ORIGINAL ARTICLE

Vitamin D supplementation and COVID-19 risk: a population-based, cohort study

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Abstract

Purpose To analyze the associations between cholecalciferol or calcifediol supplementation, serum 25-hydroxyvitamin D (25OHD) levels and COVID-19 outcomes in a large population.

Methods All individuals ≥ 18 years old living in Barcelona-Central Catalonia ($n = 4.6$ million) supplemented with cholecalciferol or calcifediol from April 2019 to February 2020 were compared with propensity score-matched untreated controls. Outcome variables were SARS-CoV2 infection, severe COVID-19 and COVID-19 mortality occuring during the first wave of the pandemic. Demographical data, comorbidities, serum 25OHD levels and concomitant pharmacological treatments were collected as covariates. Associations between cholecalciferol or calcifediol use and outcome variables were analyzed using multivariate Cox proportional regression.

Results Cholecalciferol supplementation ($n = 108{,}343$) was associated with slight protection from SARS-CoV2 infection ($n = 4352$ [4.0%] vs 9142/216,686 [4.2%] in controls; HR 0.95 [CI 95% 0.91–0.98], $p = 0.004$). Patients on cholecalciferol treatment achieving 25OHD levels ≥ 30 ng/ml had lower risk of SARS-CoV2 infection, lower risk of severe COVID-19 and lower COVID-19 mortality than unsupplemented 25OHD-deficient patients (56/9474 [0.6%] vs 96/7616 [1.3%]; HR 0.66 [CI 95% 0.46–0.93], $p = 0.018$). Calcifediol use ($n = 134{,}703$) was not associated with reduced risk of SARS-CoV2 infection or mortality in the whole cohort. However, patients on calcifediol treatment achieving serum 25OHD levels ≥ 30 ng/ml also had lower risk of SARS-CoV2 infection, lower risk of severe COVID-19, and lower COVID-19 mortality compared to 25OHD-deficient patients not receiving vitamin D supplements (88/16276 [0.5%] vs 96/7616 [1.3%]; HR 0.56 [CI 95% 0.42–0.76], $p < 0.001$).

Conclusions In this large, population-based study, we observed that patients supplemented with cholecalciferol or calcifediol achieving serum 25OHD levels ≥ 30 ng/ml were associated with better COVID-19 outcomes.

Keywords Cholecalciferol · Calcifediol · Vitamin D · COVID-19 · SARS-CoV2

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Introduction

Infection with the new coronavirus SARS-CoV2 is characterized by an important clinical variability, which suggests that there are important host-related factors that impact in COVID-19 outcomes. One of these factors has been postulated to be vitamin D deficiency [1, 2], which is a prevalent condition worldwide [3]. Vitamin D is now being recognized as an hormonal system with many extra-skeletal actions, including important effects on the immunological system [4–6].

Several clinical trials and two meta-analysis have shown that cholecalciferol or ergocalciferol supplementation may help prevent acute respiratory infections [7, 8]. However, evidence that these drugs are helpful to treat or prevent SARS-CoV2 infection is still controversial [9].

Observational studies have described an association between low serum levels of 25-hydroxyvitamin D (25OHD) and higher risk of SARS-CoV2 infection [10–12], higher risk of severe COVID-19 or higher COVID-19 mortality [13–15]. Three meta-analysis have also concluded that there is an association between low serum levels of 25OHD and higher risk of COVID-19 mortality [16–18]. However, studies analyzing the use of cholecalciferol or calcifediol supplementation to modify COVID-19 outcomes have offered inconclusive results. While some observational studies in hospitalized patients have shown reduced COVID-19 severity or mortality in patients supplemented with cholecalciferol or calcifediol [19–21], Cereda et al. described a trend to an increased mortality in patients supplemented with calcifediol [22].

The effects of cholecalciferol or calcifediol as a treatment for hospitalized COVID-19 patients have also been studied in three low-powered clinical trials, without observing any significant reduction in COVID-19 mortality [23–25].

To the best of our knowledge, there are not published results describing the effects, at the population level, of cholecalciferol or calcifediol supplementation on COVID-19 outcomes. To ascertain whether vitamin D supplementation protects against SARS-CoV2 infection or from COVID-19 adverse outcomes, we designed this large observational study. We have already shown that calcitriol supplementation, the active form of vitamin D, mainly used in patients with advanced renal failure, was associated with important reductions in COVID-19 mortality [26]. Now, we present the results of cholecalciferol or calcifediol supplementation showing also beneficial effects of these drugs in patients that reach normal serum 25OHD levels.

Methods

Study design and population included

A retrospective cohort was built using the databases of the public healthcare system in Catalonia. We analyzed all individuals ≥ 18 years old living in Barcelona and Central Catalonia regions on 25 February 2020, date of the first positive PCR for SARS-CoV2 in our country ($n = 4,643,139$).

In this population, we performed three independent studies to investigate the association of cholecalciferol or calcifediol supplementation with COVID-19 outcomes:

Comparison of COVID outcomes between supplemented patients and propensity score-matched controls: We identified all patients receiving cholecalciferol ($n = 201,445$) or calcifediol ($n = 207,136$) supplementation from 1 April 2019 to 28 February 2020 and patients not receiving any vitamin D supplement (4,267,430) during the same period. Since chronic kidney disease (CKD) is a strong predictor of worse prognosis in COVID-19 [27], subjects without an available serum creatinine determination performed between 1 October 2018 and 28 February 2020 were excluded from the study. After propensity score matching (see below), 108,343 patients on cholecalciferol, 216,686 matched controls (cholecalciferol controls), 134,703 patients on calcifediol and 269,406 matched controls (calcifediol controls) were selected for the analysis. *Association between mean daily cholecalciferol or calcifediol dose and COVID-19 outcomes:* All patients receiving cholecalciferol or calcifediol supplementation from 1 November 2019 to 28 February 2020, with an available serum creatinine level ($n = 165,588$ and 132,590, respectively), were selected for this analysis. This shorter period of time was chosen to minimize the effects of eventual changes in the dose of these drugs. *Comparison of COVID-19 outcomes between cholecalciferol- or calcifediol-supplemented patients with a sufficient vitamin D status (serum 25OHD > 30 ng/ml) and unsupplemented vitamin D-deficient (serum 25OHD < 20 ng/ml) patients:* In order to reduce the variability in serum 25OHD levels due to seasonal sun exposure, we only analyzed serum levels determined between 1 November 2019 and 28 February 2020. All patients of the cohort that had a serum 25OHD determination in this period of time ($n = 85,158$) were included in this analysis.

