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ORIGINAL ARTICLE

Chronic cholecalciferol supplementation and adequate vitamin D status are associated with reduced risk of Long COVID

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Abstract

Introduction Vitamin D deficiency has been associated with adverse acute and long-term COVID-19 outcomes. However, data on its supplementation on post-acute recovery are still limited. This retrospective observational study investigated the role of chronic cholecalciferol supplementation and 25(OH) vitamin D concentrations on Long COVID risk.

Methods We included patients with COVID-19 between March 2020 and May 2021 previously followed at our Endocrine Department. Long COVID was defined according to NICE criteria. Patients chronically supplemented with cholecalciferol were compared with matched controls for age, sex, comorbidities, and COVID-19 severity.

Results A total of 132 patients were evaluated; 50 (38%) received cholecalciferol supplementation and 30 (22.7%) developed Long COVID. Long COVID occurred less frequently among supplemented patients (14% vs. 28%, $p=0.086$), particularly with daily-doses ≥ 750 IU (12.5% vs. 28.6%, $p=0.051$) and ≥ 1000 IU (10.5% vs. 27.6%, $p=0.039$). Baseline 25(OH) D concentrations were significantly lower in those with Long COVID ($p=0.02$), and showed a significant predictive performance for Long COVID (AUROC 67.3%, $p=0.023$). In the matched cohort ($n=86$), Long COVID occurred less frequently among supplemented patients (11.6% vs. 32.6%, $p=0.036$) and similar findings were confirmed in the matched subgroup receiving ≥ 1000 IU/day (8.5% vs. 34.2%, $p=0.018$). Multivariate analyses showed a protective role of cholecalciferol supplementation on Long COVID in both entire and matched cohorts.

Conclusions Chronic cholecalciferol supplementation, particularly at daily doses ≥ 750–1000 IU, was associated with a significantly lower risk of Long COVID. Ensuring adequate vitamin D status may represent a potential useful preventive strategy for post-COVID sequelae.

Keywords Vitamin D · Long COVID · COVID-19 · Cholecalciferol · SARS-CoV-2

Introduction

Vitamin D has been well demonstrated to modulate both innate and adaptive immune responses. Consistent evidence has linked vitamin D deficiency to worse clinical outcomes during the COVID-19 pandemic [1–7] . Beyond its impact on the acute phase of the disease, the extra-skeletal effects of this hormone have also been implicated in post-acute recovery and the development of Long COVID syndrome.

Long COVID is a multisystem condition characterized by neurocognitive, cardiorespiratory, constitutional, and musculoskeletal sequelae [8–10]. A potential role of vitamin D in modulating recovery after acute COVID-19 has been hypothesized based on its known effects on musculoskeletal health and pain modulation [11, 12], neurological function and disorders [13, 14], as well as respiratory recovery following pneumonia [15, 16]. For the first time in the literature, we previously reported that patients with Long COVID were characterized by significantly lower 25(OH) vitamin D concentrations, assessed six months after hospital discharge, compared with those without Long COVID, particularly among individuals experiencing neurocognitive symptoms [17]. Our findings were subsequently confirmed by several studies conducted worldwide, which consistently demonstrated lower 25(OH) vitamin D concentrations in

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patients with Long COVID or persistent post-acute symptoms compared with control subjects [18–22], especially among those presenting neurological manifestations [19, 20]. To date, only a few seminal studies have investigated the potential role of vitamin D supplementation in reducing the occurrence of Long COVID, generally reporting promising, although sometimes null, effects [23–27]. These inconsistent results can, at least in part, be attributed to methodological limitations and heterogeneity across studies, including the administration of vitamin D supplementation regardless of baseline vitamin D status, the short duration of supplementation (mostly limited to a few months), the lack of comprehensive multisystemic assessments to accurately characterize Long COVID, and the variable timing of follow-up evaluations for post-acute symptoms. Indeed, the benefits of vitamin D may be attenuated or not apparent when supplementation is administered in vitamin D–replete populations or over short time periods [2, 28].

