated with longer hospital and ICU length of stay, lung and other organ injury, prolonged mechanical ventilation, and death (14).

Recent studies suggested that vitamin D supplementation might reduce the risk of influenza and SARS-CoV-2-related infections and deaths by decreasing proinflammatory cytokine production, thereby, diminishing the risk of developing a cytokine storm, which ultimately leads to ARDS development (15). A 2017 meta-analysis of data from 25 RCTs of vitamin D supplementation for the prevention of acute respiratory infections revealed a protective effect of this intervention, although the risk reduction was small. Protection was associated with administration of daily doses of 400 to 1000 IU for up to 12 months (16).

Considering the aforementioned data, we evaluated the effect of 25(OH)D₃ (calcifediol) treatment and baseline 25-hydroxyvitamin D (25(OH)D) serum levels on COVID-19-related severe outcomes: ICU admission and mortality rate among patients hospitalized for COVID-19 in a population-based study on patients admitted to COVID-19 wards in Hospital del Mar, Barcelona, Spain.

Materials and Methods

Study Design

Clinical data from patients with COVID-19, hospitalized randomly at 1 of the 8 COVID-19 units in Hospital del Mar (Barcelona, Spain), were collected from March to May 2020 in the Barna-COVIDIOL cohort—a prospective, nonselected, observational, clinical cohort study. Each COVID ward from Hospital del Mar was assigned to a single medical unit during the first pandemic outbreak in Barcelona. The study protocol was approved by the ethics committee of Parc de Salut Mar (Exp Nos. 2020/9287 and 2021/9751) and it was conducted in accordance with the Declaration of Helsinki and the Good Clinical Practice guidelines of the International Conference on Harmonization. All patients were verbally informed about the treatment options. Verbal consent was registered in the hospital's electronic medical record system.

Participants

Patients with SARS-CoV-2 infection with chronic conditions and/or severe COVID-19 symptomatology were enrolled at admission. Patients who met one of these conditions were admitted in one of the COVID-19 wards:

Both options apply if the SARS-CoV-2 polymerase chain reaction was positive:

- 1) Severe pneumonia was confirmed by x-ray or computed tomography scan CURB-65 greater than 2, defined as disease severity scoring of the combination of confusion, urea, respiratory rate, blood pressure, and age 65 or older.

Oxygen saturation less than 90%

Respiratory rate greater than 30

Modified Early Warning score (MEWS) 3 to 4, defined as physiological parameters including systolic blood pressure, pulse rate, respiratory rate, temperature, and level of consciousness

- 2) Mild pneumonia confirmed by x-ray or computed tomography scan CURB-65 less than or equal to 1

Oxygen saturation 90% or greater

Respiratory rate 30 or less

MEWS score less than 3

Chronic diseases were defined as chronic obstructive pulmonary disease, chronic kidney disease (CKD), obesity, diabetes, and age older than 65 years.

Patients were followed from the first day of admission to their COVID ward up to the date of medical discharge or death. Eligible participants were patients aged 18 years or older who tested polymerase chain reaction positive for SARS-CoV-2. A total of 930 individuals were included in the Barna-COVIDIOL cohort (Fig. 1). Of these, 92 patients had been recently treated (< 3 months) with calcifediol and were excluded from the study. Ultimately, 838 patients were included in the intention-to-treat (ITT) analysis: A total of 447 received calcifediol at admission to the hospital and 391 were not treated (control group). Fifty-three patients in the control group who required the ICU also received calcifediol during ICU admission (same dosage and schedule as for patients treated at admission) at the discretion of the treating physicians and outside the original protocol.

Clinical samples for SARS-CoV-2 testing were obtained and analyzed according to World Health Organization guidelines (Laboratory testing for 2019 novel coronavirus [2019-nCoV] in suspected human cases: <https://www.who.int/publications/i/item/10665-331501> (interim guidance March 19, 2020) (17). All hospitalized patients received the hospital's standard-of-care therapy (applicable at the time of the study), consisting of hydroxychloroquine