Data sources

Given Catalonia's universal health and medication coverage, we were able to utilize electronic databases to

examine the association of cholecalciferol and calcifediol use with COVID-19 outcomes in a real world setting. We used anonymized data provided by the Catalan Agency for Health Quality and Evaluation (AQUAS) within the framework of the Data Analytics Program for Health Research and Innovation (PADRIS). PADRIS databases include information on demographics (age and sex), diagnoses, laboratory data, drugs supplied by pharmacies, Primary Care physician diagnoses, laboratory results and diagnoses, procedures and outcomes of medical admissions in the public hospitals in Catalonia. This project was approved in a public call for grants for using PADRIS databases in research projects on COVID-19.

Identification of patients on cholecalciferol or calcifediol supplementation

Patients who had been supplied in pharmacies with drugs of the Anatomical Therapeutic Chemical Classification System groups A11CC05, A12AX, M05BB03, M05BB07, M05BB08, M05BB09, A11CC06 or A11CC55 from 1 April 2019 to 28 February 2020 were analyzed. The sum of Defined Daily Doses (DDD) of cholecalciferol or the sum of calcifediol doses supplied from 1 November 2019 to 28 February 2020 were identified, transformed into micrograms, and the mean daily cholecalciferol or calcifediol dose received per patient, in micrograms, was calculated. Patients receiving formulations containing > 250 μg of cholecalciferol (12.5 DDD) or > 250 μg of calcifediol per dose were considered as receiving bolus doses.

Identification of control subjects through propensity score matching

We performed two independent propensity score matching to build the control groups for cholecalciferol and calcifediol using the 'Matching' package in R [28] as described [26]. First, we used multivariate logistic regression to model receiving or not each drug as a function of the following covariates: sex, age, fifteen comorbidities identified from the International Classification of Diseases (ICD-10) diagnostic codes issued by family physicians (Supplementary Table 1), estimated glomerular filtration rate (eGFR), history of cigarette smoking, nursing home residence and use of seven classes of drugs that could potentially affect the prognosis (Supplementary Table 1). Estimated glomerular filtration rate was obtained from serum levels of creatinine, sex and age according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [29]. Propensity scores were matched using the nearest-neighbor matching method without replacement at a 1:2 ratio of treated subjects and controls. A caliper of 0.2 of the standard deviation of the propensity score logit was established as the maximum

tolerated difference between matched patients. To examine the balance of each covariate between the treatment and the control group, the standardized mean difference was calculated before and after matching using Tableone package in R [30]. We considered the groups well balanced if the standardized mean difference was < 0.10 for each covariate.

Serum levels of 25-hydroxyvitamin D

Serum levels of 25OHD determined in the laboratories of the catalan public health system between 1 November 2019 and 28 February 2020 in the whole cohort were obtained from PADRIS databases. A deficient vitamin D status was defined as a serum 25OHD level < 20 ng/mL and a sufficient vitamin D status was defined as a serum 25OHD level ≥ 30 ng/mL.

Outcome variables

We analyzed the occurence of SARS-CoV2 infection, COVID-19 hospitalization, intensive care admission, the procedures during hospitalization and mortality during the first wave of the pandemic. Four main outcome variables were defined, with different timings due to the natural course of the disease:

SARS-CoV2 infection

Positive PCR result for SARS-CoV2 or a clinical diagnosis made by a Primary Care physician, or a hospital discharge report stating a diagnosis of COVID-19 (ICD-10 codes used are displayed in Supplementary Table 1), from 25 February 2020 to 30 April 2020. Time (in days) from 24 February 2020 until a positive PCR or a clinical diagnosis (the first event) was used for survival analysis. Censored time for those individuals without the event was the time from 24 February to 30 April 2020.

COVID-19 mortality

Patients diagnosed with COVID-19 infection resulting in death between 25 February and 15 May 2020. Patients with COVID-19 admitted to hospital before 16 May 2020 and dying before 7 June were also included. Time (in days) from 24 February 2020 to COVID-19 death was used for survival analysis. Censored time for those individuals without the event was the time from 24 February to 7 June 2020.

Severe COVID-19

Composite outcome of COVID-19 mortality, as already defined, or COVID-19 hospital admission needing non-invasive mechanical ventilation, orotracheal intubation, mechanical ventilation or intensive care unit admission from 25 February

2020 to 15 May 2020. Time (in days) from 24 February 2020 until hospital admission (if severe COVID-19 developed during hospitalization) or time (in days) from 24 February 2020 until COVID-19 death was used for survival analysis. Censored time for those individuals without the event was the time from 24 February to 7 June 2020.

Statistical analysis

Continous variables are reported as mean and standard deviation and qualitative variables are summarized by frequencies and percentages. Basal differences between treated and untreated groups were assessed using Student's t test or chi-square test and standardized mean differences.

Once the control groups were established, associations between cholecalciferol or calcifediol supplementation and outcome variables were further analyzed using unadjusted and multivariate Cox proportional hazards regression models. All the variables that approached statistical significance ($p < 0.2$) were initially selected for inclusion in the adjusted analyses. Multivariable models were constructed by means of a stepwise forward inclusion procedure and only the significant variables were retained in the final model. Unadjusted and adjusted hazard ratios and their 95% confidence intervals are reported.