The aim of our study was to investigate the impact of chronic vitamin D supplementation, administered according to routine clinical practice and in line with international and national guidelines [2, 28–31], on the occurrence of Long COVID in patients who had previously experienced acute COVID-19.

Materials and methods

Study design

This was an observational cross-sectional retrospective study performed in a tertiary health care centre, the IRCCS Ospedale San Raffaele, Milan, Italy. The study-protocol complies with the Declaration of Helsinki and was approved by the hospital ethics committee (protocol no. CET 178–2023 ABIO/NC/06 ClinicalTrials.gov ID NCT06452082). Signed informed consent was obtained from all patients participating in this study. We included only adult (age ≥ 18 years) patients with a confirmed diagnosis of COVID-19 from March 2020 to May 2021 and who were previously admitted in years 2019 and 2020 in our Hospital Endocrine Outpatient Department for general endocrine consultations.

A comprehensive assessment of the acute COVID-19 outcomes and the post-acute recovery was specifically collected from all patients though telephone interviews conducted by trained and dedicated personnels. Interviewers had access to patients' clinical information recorded during endocrine visits to ensure consistency and completeness of data collection. On the basis of the National Institute for Health and Care Excellence (NICE) guidelines [8, 32], Long COVID was defined by the concomitant presence of at least 2 or more symptoms and signs (complete list reported below under "Data Collection") at six months after acute disease recovery that were not explained by an alternative medical diagnosis and could only be attributed to the previous COVID-19. As defined by NICE guidelines, none of the symptoms and signs characterizing Long COVID were present before the acute disease.

Only patients fulfilling the following inclusion and exclusion criteria were enrolled: (1) we excluded patients who were admitted to the intensive care unit (ICU) for COVID-19 management in order to minimize the possible bias due to the reported influence of worse acute disease severity in increasing the risk of post-COVID symptom persistence [8, 32]; (2) only patients with available medical data recorded upon hospital endocrine visits and detailed information about the COVID-19 acute presentation and the recovery in the first six months after acute disease were enrolled; (3) patients with the following comorbidities and concomitant active therapies influencing vitamin D metabolism including chronic kidney disease (CKD), active neoplasia, chronic glucocorticoid and antiepileptic treatments were excluded; (4) patients immunized with at least one anti-SARS-CoV-2 vaccination dose before the occurrence of acute COVID-19 or in the first six months after acute disease were not enrolled.

Patients treated with vitamin D supplementation were also subsequently matched on a 1:1 ratio for age, sex, concomitant comorbidities, and acute COVID-19 severity with patients not supplemented in order to assess the impact of vitamin D supplementation on Long COVID occurrence. In addition, specific analyses were performed to investigate the impact of cholecalciferol supplementation with a median daily dose ≥ 1000 International Units (IU), which are the dosages recommended by most guidelines to effectively prevent vitamin D deficiency and also to be potentially associated with extra-skeletal effects in adult subjects [2, 28].

The study enrolment flow chart is summarized in detail in Fig. 1.

Data collection

Data recorded upon endocrine visits were collected directly by patient interview or from medical chart review. Data assessing the acute COVID-19 outcomes and the post-acute recovery were collected from patients interviewed by telephone. All the data were entered on a dedicated electronic case record form specifically developed for the study. Before the analysis, data were cross-checked with medical charts and verified by data managers and clinicians for accuracy. The following variables were collected: age, gender, body mass index (BMI) calculated as the ratio of weight in kilograms divided by height in meters squared and obesity was defined as BMI > 30 kg/m^2. We collected comorbidities