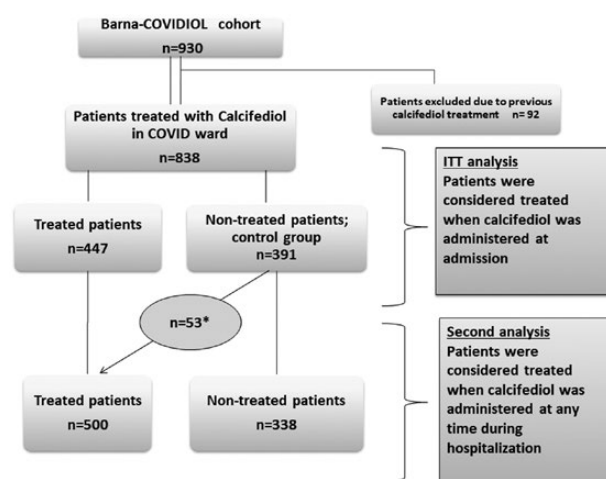


Figure 1. Flowchart showing the number of patients recruited in the Barna-COVIDIOL cohort allocated according to calcifediol treatment at admission-intention-to-treat (ITT) or during hospitalization. *Fifty-three patients from the control group started calcifediol during intensive care unit admission.

400 mg/24 hours the first day and 200 mg/24 hours for 4 days with azithromycin 500 mg/24 hours for 3 days, plus ceftriaxone 1 or 2 g/24 hours for 7 days when there was bacterial superinfection. Patients with severe or critical pulmonary inflammation or clinical suspicion of cytokine storm were additionally treated with dexamethasone bolus (20 mg/day \times 4 days) according to hospital guidelines.

SARS-CoV-2 patients were placed in 1 of the 8 COVID-19 wards according to bed availability at admission time. All COVID-19 wards followed the same hospital standard of care protocol for COVID-19 treatment except for calcifediol, which was prescribed to patients in 5 wards, whereas no such therapy was given to patients in the other 3 wards. Oral calcifediol treatment (Hidroferol Faes-Farma) in soft capsules was administered as follows: first dose of 2 capsules (266 μ g/capsule) at baseline (day 0), a second dose of 1 capsule at day 3, and subsequent doses of 1 capsule given at days 7, 15, and 30. The nontreated control group consisted of patients who did not receive calcifediol at the time of hospitalization.

Outcomes

The outcomes of the study were admission into the ICU (yes/no) and mortality (yes/no). The effect of calcifediol administration was studied in a prospective, open-label study. The consequences of baseline vitamin D status were studied in a subset of 678 patients for whom baseline serum 25-hydroxyvitamin D (25(OH)D) concentrations were measured.

Variables

Serum 25-hydroxyvitamin D levels

Serum levels of 25(OH)D were measured at baseline by competitive immunoluminometric direct assay with direct-coated magnetic microparticles (coefficient of variation < 10%) (Elecsys 25OHD total II, model 07028148190; Cobas e801 system, Roche Diagnostics GmbH).

Obtained baseline levels were linearized using the decimal logarithm (logarithm with base 10 or log₁₀) to fit the multivariable regression analysis. Moreover, participants were classified according their 25(OH)D levels: Patients with a serum concentration below 20 ng/mL were considered to be vitamin D deficient, whereas those with a serum concentration 20 ng/mL or greater were considered to be vitamin D replete (18, 19).

Risk factors and comorbidities

At the time of recruitment, several clinical variables and risk factors were registered from our hospital's and/or primary care medical records: age, sex, high blood pressure,

obesity (body mass index \geq 30), dyslipidemia, cardiovascular disease including ischemic heart disease, congestive heart failure, arrhythmia, heart valve disorders, and stroke, chronic kidney disease (CKD), type 1 or type 2 diabetes mellitus, respiratory-related diseases including chronic obstructive pulmonary disease and asthma, previous or current cancer, persistent viral infections including HIV, hepatitis C virus, and hepatitis B virus, and autoimmune diseases including lupus, psoriasis, and rheumatoid arthritis.

Statistical Analysis

Descriptive statistics were used for demographic, laboratory, and clinical prognostic factors related to COVID-19. Comparisons between groups for quantitative variables were performed by ITT analysis using *t* test or Kruskal-Wallis test. Chi-square tests were used for qualitative variables and cross tabs to estimate the odds ratio (OR). Multivariate logistic regressions were used to estimate adjusted OR or β coefficient, and 95% CIs for the probability of admission to the ICU or mortality. Baseline levels of 25(OH)D were linearized by applying the base 10 logarithm. Age, sex, and COVID-19 risk factors (ie, high blood pressure, dyslipidemia, cardiovascular disease, obesity, previous or current cancer, CKD, chronic infections, autoimmune conditions, chronic respiratory diseases, and type 2 diabetes mellitus) were selected as confounders. Statistical analysis was performed with R for Windows, version 3.5.3 (haven, stats, gam, and compareGroups packages) and SPSS Statistics, version 22.0. *P* values lower than .05 were considered significant.