Taking into account that vitamin D supplementation may be prescribed to treat a low vitamin D status, we also compared the outcome variables between patients with sufficient vitamin D status, while being vitamin D-supplemented, with patients deficient in vitamin D and not supplemented, also using multivariate Cox regression analysis. Finally, the associations between the mean daily cholecalciferol or mean daily calcifediol dose and COVID-19 outcomes were also analyzed using multivariate Cox regression analysis.

For all statistical tests, a p value < 0.05 was used for statistical significance.

Descriptive statistics and survival analysis were carried out using SPSS version 25.0 for Windows (SPSS, Chicago, IL, USA), and Survival and Survminer packages in R [31, 32].

Ethical issues and confidenciality

All data were treated anonymously in order for this study to comply with the provisions of Spanish and European laws on Protection of Personal Data. The study was approved by the ethics committee of the Corporació Sanitària Parc Taulí-Universitat Autònoma de Barcelona.

Results

COVID-19 outcomes in cholecalciferol-supplemented patients versus propensity score-matched controls

A total of 201,445 patients ≥ 18 years old were identified as being on cholecalciferol treatment between 1 April 2019 and 28 February 2020 in Barcelona and Central Catalonia regions. After propensity-score matching, 108,343 patients on cholecalciferol and 216,686 matched control patients were included in the analysis.

Clinical characteristics of the patients treated with cholecalciferol and their matched controls are shown in Table 1. Mean age was 70 years and more than 83% were women. SARS-CoV2 infection was diagnosed in 4352 patients supplemented with cholecalciferol (2113 of them [48.6%] confirmed by PCR) and in 9142 untreated controls (4300 of them [47.0%] confirmed by PCR). Cholecalciferol use was associated with a mild, but significant, lower risk of SARS-CoV2 infection, both in the univariate and in the multivariate Cox regression analysis ($n = 4352$ [4.0%] versus $n = 9142$ [4.2%] in controls; HR 0.95 [CI 95% 0.91–0.98], $p = 0.004$). This mild reduction in the infection rate was not observed in the subgroup of patients diagnosed by PCR (HR 0.98 [0.93–1.04; $p = 0.51$]). We did not observe any significant association between cholecalciferol supplementation and COVID-19 severity ($n = 798$ [0.7%] versus $n = 1650$ [0.8%] in controls) or COVID-19 mortality (n = 716 [0.7%] versus $n = 1492$ [0.7%] in controls), when comparing with matched untreated controls (Table 2).

Patients receiving bolus cholecalciferol had lower COVID-19 mortality (100/20,715; [0.5%]) than patients on daily cholecalciferol treatment (616/87628; [0.7%]). However, in the multivariate Cox regression analysis, receiving bolus cholecalciferol was not significantly associated with lower COVID-19 severity nor mortality.

We analyzed the cholecalciferol DDD received in the 165,588 patients that had been supplied between 1 November 2019 and 28 February 2020. Mean daily cholecalciferol doses were similar in patients who became infected or died due to COVID-19 than in patients without these outcomes (Table 3). However, in the multivariate Cox regression analysis, the mean daily dose of cholecalciferol, measured in 10 μg intervals (equivalent to 400 IU), was associated with small but significant reductions in the risk of SARS-Cov2 infection (HR 0.97 [CI 95% 0.95–0.99], $p = 0.007$), in SARS-CoV2 infection confirmed by PCR (HR 0.94 [CI 95% 0.91–0.97], $p < 0.001$), in severe COVID-19 (HR 0.92 [CI 95% 0.87–0.97], $p = 0.002$) and in COVID-19 mortality (HR 0.90 [CI 95% 0.85–0.96], $p = 0.001$) (Table 3).

Table 1 Clinical characteristics of patients on cholecalciferol or calcifediol treatment and their matched controls

	Cholecalciferol treated ($n=108,343$)	Cholecalciferol matched ($n=216,686$)	SMD	Calcifediol treated ($n=134,703$)	Calcifediol matched ($n=269,406$)	SMD
Variables used for matching						
Female gender, n (%)	90,417 (83.5)	181,414 (83.7)	0.007	105,229 (78.1)	209,824 (77.9)	0.006
Age, mean (SD)	70.0 (14.0)	70.0 (14.6)	0.001	68.8 (14.9)	68.8 (15.1)	0.004
Nursing home residence, n (%)	2884 (2.7)	5649 (2.6)	0.003	3548 (2.6)	6989 (2.6)	0.002
Cigarette smoking, n (%)	23,329 (21.5)	46,003 (21.2)	0.007	32,507 (24.1)	64,571 (24.0)	0.004
Comorbidities						
Hypertension, n (%)	59,157 (54.6)	116,565 (53.8)	0.016	75,058 (55.7)	148,784 (55.2)	0.010
Obesity, n (%)	39,941 (36.9)	78,711 (36.3)	0.011	51,547 (38.3)	102,367 (38.0)	0.006
Diabetes, n (%)	23,899 (22.1)	46,978 (21.7)	0.009	33,284 (24.7)	66,443 (24.7)	0.001
Heart failure, n (%)	11,572 (10.7)	22,939 (10.6)	0.003	14,692 (10.9)	29,260 (10.9)	0.001
COPD, n (%)	14,838 (13.7)	29,376 (13.6)	0.004	18,431 (13.7)	36,947 (13.7)	0.001
Asthma, n (%)	11,791 (10.9)	23,332 (10.8)	0.004	13,983 (10.4)	27,894 (10.4)	0.001
eGFR, mean (SD)	78.35 (20.65)	78.46 (20.96)	0.005	78.01 (22.91)	78.01 (21.62)	<0.001
Cerebrovascular disease, n (%)	7949 (7.3)	15,674 (7.2)	0.004	10,234 (7.6)	20,346 (7.6)	0.002
Dementia, n (%)	6517 (6.0)	12,783 (5.9)	0.005	7841 (5.8)	15,590 (5.8)	0.001
Malignant neoplasia, n (%)	28,731 (26.5)	57,158 (26.4)	0.003	32,604 (24.2)	65,240 (24.2)	<0.001
Liver cirrhosis, n (%)	1426 (1.3)	2846 (1.3)	<0.001	1641 (1.2)	3346 (1.2)	0.002
Osteoporosis, n (%)	19,332 (17.8)	37,668 (17.4)	0.012	19,052 (14.1)	36,908 (13.7)	0.013
Past femur fracture, n (%)	1746 (1.6)	2939 (1.4)	0.021	1676 (1.2)	2877 (1.1)	0.016
Dyslipidemia, n (%)	57,688 (53.2)	115,410 (53.3)	<0.001	70,817 (52.6)	142,020 (52.7)	0.003
Ischemic heart disease, n (%)	7204 (6.6)	14,097 (6.5)	0.006	10,331 (7.7)	20,784 (7.7)	0.002
Peripheral arteriopathy, n (%)	3085 (2.8)	6064 (2.8)	0.003	4679 (3.5)	9372 (3.5)	<0.001
Use of drugs						
Proton pump inhibitors	49,488 (45.7)	98,961 (45.7)	<0.001	58,522 (43.4)	118,032 (43.8)	0.007
Oral corticosteroids, n (%)	11,986 (11.1)	24,149 (11.1)	0.003	11,098 (8.2)	22,562 (8.4)	0.005
DPP4-inhibitors, n (%)	2659 (2.5)	5194 (2.4)	0.004	4220 (3.1)	8440 (3.1)	<0.001
Statins, n (%)	33,471 (30.9)	66,992 (30.9)	<0.001	43,130 (32.0)	86,714 (32.2)	0.004
ACE inhibitors, n (%)	26,353 (24.3)	52,413 (24.2)	0.003	32,101 (23.8)	64,087 (23.8)	0.001
ARB, n (%)	18,988 (17.5)	37,532 (17.3)	0.005	25,486 (18.9)	50,685 (18.8)	0.003
Immunosuppressants, n (%)	3095 (2.9)	6082 (2.8)	0.003	3004 (2.2)	5941 (2.2)	0.002