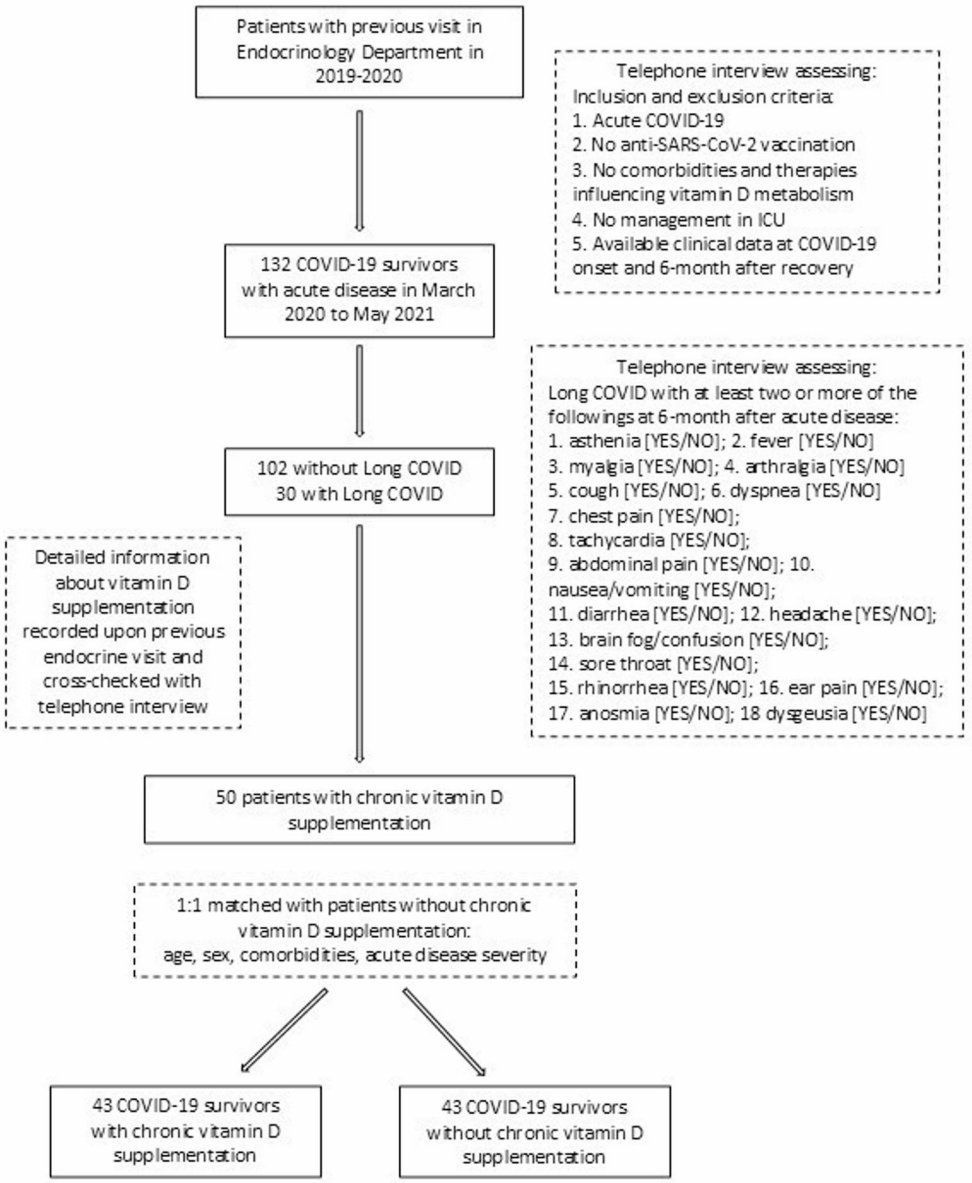

Fig. 1 Study enrolment flow chart

including history of arterial hypertension, diabetes mellitus, cardiovascular disease, CKD, history of active neoplasia, and COVID-19 outcomes occurred during the acute disease including hospitalization and admission to ICU. A comprehensive assessment of constitutional, neurocognitive, cardiorespiratory, gastrointestinal, ear nose and throat, and sensory health systems was performed collecting symptoms and signs present or not at six months after disease recovery and used in the present study for Long COVID diagnosis: constitutional (asthenia, fever, myalgia, arthralgia), cardiorespiratory (cough, dyspnea, chest pain, tachycardia), gastrointestinal (abdominal pain, nausea/vomiting, diarrhea), neurocognitive (headache, brain fog/confusion), ear nose and throat (sore throat, rhinorrhea, ear pain), and sensory (anosmia and dysgeusia).