Results

Patient Characteristics

A total of 838 participants were included in the ITT analysis. Of these, 447 patients were treated with calcifediol at admission and 391 were not treated. Fifty-three patients in this nontreated group started calcifediol during ICU admission. These patients were allocated to the treated group in a second analysis (see Fig. 1). Patient characteristics, stratified by calcifediol treatment at admission (ITT), are reported in Table 1A. Significant differences were found in baseline 25(OH)D levels between groups where the treated group had a median higher than the nontreated group (13 [8-24] vs 0.12 [8-19], respectively; *P* < .026). No other significant baseline differences were found in demographic characteristics, features, and illness condition between the groups.

A total of 678 patients in the cohort had baseline 25(OH)D levels measured, with a median (quartile [Q]1-Q3) of 13 ng/mL (8-22 ng/mL). Of these, 478 (70.5%) had

25(OH)D levels of less than 20 ng/mL: 270 (56.4%) in the treated group and 208 (43.5%) in the untreated group.

Effects of Calcifediol Treatment and Baseline 25-Hydroxyvitamin D Levels on Intensive Care Unit Admission

ICU assistance was required by 102 participants (12.2%). Out of 447 patients treated with calcifediol (at admission), 20 (4.5%) required the ICU, whereas out of 391 patients not treated with calcifediol at admission, 82 (21.0%) required the ICU (OR: 0.18; 0.11-0.29, $P < .001$). Patients admitted to the ICU had significantly lower baseline 25(OH)D levels compared to patients who remained in COVID wards (median [Q1-Q3]: ICU patients 10 ng/mL

[7-14 ng/mL]; no ICU 13 ng/mL [8-23 ng/mL], $P < .001$). Obesity was significantly more frequent in ICU patients (Table 1B). Logistic regression of calcifediol treatment on ICU admission, adjusted by age, sex, linearized 25(OH)D levels at baseline, and comorbidities showed that treated patients had an 87% reduced risk of requiring the ICU (OR 0.13; 95% CI, 0.07 to 0.23) (see Table 2). Baseline 25(OH)D levels inversely correlated with risk of ICU admission (β coefficient -1.72 ; 95% CI, -2.78 to -0.71); $P = .001$). The same type of analysis was performed using categorized 25(OH)D levels (< 20 ng/mL or ≥ 20 ng/mL), showing that patients with adequate baseline levels (≥ 20 ng/mL) had a decreased risk of ICU admission of 70% compared to patients with deficient 25(OH)D levels (OR 0.30; 95% CI, 0.14 to 0.65) (see Table 2). Obesity was also significantly

Table 1A. Patient characteristics stratified according to calcifediol treatment at admission

Calcifediol	Treated N = 447 (53.3%)	Nontreated N = 391 (46.7%)	P
Mean age \pm SD, y	61.81 \pm 15.5	62.41 \pm 17.2	NS
Sex, % male	264 (59.1%)	231 (59.1%)	NS
Baseline levels of 25(OH)D, median ng/mL, Q1-Q3	13 (8-24)	12 (8-19)	.026
HBP, No., %	189 (42.3%)	182 (46.5%)	NS
Dyslipidemia, No., %	122 (27.3%)	120 (30.7%)	NS
CAD, No., %	75 (16.8%)	76 (19.4%)	NS
Obesity, No., %	35 (9.0%)	33 (7.4%)	NS
Cancer ^a , No., %	35 (7.8%)	35 (9.0%)	NS
Chronic kidney disease, No., %	29 (6.5%)	35 (9.0%)	NS
Chronic infections, No., %	19 (4.3%)	16 (4.1%)	NS
Autoimmune conditions, No., %	11 (2.5%)	11 (2.8%)	NS
Chronic respiratory diseases, No., %	69 (15.4%)	86 (22.0%)	NS
Type 2 diabetes mellitus, No., %	93 (20.8%)	76 (19.4%)	NS

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CAD, cardiovascular disease; HBP, high blood pressure; NS, nonsignificant; Q, quartile.

^aPrevious or current cancer.