SMD standardized mean difference, *SD* standard deviation, *COPD* chronic obstructive pulmonary disease, *eGFR* estimated glomerular filtration rate, *ACE* angiotensin convertint enzyme, *ARB* angiotensin-II receptor blockers, *DPP4* dipeptidyl peptidase-4

COVID-19 outcomes in calcifediol-supplemented patients versus propensity score-matched controls

A total of 207,136 patients ≥ 18 years old were identified as being on calcifediol treatment between 1 April 2019 and 28 February 2020 in Barcelona-Central Catalonia region. After propensity-score matching, 134,703 patients on calcifediol and 269,406 matched control patients were included in the study. Calcifediol was supplied as bolus doses in 99.4% of patients or as a daily drop formulation in 0.6% of patients.

Clinical variables in patients treated with calcifediol and their respective control group are shown in Table 1. Mean age of patients was 69 years, with a high proportion of women and similar comorbidities to the patients treated with cholecalciferol.

SARS-CoV2 infection was diagnosed in 5662 patients supplemented with calcifediol (2607 of them [46.0%] confirmed by PCR) and in 11,401 untreated controls (5413 of them [47.5%] confirmed by PCR). We did not observe any significant association between calcifediol supplementation and the risk of SARS-CoV2 infection ($n=5662$ [4.2%] versus 11,401 [4.2%] in matched controls), nor between calcifediol use and COVID-19 severity ($n=1037$ [0.8%] versus $n=2073$ [0.8%] in controls) or mortality ($n=934$ [0.7%] versus $n=1859$ [0.7%] in controls) (Table 4).

Among calcifediol treated patients, 132,590 had received the drug between 1 November 2019 and 28 February 2020. In the multivariate Cox regression analysis, the mean daily calcifediol dose received in this period, measured in 10 μg intervals, was not associated with the risk of SARS-CoV2

Table 2 Variables associated with COVID-19 outcomes in patients on cholecalciferol treatment (n=108,343) and matched controls (n=216,686)