Detailed information about vitamin D supplementation were specifically recorded upon the endocrine visits and were cross-checked with patients during telephone interviews. To homogenise the investigation of the vitamin D supplementation role, for this study, we considered only patients with or without non-active vitamin D formulations, in particular vitamin D3 cholecalciferol. Thus, the vitamin D supplemented patients' group only included those who were treated, for at least 24 months before the hospital endocrine visit, with cholecalciferol as for clinical practice and recommended guidelines [2, 28–31]. Data about type of supplementation, dosages and starting time were routinely and systematically collected. Serum 25(OH) vitamin D concentrations were recorded upon hospital endocrine visits or, alternatively, if not available, they were retrospectively and

specifically collected for the study purpose from electronic regional health-care information system if performed up to 12 months before the hospital visit. 25(OH) vitamin D concentrations were measured using electrochemiluminescence immunoassays (ng/mL) and vitamin D deficiency was defined with concentrations below 20 ng/mL [2].

Statistical analysis

Descriptive statistics were obtained for all study variables. Categorical variables were summarized as counts and percentages. Kolmogorov–Smirnov normality test was performed ($p<0.05$) and continuous variables were expressed as medians and interquartile range (IQR) (25th–75th percentile). Fisher exact test or $\chi 2$ test and the Wilcoxon signed-rank test or the Kruskal–Wallis test were used to determine the statistical significance of differences in proportions and medians, respectively. Multiple logistic regression analyses were used to estimate the odds ratio (OR) with 95% confidence interval (CI) of the different assessed variables for the Long COVID outcome occurrence risk. The performance of 25(OH) vitamin D concentrations in predicting the Long COVID outcome was estimated using the area under the receiver operating characteristic curve (AUROC) with the 95% CI, and the optimal cut-off value was chosen to maximize the sum of the sensitivity and specificity on the Youden index. All statistical tests were two-sided. P value of <0.05 was considered statistically significant. Statistical analysis was conducted using IBM SPSS Statistics (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.) and GraphPad Prism (GraphPad Software for Windows, version 9.0.0, San Diego, California USA).

Table 1 Comparisons of demographics, clinical characteristics and COVID-19 outcomes between patients treated or not with cholecalciferol in the entire cohort

	Entire cohort	Vitamin D group	Non-Vitamin D group	*p* value*
N. of patients	132	50	82	
Age, years	56 (50–65)	63 (53–70)	55 (47–62)	**<0.001**
Male sex, n. (%)	65 (49%)	13 (26%)	52 (63%)	**<0.001**
BMI, kg/m^2	26.8 (24.2–30.9)	27.1 (24.5–28.9)	26.7 (23.8–31.2)	0.95
Obesity, n. (%)	39 (29%)	14 (28%)	25 (30%)	0.84
Arterial hypertension, n. (%)	48 (36.4%)	20 (40%)	28 (34%)	0.58
Cardiovascular Disease, n. (%)	9 (6.8%)	4 (8%)	5 (6%)	0.73
Diabetes mellitus, n. (%)	12 (9.1%)	6 (12%)	6 (7.3%)	0.37
COPD, n. (%)	10 (7%)	5 (10%)	5 (6.1%)	0.5
25(OH) vitamin D concentrations, ng/mL	21 (15–28)	28 (25–31)	15 (12–19)	**<0.001**
Vitamin D deficiency, n. (%)	44/96 (46%)	4/45 (8.8%)	40/51 (78%)	**<0.001**
Hospitalization for acute COVID-19, n. (%)	92 (69.7%)	30 (60%)	62 (76%)	0.079
Long COVID, n. (%)	30 (22.7%)	7 (14%)	23 (28%)	0.086

n. number, BMI body mass index, COPD chronic obstructive pulmonary disease * p values are referred to analysis of differences between patients supplemented or not with cholecalciferol.