Table 1B. Patient characteristics according to intensive care requirements

Patients with ICU requirement	Yes N = 102 (12.2%)	No N = 736 (87.8%)	P
Mean age \pm (SD), y	61.20 \pm 14.7	62.21 \pm 16.6	NS
Sex, % male	67 (65.7%)	428 (58.2%)	NS
Baseline levels of 25(OH)D, median ng/mL, Q1-Q3	10 (7-14)	13 (8-23)	$< .001$
HBP, No., %	49 (48%)	322 (43.8%)	NS
Dyslipidemia, No., %	25 (24.5%)	217 (29.5%)	NS
CAD, No., %	21 (20.6%)	130 (17.7%)	NS
Obesity, No., %	14 (13.7%)	54 (7.3%)	.027
Cancer ^a , No., %	10 (9.8%)	60 (8.2%)	NS
Chronic kidney disease, No., %	3 (2.9%)	61 (8.3%)	NS
Chronic infections, No., %	3 (2.9%)	32 (4.3%)	NS
Autoimmune conditions, No., %	5 (4.9%)	17 (2.3%)	NS
Chronic respiratory diseases, No., %	19 (18.6%)	136 (18.5%)	NS
Type 2 diabetes mellitus, No., %	25 (24.5%)	144 (19.6%)	NS

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CAD, cardiovascular disease; HBP, high blood pressure; ICU, intensive care unit; NS, nonsignificant; Q, quartile.

^aPrevious or current cancer.

associated with ICU admission (OR 2.34; 95% CI, 1.10 to 4.93) (see [Table 2](#)).

Effects of Calcifediol Treatment and Baseline 25-Hydroxyvitamin D Levels on Mortality

Out of 838 hospitalized patients included in the ITT analysis, 83 died (10%). Of these, 31.3% died during ICU admission. First, we calculated the mortality risk according to the initial ITT: Out of 447 patients who received calcifediol at admission, 21 died (4.7%), whereas of the control group (n = 391), 62 (15.9%) died (OR: 0.26; 0.15-0.43, $P < .001$). Logistic regression analysis adjusted by 25(OH)D levels,

age, sex, and comorbidities showed that calcifediol treatment reduced the mortality risk by more than 70% (OR 0.21; 95% CI, 0.10 to 0.43) ([Table 3](#)). In a second analysis, the patients who received calcifediol at hospital admission (n = 447) were combined with the ones starting with this drug when admitted to the ICU (n = 53): Of these 500 patients, 36 died (7.2%) vs 47 who died (13.9%) among the 338 patients who never received calcifediol (OR: 0.48; 0.30-0.76, $P = .001$). Logistic regression analysis adjusted by 25(OH)D levels, age, sex, and comorbidities showed a reduction of mortality risk in treated individuals (OR 0.52; 95% CI, 0.27 to 0.99) ([Table 4](#)). An additional analysis was performed excluding those 53 patients who started

Table 2. Logistic regression analysis for the association of calcifediol treatment and 25-hydroxyvitamin D baseline serum levels with intensive care unit requirement

Using linearized baseline 25(OH)D levels (n = 678)				
	OR	Low CI	High CI	P
Calcifediol treatment	0.13	0.07	0.23	< .001
Linear 25(OH)D	0.18	0.06	0.50	.001
Obesity	2.34	1.10	4.93	.026
Using categorized baseline 25(OH)D levels (n = 678)				
	OR	Low CI	High CI	P
Calcifediol treatment	0.13	0.07	0.24	< .001
25(OH)D, ≥ 20 ng/mL	0.30	0.14	0.65	.002
Obesity	2.10	1.00	4.40	.048

Adjusted by age, sex, 25OHD levels, and COVID-19 risk factors.

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; Calcifediol treatment, minimum of 2 capsules before intensive care unit admission; Linear 25(OH)D, baseline values of 25(OH)D linearized by base 10 logarithm; Obesity, body mass index ≥ 30 ; OR, odd ratio.

Table 3. Logistic regression analysis for the association of calcifediol treatment at admission and 25-hydroxyvitamin D baseline serum levels with mortality. Original intention-to-treat analysis

Using linearized baseline 25(OH)D levels (n = 678)				
	OR	Low CI	High CI	P
Calcifediol treatment	0.21	0.10	0.43	< .001
Linear 25OHD	0.09	0.02	0.39	.001
Age, y	1.11	1.07	1.15	< .001
Autoimmune conditions	5.78	1.32	25.30	.02
Obesity	3.48	1.30	9.33	.013
Using categorized baseline 25(OH)D levels (n = 678)				
	OR	Low CI	High CI	P
Calcifediol treatment	0.22	0.11	0.44	< .001
25(OH)D, ≥ 20 ng/mL	0.30	0.11	0.78	.015
Age, y	1.11	1.08	1.15	< .001
Autoimmune conditions	6.17	1.37	27.72	.018
Obesity	2.94	1.10	7.87	.031

Adjusted by age, sex, 25(OH)D levels, and COVID-19 risk factors.