	SARS-CoV2 infection[1]				Severe COVID-19[2]				COVID-19 mortality			
	Univariate[3] HR (CI 95%)	p	Multivariate[4] HR (CI 95%)	p	Univariate[3] HR (CI 95%)	p	Multivariate[4] HR (CI 95%)	p	Univariate[3] HR (CI 95%)	p	Multivariate[d] HR (CI 95%)	p
Cholecalciferol treatment	0.95 (0.92–0.99)	0.007	0.95 (0.91–0.98)	0.004	0.97 (0.89–1.05)	0.439			0.96 (0.88–1.05)	0.366		
Female sex[5]	0.88 (0.84–0.92)	<0.001			0.42 (0.39–0.46)	<0.001	0.52 (0.48–0.57)	<0.001	0.42 (0.38–0.46)	<0.001	0.56 (0.50–0.62)	<0.001
Age[5]	1.14 (1.13–1.16)	<0.001	0.94 (0.92–0.95)	<0.001	2.38 (2.28–2.48)	<0.001	1.48 (1.41–1.55)	<0.001	2.90 (2.77–3.03)	<0.001	1.79 (1.69–1.89)	<0.001
Cigarette smoking	0.93 (0.89–0.97)	<0.001			0.97 (0.88–1.07)	0.554			0.93 (0.83–1.03)	0.147	1.28 (1.13–1.45)	<0.001
Nursing home residence	8.89 (8.51–9.29)	<0.001	6.25 (5.91–6.60)	<0.001	17.49 (16.06–19.05)	<0.001	6.20 (5.59–6.87)	<0.001	20.25 (18.55–22.11)	<0.001	6.10 (5.49–6.78)	<0.001
Hypertension	1.12 (1.08–1.16)	<0.001			2.37 (2.17–2.60)	<0.001			2.61 (2.37–2.88)	<0.001		
Obesity	1.06 (1.03–1.10)	0.001	1.10 (1.06–1.14)	<0.001	1.23 (1.13–1.33)	<0.001	1.25 (1.15–1.36)	<0.001	1.19 (1.09–1.30)	<0.001	1.26 (1.15–1.38)	<0.001
Diabetes	1.22 (1.17–1.27)	<0.001	1.06 (1.02–1.11)	0.004	2.27 (2.09–2.46)	<0.001	1.33 (1.22–1.45)	<0.001	2.35 (2.15–2.55)	<0.001	1.36 (1.25–1.49)	<0.001
Heart failure	1.97 (1.89–2.06)	<0.001	1.33 (1.27–1.40)	<0.001	4.11 (3.77–4.47)	<0.001	1.35 (1.23–1.48)	<0.001	4.55 (4.17–4.96)	<0.001	1.36 (1.23–1.50)	<0.001
COPD	1.42 (1.36–1.49)	<0.001	1.11 (1.06–1.16)	<0.001	2.46 (2.25–2.69)	<0.001	1.17 (1.07–1.29)	<0.001	2.54 (2.31–2.78)	0.001	1.13 (1.02–1.25)	0.021
Asthma	1.25 (1.19–1.31)	<0.001	1.11 (1.06–1.17)	<0.001	1.11 (0.98–1.26)	0.094			1.03 (0.90–1.17)	0.705		
eGFR	0.92 (0.92–0.93)	<0.001	0.97 (0.96–0.98)	<0.001	0.70 (0.69–0.71)	<0.001	0.88 (0.86–0.90)	<0.001	0.68 (0.67–0.69)	<0.001	0.88 (0.86–0.91)	<0.001
Cerebrovascular disease	1.69 (1.61–1.78)	<0.001	1.23 (1.17–1.30)	<0.001	2.85 (2.57–3.16)	<0.001	1.20 (1.08–1.33)	0.001	3.10 (2.79–3.44)	0.001	1.22 (1.09–1.36)	<0.001
Dementia	3.56 (3.41–3.72)	<0.001	1.78 (1.68–1.87)	<0.001	7.63 (7.01–8.31)	<0.001	2.18 (1.97–2.41)	<0.001	8.78 (8.05–9.58)	<0.001	2.22 (2.00–2.45)	<0.001
Malignant neoplasia	1.11 (1.07–1.15)	<0.001	1.10 (1.06–1.14)	<0.001	1.52 (1.40–1.65)	<0.001	1.13 (1.04–1.23)	0.006	1.58 (1.45–1.72)	<0.001	1.16 (1.06–1.27)	0.002
Liver cirrhosis	1.06 (0.91–1.22)	0.460			1.09 (0.78–1.52)	0.616			0.96 (0.66–1.40)	0.848		
Osteoporosis	0.91 (0.87–0.95)	<0.001	0.89 (0.84–0.93)	<0.001	0.97 (0.87–1.07)	0.511			0.99 (0.88–1.10)	0.814		
Past femur fracture	0.95 (0.92–0.99)	0.007	1.23 (1.11–1.36)	0.007	0.97 (0.89–1.05)	0.439			4.32 (3.62–5.16)	<0.001	1.24 (1.03–1.48)	0.022
Dyslipidemia	0.89 (0.86–0.92)	<0.001	0.92 (0.88–0.95)	<0.001	1.11 (1.03–1.21)	0.008			1.11 (1.02–1.20)	0.018	0.91 (0.84–0.99)	0.038
Ischemic heart disease	1.33 (1.25–1.42)	<0.001	1.09 (1.02–1.16)	0.012	2.10 (1.87–2.36)	<0.001			2.18 (1.93–2.47)	<0.001		
Peripheral arteriopathy	1.37 (1.25–1.49)	<0.001			2.68 (2.30–3.12)	<0.001			2.81 (2.40–3.29)	<0.001		
Use of PPI	1.41 (1.37–1.46)	<0.001	1.17 (1.12–1.21)	<0.001	2.40 (2.21–2.61)	<0.001	1.14 (1.04–1.24)	0.005	2.53 (2.31–2.77)	<0.001	1.14 (1.03–1.25)	0.008
Use of oral corticosteroids	1.63 (1.55–1.70)	<0.001	1.39 (1.33–1.46)	<0.001	2.61 (2.38–2.86)	<0.001	1.79 (1.63–1.98)	<0.001	2.62 (2.38–2.89)	<0.001	1.79 (1.61–1.98)	<0.001
Use of DPP4-inhibitors	1.15 (1.04–1.27)	0.008			1.89 (1.56–2.29)	<0.001			1.84 (1.50–2.26)	<0.001		
Use of statins	0.84 (0.81–0.87)	<0.001	0.87 (0.83–0.91)	<0.001	1.04 (0.96–1.14)	0.340			1.02 (0.93–1.12)	0.683		

Table 2 (continued)

	SARS-CoV2 infection[1]				Severe COVID-19[2]				COVID-19 mortality			
	Univariate[3] HR (CI 95%)	p	Multivariate[4] HR (CI 95%)	p	Univariate[3] HR (CI 95%)	p	Multivariate[4] HR (CI 95%)	p	Univariate[3] HR (CI 95%)	p	Multivariate[d] HR (CI 95%)	p
Use of ACE inhibitors	0.91 (0.88–0.95)	<0.001	0.85 (0.81–0.89)	<0.001	1.18 (1.08–1.29)	<0.001	0.87 (0.80–0.95)	0.003	1.21 (1.10–1.33)	<0.001	0.88 (0.80–0.97)	0.007
Use of ARB	0.92 (0.88–0.96)	<0.001	0.88 (0.84–0.93)	<0.001	1.27 (1.15–1.40)	<0.001		<0.001	1.29 (1.16–1.43)	<0.001		<0.001
Use immunosuppressants	1.08 (0.98–1.19)	0.134		0.134	1.61 (1.33–1.95)	<0.001	1.49 (1.22–1.82)	<0.001	1.50 (1.22–1.85)	<0.001	1.54 (1.24–1.91)	<0.001

eGFR estimated glomerular filtration rate (ratios are calculated for every 10 ml increase of creatinine clearance), *HR* hazard ratio, *CI 95%* confidence interval 95%, *COPD* chronic obstructive pulmonary disease, *PPI* proton pump inhibitors, *DPP4* dipeptidyl peptidase-4, *ACE* angiotensin-converting enzyme, *ARB* angiotensin-II receptor blockers