P values reported in bold are those statically significant

Results

Demographics, vitamin D supplementation and outcomes

A total of 132 patients with available information during the 6-months recovery after acute COVID-19 collected by telephone interview and who did fulfil the above-mentioned criteria, were included in this study. Demographic characteristics of patients' cohort are summarized in Table 1. Median [IQR] age was 56 [50–65] years and 65 (49%) were male. The main comorbidities were history of arterial hypertension (36.4%) and diabetes (9.1%). Median BMI value was 26.8 [24.2–30.9] kg/m^2 and 39 patients were obese (29%). At baseline visit, 50 patients (38%) were treated with vitamin D cholecalciferol chronic supplementation that was subsequently maintained for the entire study-period. Forty-eight patients of them were treated with a median daily dose of cholecalciferol $\geq$ 750 IU, and 38 with median daily dose $\geq$ 1000 IU. Median time of supplementation was 38 [28–83] months. A biochemical assessment of serum circulating 25(OH) vitamin D concentrations was available in 96 patients (45 supplemented receiving cholecalciferol and 51 without) and median values were 21 [15–28] ng/mL. The median time interval between hospital endocrine visit and the occurrence of acute SARS-CoV-2 infection was 4.4 months [1.8–7.5], while the median interval between serum 25(OH) vitamin D measurement and infection was 5.5 months [2–8.8]. Among these 96 patients, a status of vitamin D deficiency was observed in 44 of them (45.8%), and its prevalence was significantly higher in patients without supplementation (40/51 78%) as compared to those supplemented (4/45 8.8%) ($p<0.001$). Serum 25(OH) vitamin D concentrations were significantly higher in patients receiving cholecalciferol as compared to those without supplementation (28 [25-31] vs 15 [12-19] ng/mL; $p<0.001$). Nine-two patients (92/132 69.7%) were hospitalized for acute COVID-19 and, on the basis on symptoms and signs present six months after acute disease, Long COVID was diagnosed in 30 patients (30/132 22.7%) (Table 1).

Long COVID and predictive factors

Long COVID was diagnosed in 30 patients (30/132 22.7%). No statistically significant differences were observed between patients with and without Long COVID regarding age, gender, comorbidities and hospitalization need (Table 2). Patients with Long COVID were characterized by a statistically significant higher BMI as compared to those without Long COVID (29 [25-35.7] vs. 26.5 [24-29.9], $p=0.041$), and obesity was more prevalent in Long COVID patients (14/30 46% vs. 25/102 24%, $p=0.024$) (Table 2). A lower occurrence rate of Long COVID was observed in patients supplemented with cholecalciferol (7/50 14% vs. 23/82 28%, $p=0.086$), especially in those receiving a daily dose≥1000 IU (total n.38) (4/38 10.5% vs. 26/94 27.6%, $p=0.039$), and in those with a daily dose≥750 IU (total n.48) (6/48 12.5% vs. 24/84 28.6%, $p=0.051$). Circulating 25(OH) vitamin D concentrations were available in 18 (60%) patients with Long COVID and in 78 (76%) without. Patients presenting with Long COVID were characterized by statistically significant lower 25(OH) vitamin D concentrations as compared to those without, and by a non-significant trend toward higher prevalence of vitamin D deficiency (Table 2).