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; Calcifediol treatment, minimum of 2 capsules before intensive care unit admission; Linear 25(OH)D, baseline values of 25(OH)D linearized by base 10 logarithm; Obesity, body mass index ≥ 30 ; OR, odd ratio.

calcifediol treatment during ICU admission: Out of 447 patients treated with calcifediol, 21 (4.7%) died, while out of 338 patients of the nontreatment group, 47 (13.9%) died (OR: 0.30; 0.18-0.52, $P < .001$). Logistic regression analysis adjusted by 25(OH)D levels, age, sex, and comorbidities showed that calcifediol treatment was associated with decreased mortality (OR 0.28; 95% CI, 0.13 to 0.60) compared to nontreated patients (Table 5).

COVID-19 patients who died had lower baseline 25(OH)D levels compared to patients who survived (median

[Q1-Q3]: exitus patients 9 ng/mL [6-13.5]; discharged alive patients 13 ng/mL [8;22.7], $P < .001$). Adjusted results showed that baseline 25(OH)D levels were significantly associated with mortality where adequate 25(OH)D levels reduced the mortality risk about 70% (see Tables 3 and 4). Linear 25(OH)D levels at baseline inversely correlated with mortality with a β coefficient of -2.32 (95% CI, -3.77 to -0.99 ; $P < .001$). Age, autoimmune disorders, and obesity were identified as risk factors for mortality (see Tables 3 and 4).

Table 4. Logistic regression analysis for the association of calcifediol and baseline 25-hydroxyvitamin D serum levels with mortality. Treated group: all patients treated during hospitalization; nontreated group: patients never treated

Using linearized baseline 25(OH)D levels (n = 678)

	OR	Low CI	High CI	P
Calcifediol treatment	0.52	0.27	0.99	.048
Linear 25(OH)D	0.10	0.02	0.38	.001
Age, y	1.11	1.07	1.14	< .001
Autoimmune conditions	7.01	1.58	31.13	.010
Obesity	4.42	1.74	11.21	.002

Using categorized baseline 25(OH)D levels (n = 678)

	OR	Low CI	High CI	P
Calcifediol treatment	0.55	0.29	1.04	.066
25(OH)D, ≥ 20 ng/mL	0.29	0.11	0.74	.010
Age, y	1.11	1.07	1.15	< .001
Autoimmune conditions	6.90	1.53	31.06	.012
Obesity	3.71	1.45	9.50	.006

Adjusted by age, sex, 25(OH)D levels, and COVID-19 risk factors.

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; Calcifediol treatment, minimum of 2 capsules before intensive care unit admission; Linear 25(OH)D, baseline values of 25(OH)D linearized by base 10 logarithm; Obesity, body mass index ≥ 30 ; OR, odd ratio.

Table 5. Logistic regression analysis for the association of calcifediol treatment at admission and baseline 25-hydroxyvitamin D levels with mortality. Patients treated during intensive care unit admission were excluded from analysis

Using linearized baseline 25(OH)D levels (n = 631)

	OR	Low CI	High CI	P
Calcifediol treatment	0.28	0.13	0.60	.001
Linear 25(OH)D	0.10	0.021	0.45	.003
Age, y	1.13	1.09	1.18	< .001
Autoimmune conditions	6.14	1.08	34.89	.041

Using categorized baseline 25(OH)D levels (n = 631)

	OR	Low CI	High CI	P
Calcifediol treatment	0.29	0.14	0.62	.001
25(OH)D, ≥ 20 ng/mL	0.39	0.14	1.06	.065
Age, y	1.13	1.09	1.18	< .001
Autoimmune conditions	5.81	1.01	33.18	.048

Adjusted by age, sex, 25(OH)D levels, and COVID-19 risk factors.

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; Calcifediol treatment, minimum of 2 capsules before intensive care unit admission; Linear 25(OH)D, baseline values of 25(OH)D linearized by base 10 logarithm; Obesity, body mass index ≥ 30 ; OR, odd ratio.