[1] Positive PCR or clinical diagnosis of SARS-CoV2 infection

[2] Composite outcome of need for non-invasive mechanical ventilation, orotracheal intubation, mechanical ventilation, intensive care unit admission or death

[3] Unadjusted Cox regression analysis

[4] Cox regression analysis controlling for all covariates

[5] Ratios are calculated for every 10 years of age

infection, nor with COVID-19 severity or mortality (Table 3).

Since renal proximal tubular reabsortion of calcifediol may be impaired in CKD, and low serum calcifediol levels are a frequent finding in this disease [33], we also analyzed the association between calcifediol supplementation and COVID-19 outcomes in the subgroup of patients with advanced CKD (stages 4 and 5). In these patients, calcifediol supplementation was associated with a significant reduction in SARS-CoV2 infection (311/4380 [7.1%] vs 568/5533 [10.3%] in untreated controls; HR 0.77 [0.67–0.88]; $p < 0.001$ in multivariate Cox proportional hazards regression analysis) and a reduced risk of severe COVID-19 (144/4380; [3.3%] vs 260/5533 [4.7%] in untreated controls; HR 0.78 [0.63–0.96]; $p < 0.001$). A reduction in COVID-19 mortality was also observed in patients with advanced CKD supplemented with calcifediol, but without reaching statistical significance (138/4380; [3.2%] vs 245/5533; [4.4%]; HR 0.83 [0.67–1.02]; $p = 0.077$). These associations were not observed in patients with better renal function (Table 5).

Serum 25-hydroxyvitamin D levels and COVID-19 risk.

Serum 25OHD levels were determined in 85,158 patients in the whole cohort (71,972 patients on cholecalciferol or calcifediol supplementation and 13,186 patients of the untreated control groups) between 1 November 2019 and 28 February 2020.

Mean serum 25OHD levels were significantly lower in patients that developed SARS-CoV2 infection (22.7 [SD 14.1] ng/ml, $n = 3091$) than in non-infected patients (24.0 [14.1] ng/ml, $p < 0.001$). Mean serum 25OHD levels were also significantly lower in patients that developed severe COVID-19 (22.0 [SD 15.7] ng/ml [$n = 538$] versus 24.0 [14.3] ng/ml, $p = 0.004$) and in patients that died due to COVID (21.9 [15.7] ng/ml [$n = 475$] versus 24.0 [14.3] ng/ml, $p = 0.004$).

In the multivariate analysis, lower serum 25OHD levels were also associated with increased risk of SARS-CoV2 infection (HR 0.97 [CI 95% 0.94–0.99]; $p = 0.017$), but were not significantly associated with severe COVID-19 or with COVID-19 mortality.

COVID-19 outcomes in cholecalciferol-supplemented, vitamin D-sufficient patients, versus unsupplemented, vitamin D-deficient patients.

The rate of SARS-CoV2 infection was significantly lower in vitamin D-sufficient patients supplemented with cholecalciferol (309/9474 [3.3%]) than in patients vitamin D deficient not receiving vitamin D supplements (430/7616 [5.6%];

Table 3 Association between daily cholecalciferol ($n = 165{,}588$) or calcifediol dose ($n = 132{,}590$) and COVID-19 outcomes

Outcome	Cholecalciferol dose, daily mean (SD) µg	Univariate analysis[3]		Multivariate analysis[4]		Calcifediol dose, daily mean (SD) µg	Univariate analysis[3]		Multivariate analysis[4]	
		HR (CI 95%)[5]	p	HR (CI 95%)[5]	p		HR (CI 95%)[5]	p	HR (CI 95%)[5]	p
SARS-CoV2 infection[1], n (%)										
Yes	15.4 (11.9)	1.00 (0.98–1.03)	0.729	0.97 (0.95–0.99)	0.005	19.8 (10.0)	1.06 (1.03–1.09)	< 0.001	–	NS
No	15.3 (12.3)					19.3 (8.5)				
Severe COVID-19[2], n (%)										
Yes	15.1 (12.3)	0.99 (0.94–1.04)	0.986	0.92 (0.87–0.97)	0.002	20.1 (9.1)	1.06 (1.02–1.09)	0.002	–	NS
No	15.3 (12.3)					19.3 (8.6)				
COVID-19 mortality, n (%)										
Yes	15.0 (11.8)	0.98 (0.93–1.03)	0.442	0.90 (0.85–0.96)	0.001	20.2 (9.4)	1.06 (1.02–1.11)	0.002	–	NS
No	15.4 (12.3)					19.3 (8.6)				

[1]Positive PCR or clinical diagnosis of SARS-CoV2 infection

[2]Composite outcome of need for non-invasive mechanical ventilation, orotracheal intubation, mechanical ventilation, intensive care unit admission or death

[3]Unadjusted Cox regression analysis

[4]Cox regression analysis controlling for all covariates

[5]Ratios are calculated for every 10 µg increase in the mean daily cholecalciferol or calcifediol dose

Table 4 SARS-CoV2 infection, severe infection and death in patients treated with calcifediol (n=134,703) and their matched controls (269,406)

Outcome	Calcifediol treatment (n=134,703)	Matched controls (n=269,406)	Univariate analysis[3]		Multivariate analysis[4]	
			HR (CI 95%)	p	HR (CI 95%)	p
SARS-CoV2 infection[1], n (%)	5662 (4.2)	11,401 (4.2)	0.99 (0.96–1.03)	0.646	–	–
Severe COVID-19[2], n (%)	1037 (0.8)	2073 (0.8)	1.00 (0.93–1.08)	0.995	–	–
COVID-19 mortality, n (%)	934 (0.7)	1859 (0.7)	1.01 (0.93–1.09)	0.908	–	–