As a subanalysis, we evaluated the mortality of ICU-admitted patients ($n = 102$): The mortality rate of such patients who received calcifediol at time of hospitalization ($n = 20$) was 10.0% vs 28.3% of mortality in individuals who started calcifediol after ICU admission ($n = 53$) and 31% in patients never treated with calcifediol ($n = 29$). No statistically significant differences were found between groups.

Discussion

In this open-label study conducted during the first European outbreak of the deadly COVID-19 pandemic, we have observed that, in hospitalized COVID-19 patients, treatment with calcifediol reduced the risk of requirement for critical care by more than 80%. This supports the conclusion of a prior pilot trial in Cordoba (Spain) in which calcifediol treatment led to a dramatic decrease in ICU admission (OR 0.03; 95% CI, 0.003 to 0.25) (20). Furthermore, calcifediol started at the time of hospitalization (ITT analysis) reduced the mortality risk by more than 70%. Importantly, our results suggest that calcifediol administration should preferably be given prior to ARDS development, since initiation of calcifediol during ICU admission did not modify patient survival to the same extent.

Studies with cholecalciferol have generated mixed results. In a rather underpowered Brazilian RCT (21), a bolus of cholecalciferol (200 000 IU or 5 mg of vitamin D_3) administered during hospitalization because of COVID-19 did not reduce ICU admission nor the risk of death. In a small-scale study in hospitalized, frail, older COVID-19 patients, the administration of an oral supplement of 80 000 IU vitamin D_3 within hours of diagnosis also did not decrease the risk of death (22). However, vitamin D_3 supplementation in a bolus of 80 000 IU vitamin D_3 either the month before or the week following diagnosis of COVID-19 was associated with less severe COVID-19 evolution and better survival rate in frail, older patients (22). In addition, regular vitamin D_3 supplementation, at least in older individuals, in boluses administered regularly during the year prior to diagnosis, has been shown to reduce the risk of death and improve clinical outcome in older patients with COVID-19 (23, 24). In our study, patients treated with calcifediol before hospitalization were excluded from the ITT analysis because the main objective of this study was to analyze the effect of calcifediol administered at admission time.

Calcifediol, a prehormone of the vitamin D endocrine system, a substrate for the synthesis of 1,25(OH) $_2$ D, was selected, rather than more commonly used cholecalciferol or, native vitamin D_3 threshold system nutrient, because

of its excellent pharmacokinetic profile, including a high intestinal absorption (close to 100%), a time to maximum of approximately 4 hours resulting in a rapid increase in serum 25(OH)D, and a half-life of 12 to 22 days (25). The area under the curve (AUC) $_{0-72h}$ (ng/mL·h) is 2382.02 ± 665.43 (26). Moreover, based on all published comparative RCTs, oral calcifediol is more potent than oral cholecalciferol. When comparing a daily dose of less than 25 μ g of cholecalciferol (1000 IU) with similar low dosages of calcifediol, oral calcifediol is 2- to 5-fold more potent (mean of all studies, ~ 3.2) than oral cholecalciferol, although the potency of calcifediol is much higher in studies using high-dose oral of cholecalciferol, varying from 5.5 to 9 to 12 times (25). Calcifediol does not require hepatic hydroxylation, which is frequently impaired in acutely ill patients, and thus is more readily available for conversion to vitamin's D active metabolite, 1,25(OH) $_2$ D (25).

The study population had a relatively low vitamin D status as demonstrated by the median (Q1-Q3) serum 25(OH)D concentration at baseline (13 ng/mL [8-22 ng/mL]). Whether the beneficial effects of calcifediol would also be applicable in less vitamin D-deficient populations will require appropriate intervention studies.