[1]Positive PCR or clinical diagnosis of SARS-CoV2 infection

[2]Composite outcome of need for non-invasive mechanical ventilation, orotracheal intubation, mechanical ventilation, intensive care unit admission or death

[3]Unadjusted Cox regression analysis

[4]Cox regression analysis controlling for all covariates

Table 5 SARS-CoV2 infection, severe infection and death in patients treated with calcifediol and controls, according to CKD stages

Patients on stages 1–3 CKD	Calcifediol (n=130,323)	Controls (n=263,873)	Univariate analysis[3]		Multivariate analysis[4]	
			HR (CI 95%)	p	HR (CI 95%)	p
SARS-CoV2 infection[1], n (%)	5351 (4.1)	10,833 (4.1)	1.00 (0.97–1.03)	0.96	–	NS
Severe COVID-19[2], n (%)	893 (0.7)	1813 (0.7)	1.00 (0.92–1.08)	0.94	–	NS
COVID-19 mortality, n (%)	796 (0.6)	1614 (0.6)	1.00 (0.92–1.09)	0.97	–	NS
Patients on stages 4–5 CKD	Calcifediol (n=4380)	Controls (n=5533)	HR (CI 95%)	p	HR (CI 95%)	p
SARS-CoV2 infection[1], n (%)	311 (7.1)	568 (10.3)	0.68 (0.59–0.78)	<0.001	0.77 (0.67–0.88)	<0.001
Severe COVID-19[2], n (%)	144 (3.3)	260 (4.7)	0.69 (0.57–0.85)	<0.001	0.78 (0.63–0.96)	0.018
COVID-19 mortality, n (%)	138 (3.2)	245 (4.4)	0.71 (0.57–0.87)	0.001	0.83 (0.67–1.02)	0.077

CKD chronic kidney disease

[1]Positive PCR or clinical diagnosis of SARS-CoV2 infection

[2]Composite outcome of need for non-invasive mechanical ventilation, orotracheal intubation, mechanical ventilation, intensive care unit admission or death

[3]Unadjusted Cox regression analysis

[4]Cox regression analysis controlling for all covariates

HR 0.66 [CI 95% 0.57–0.77], $p < 0.001$ in multivariate Cox proportional hazards regression analysis). Similarly, vitamin D-sufficient patients on cholecalciferol supplementation had lower risk of severe COVID-19 (65/9474 [0.7%] vs 99/7616 [1.3%]; HR 0.72 [0.52–1.00]; $p = 0.050$), and lower COVID-19 mortality (56/9474 [0.6%] vs 96/7616 [1.3%]; HR 0.66 [CI 95% 0.46–0.93], $p = 0.018$) compared to vitamin D-deficient unsupplemented patients (Table 6).

COVID-19 outcomes in calcifediol-supplemented, vitamin D-sufficient patients, versus unsupplemented, vitamin D-deficient patients.

The rate of SARS-CoV2 infection was significantly lower in vitamin D-sufficient patients supplemented with calcifediol (535/16276 [3.3%]) than in patients vitamin D deficient not receiving vitamin D supplements (430/7616 [5.6%]; HR 0.69

[CI 95% 0.61–0.79], $p < 0.001$ in multivariate Cox proportional hazards regression analysis). Similarly, vitamin D-sufficient patients on calcifediol supplementation had lower risk of severe COVID-19 (n = 100/16276 [0.6%] vs n = 99/7616 [1.3%]; HR 0.61 [0.46–0.81]; $p = 0.001$), and lower COVID-19 mortality (n = 88/16276 [0.5%] vs n = 96/7616 [1.3%]; HR 0.56 [0.42–0.76]; $p < 0.001$) compared to vitamin D-deficient unsupplemented patients (Table 7).

Discussion

In this large population-based cohort, we have compared COVID-19 outcomes in patients supplemented with cholecalciferol or calcifediol versus untreated matched controls, finding only a mild reduction in the risk of SARS-CoV2 infection (diagnosed clinically or by PCR) in patients supplemented with cholecalciferol, and a small reduction in

Table 6 COVID-19 outcomes in 25OHD-sufficient, cholecalciferol-supplemented patients compared to unsupplemented, 25OHD-deficient patients

	Cholecalciferol treated and serum 25OHD ≥ 30 ng/ml (n = 9474)	Untreated controls with serum 25OHD < 20 ng/ml (n = 7616)	Univariate analysis[3]		Multivariate analysis[4]	
			HR (CI 95%)	p	HR (CI 95%)	p
SARS-CoV2 infection[1], n (%)	309 (3.3%)	430 (5.6%)	0.57 (0.50–0.66)	< 0.001	0.66 (0.57–0.77)	< 0.001
Severe COVID-19[2], n (%)	65 (0.7%)	99 (1.3%)	0.53 (0.39–0.72)	< 0.001	0.72 (0.52–1.00)	0.050
COVID-19 mortality, n (%)	56 (0.6%)	96 (1.3%)	0.47 (0.34–0.65)	< 0.001	0.66 (0.46–0.93)	0.018

25OHD 25-hydroxyvitamin D

[1]Positive PCR or clinical diagnosis of SARS-CoV2 infection

[2]Composite outcome of need for non-invasive mechanical ventilation, orotracheal intubation, mechanical ventilation, intensive care unit admission or death

[3]Unadjusted Cox regression analysis

[4]Cox regression analysis controlling for all covariates

Table 7 COVID-19 outcomes in 25OHD-suficient, calcifediol-supplemented patients compared to unsupplemented, 25OHD-suficient patients