The analysis of the Barna-COVIDIOL prospective cohort also showed that baseline serum 25(OH)D was significantly and inversely related to ICU requirement and mortality. Several observational studies have pointed to a relationship between vitamin D deficiency and COVID-19-related mortality and/or disease severity (27-30), including a large cross-sectional study performed across 20 European countries in which a negative correlation between 25(OH)D serum levels and mortality was observed (8). Panagiotou et al also reported a significantly greater prevalence of vitamin D deficiency in COVID-19 patients admitted to the ICU compared to those that not required critical care (31). However, other studies failed to find an association between vitamin D-deficient status and poor disease outcomes (31-34). Furthermore, we should take into consideration that lower 25(OH)D levels at baseline might reflect underlying comorbidities or an inflammatory state due to COVID-19 since 25(OH)D levels may decrease by up to 40% in states of systemic inflammation (35, 36). Therefore, it is plausible that patients with deficient vitamin D levels secondary to an inflammatory state are more likely to be admitted to the ICU. Recent publications have reviewed vitamin's D plausible immunomodulatory mechanisms of actions on SARS-CoV-2 infection, which are likely to contribute to our study results (8, 20). Most likely, calcifediol interferes with COVID-19-induced ARDS development. Indeed, ARDS is the most common indication for admitting a patient with COVID-19 to the ICU (35). This life-threatening condition

is the consequence of an inflammatory and diffuse alveolar injury of acute onset that leads to bilateral lung infiltration and severe hypoxemia. The pathogenesis of ARDS is closely linked to an exacerbated proinflammatory cytokine response of the host (36), and it is precisely in this setting where we speculate that vitamin D exerts its main beneficial effects. The adaptive immune system can be modulated by $1,25(\text{OH})_2\text{D}$, most importantly by modifying the phenotype of dendritic cells (responsible for antigen presentation to T cells), leading to a decrease in proinflammatory T cells subtype proliferation, while enhancing the production of regulatory T cells (37). Proinflammatory cytokine release in macrophages is also decreased by $1,25(\text{OH})_2\text{D}$ (38). Ultimately these effects are thought to curb the inflammatory cascade that leads to the cytokine and chemokine storm associated with the pathogenesis of ARDS.

There might be other mechanisms by which vitamin D might protect against ARDS (39). It downregulates the renin-angiotensin system, a proinflammatory endocrine axis implicated in ARDS pathogenesis through the decrease of renin activity and by increasing angiotensin-converting enzyme 2 and controlling the alteration of the coagulation cascade. Intra-alveolar fibrin clots are particularly typical in coronavirus-provoked ARDS, and in addition to its inhibitory effects on the renin-angiotensin system, several in vitro studies also suggest that $1,25(\text{OH})_2\text{D}$ displays direct antithrombotic effects. Finally, vitamin D appears to also play a role in the maintenance and repair of the respiratory epithelium, thus, potentially helping in the prevention and resolution of acute lung injury.

The present study also confirmed that age and obesity are additional risk factors. Indeed, both factors have been linked to COVID-19 severity and poor outcomes in multiple studies and at the same time are well-established risk factors for vitamin D deficiency (40). Our analysis also included a logistic regression analysis eliminating the potential confounding effect of age and obesity.

There are several limitations in the present study. First, it was not placebo controlled and no electronic/statistical randomization was performed and hence, it cannot be considered an RCT. However, because incoming patients were assigned to a ward based on bed availability and therefore were not selected, we can consider the effect of calcifediol close to an open-label clinical study performed in real-life conditions. Second, statistical differences in baseline $25(\text{OH})\text{D}$ levels were found between treated and nontreated patients in the ITT analysis, even though these differences cannot be considered clinically relevant. Of note, all analyses were adjusted by these $25(\text{OH})\text{D}$ levels as well as other confounder variables. Another limitation is that serum $25(\text{OH})\text{D}$ was not measured during follow-up. No dose response curve was tested, so we cannot define

the minimal required dose and there was also no comparison with the more commonly used forms of vitamin D. However, considering the administered doses and the drug's pharmacokinetic profile, we assume it replenished $25(\text{OH})\text{D}$ deposits in all treated patients. Finally, severity scales were not analyzed.

In spite of its weaknesses, the study was adequately powered to detect possible effects on the essential hard end points of ICU admission and mortality.

In summary, calcifediol administered shortly after hospitalization markedly reduced the requirement for ICU admission and decreased mortality by more than 50%. Moreover, baseline $25(\text{OH})\text{D}$ levels negatively correlated with ICU admission and mortality. These findings point to the relevance of attaining an adequate $25(\text{OH})\text{D}$ status as soon as possible in the setting of SARS-CoV-2 infection. This is particularly attractive in the current epidemiologic situation as $25(\text{OH})\text{D}$ deficiency is an easily modifiable factor. Nonetheless, additional studies are necessary to fully elucidate the effects of circulating $25(\text{OH})\text{D}$ levels and $25(\text{OH})\text{D}$ treatment on COVID-19 disease severity in other populations with different baseline vitamin D status.

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