	Calcifediol treated and serum 25OHD ≥ 30 ng/ml (n = 16,276)	Untreated controls with serum 25OHD < 20 ng/ml (n = 7616)	Univariate analysis[3]		Multivariate analysis[4]	
			HR (CI 95%)	p	HR (CI 95%)	p
SARS-CoV2 infection[1], n (%)	535 (3.3%)	430 (5.6%)	0.58 (0.51–0.66)	< 0.001	0.69 (0.61–0.79)	< 0.001
Severe COVID-19[2], n (%)	100 (0.6%)	99 (1.3%)	0.47 (0.36–0.62)	< 0.001	0.61 (0.46–0.81)	0.001
COVID-19 mortality, n (%)	88 (0.5%)	96 (1.3%)	0.43 (0.32–0.57)	< 0.001	0.56 (0.42–0.76)	< 0.001

25OHD 25-hydroxyvitamin D

[1]Positive PCR or clinical diagnosis of SARS-CoV2 infection

[2]Composite outcome of need for non-invasive mechanical ventilation, orotracheal intubation, mechanical ventilation, intensive care unit admission or death

[3]Unadjusted Cox regression analysis

[4]Cox regression analysis controlling for all covariates

the rates of infection and COVID-19 mortality associated with the use of higher cholecalciferol doses. Since vitamin D supplementation may be prescribed as a result of a low vitamin D status, we also compared COVID-19 outcomes between supplemented, vitamin D-sufficient patients with unsupplemented, vitamin D-deficient patients showing a significant reduction in the risk of infection, hospitalization and COVID-19 mortality in patients with a normal vitamin D status supplemented either with cholecalciferol or calcifediol. Overall, our results suggest that reaching a sufficient vitamin D status in patients supplemented with these vitamin D metabolites is associated with a reduced risk of SARS-CoV2 infection and lower COVID-19 mortality.

There are several pathophysiological mechanisms that could explain the benefits of vitamin D against COVID-19. Calcitriol, the hormonal form of vitamin D, can protect against infections by increasing the production of LL-37, β-defensin2 and nitric oxide in respiratory epithelia [34] and it has been shown to reduce the incidence of adult respiratory distress syndrome in experimental models of lipopolysaccharide-induced acute lung injury [35–37].

From the methodological point of view, our study differs from previously published studies, where the effects of vitamin D supplementation were mostly analyzed in hospitalized COVID-19 patients [20–25]. To the best of our knowledge, this is the largest study to analyze the association of cholecalciferol or calcifediol supplementation with COVID-19 outcomes at the population level. This has allowed to detect small differences in outcomes after adjusting for multiple covariates. Our study also differs from that of Loucera et al., a study that compares COVID-19 inhospital mortality in patients under vitamin D supplementation with unsupplemented, propensity score-matched, hospitalized COVID-19 patients, since it only analyzed hospitalized patients and not all the population at risk [38].

Our study has some similarities with that of Meltzer et al. [11], who also combined the results of serum 25OHD levels and the vitamin D supplied to categorize 489 patients from an urban academic center as likely vitamin D sufficient or

likely deficient; however, in that study, the authors only analyzed the risk of SARS-CoV2 infection, but not the risk of severe COVID-19 or COVID-19 mortality.

We also found a lower risk of SARS-CoV2 infection and reduced COVID-19 mortality in patients in stages 4–5 CKD supplemented with calcifediol. These results are similar, but of lower magnitude, than those observed in patients treated with calcitriol [26]. The better results obtained with calcitriol may be a consequence of its greater potency, being the active metabolite of vitamin D, not requiring any hydroxylation process in cytochromes and having a high affinity for the vitamin D receptor [39]. However, since renal proximal tubular cells express both ACE2 and mitochondrial 1-hydroxylase [40, 41], and they are commonly infected and damaged in severe COVID-19 [42, 43], it is also possible that during SARS-CoV2 infection, an acute decrease in renal calcitriol synthesis takes place that cannot be restored through cholecalciferol or calcifediol supplementation.

We think our study has some strengths, including the assessment of COVID-19 outcomes in a large population under supplementation with cholecalciferol or calcifediol and the use of a matched cohort of controls. This study also has some limitations. First, there are the inherent limitations of an observational cohort. Although we were comprehensive in analyzing many covariables, it is possible that there are still important variables not considered in the matching process that may disbalance the treated and control groups. Second, our data were obtained from the registries of the health administration of the government of Catalonia, which are fed by the diagnoses issued by family physicians, hospital discharge reports, laboratory data of public hospitals, or medicines supplied by pharmacies, with the inherent limitations of administrative data. Finally, we decided to focus our analysis on the first wave of the pandemic, with higher number of severe cases and mortality. However, the diagnosis of SARS-CoV2 in that phase could not be ascertained with PCR in all the cases, and some patients received a clinical diagnosis without a confirmatory microbiological confirmation.

In conclusion, in this large, population-based study, we observed that cholecalciferol or calcifediol supplementation seem to be beneficial against SARS-CoV2 infection, COVID-19 severity and COVID-19 mortality in patients achieving serum 25OHD levels ≥ 30 ng/ml.

Data availability statement All data can be offered to researchers upon request.

Declarations

Conflicts of interest The authors declare no conflict of interest. The Catalan Agency for Health Quality and Evaluation (AQUAS), a governmental office, offered a data analyst for extracting all the data necessary for the development of this project. They had no role in the design of the study, analyses, interpretation of data; in the writing of the manuscript, or in the decision to publish these results.

Institutional review board statement This study was approved by the Institutional Ethics Committee of the Corporació Sanitària Parc Taulí—Universitat Autònoma Barcelona (Project number 2020588).

Informed consent Not applicable.

Patient and public involvement It was not appropriate or possible to involve patients or the public in the design, or conduct, or reporting, or dissemination plans of our research.

Transparency statement The lead author (J. Oristrell) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported. No important aspects of the study have been omitted, and there were no significant discrepancies from the study as it was planned.

Dissemination declaration Dissemination to the patients supplemented with vitamin D compounds is not applicable.

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