Patients supplemented with cholecalciferol were significantly older, more frequently female, and characterized by a non-significant trend toward lower needs of hospitalization for acute COVID-19 management (Table 1). Significant higher 25(OH) vitamin D concentrations and lower prevalence of vitamin D deficiency were found in patients receiving cholecalciferol as compared to those without (Table 1). No significant differences regarding age, BMI and comorbidities were observed (Table 1). ROC analyses showed that the global performances of 25(OH) vitamin D concentrations to predict Long COVID was significant with the best youden index of 18.5 ng/mL (63% sensitivity, 67% specificity and AUROC 67.3%, $p=0.023$, CI 0.56–0.78) (Fig. 2a). Multiple regression analyses including demographic characteristics, comorbidities, and cholecalciferol supplementation (analysis model 1) or 25(OH) vitamin D concentrations (analysis model 2) were performed to estimate the predictor risk factors for Long COVID occurrence. In model 1 analysis, cholecalciferol supplementation and obesity resulted as the only variables significantly and independently associated with the Long COVID occurrence (Table 3a). In model 2 analysis, lower 25(OH) vitamin D concentrations and obesity resulted as the only variables significantly and independently associated with the Long COVID occurrence (Table 3b).

Table 2 Demographics, clinical characteristics and cholecalciferol supplementations of patients with and without Long COVID in the entire cohort

	Long COVID (*n*.30)	Non-Long COVID (*n*.102)	*P* value
Age, years	55 (48–63)	58 (50–66)	0.81
Male gender, n. (%)	15 (50%)	50 (49%)	>0.99
BMI, kg/m^2	29 (25-35.7)	26.5 (24-29.9)	**0.041**
Obesity, n. (%)	14 (46%)	25 (24%)	**0.024**
Arterial hypertension, n. (%)	13 (43%)	35 (34%)	0.39
Cardiovascular disease, n. (%)	3 (10%)	6 (5.8%)	0.42
Diabetes mellitus, n. (%)	4 (13%)	8 (7.8%)	0.47
COPD, n. (%)	4 (13%)	6 (5.7%)	0.24
Hospitalization for acute COVID-19, n. (%)	22 (73%)	70 (68%)	0.82
25(OH) vitamin D concentrations, ng/mL	17.5 (14–21)	24 (15–29)	**0.023**
Vitamin D deficiency, n. (%)	12/18 (67%)	32/78 (41%)	0.067
Cholecalciferol supplementation, n. (%)	7 (23%)	43 (42%)	0.086
Cholecalciferol supplementation≥750 IU, n. (%)	6 (20%)	42 (41%)	0.051
Cholecalciferol supplementation≥1000 IU, n. (%)	4 (13%)	34 (33%)	**0.039**

n. number, BMI body mass index, COPD chronic obstructive pulmonary disease, IU international units

P values reported in bold are those statistically significant

Vitamin D supplementation matched analyses and Long COVID

Patients supplemented with cholecalciferol were matched on a 1:1 ratio for age, sex, concomitant comorbidities, and acute COVID-19 severity with those without vitamin D supplementation. A total of 86 patients were included in the analyses, 43 supplemented and 43 without supplementation. Demographic characteristics of patients' cohort are summarized in Table 4. Twenty-six (26/86 30.2%) were male and 28 patients were obese (28/86 32.6%). Forty-one patients were treated with a median daily dose of cholecalciferol≥750 IU, and 35 with median daily dose≥1000 IU. A biochemical assessment of serum circulating 25(OH) vitamin D concentrations was available in 68 patients (40 supplemented with cholecalciferol and 28 without) and median values were 24 [15–29] ng/mL. In this matched cohort as well, prevalence of vitamin D deficient status was significantly higher in patients without supplementation as compared to those supplemented, and serum 25(OH) vitamin D concentrations were significantly higher in patients receiving cholecalciferol as compared to those without supplementation (Table 4). Fifty-six patients (56/86 65.1%) were hospitalized for acute COVID-19 and Long COVID was diagnosed in 19 patients (19/86 22.1%) (Table 4). In this matched cohort, no statistically significant differences were observed between patients with and without Long COVID regarding age, gender, BMI, comorbidities and hospitalization

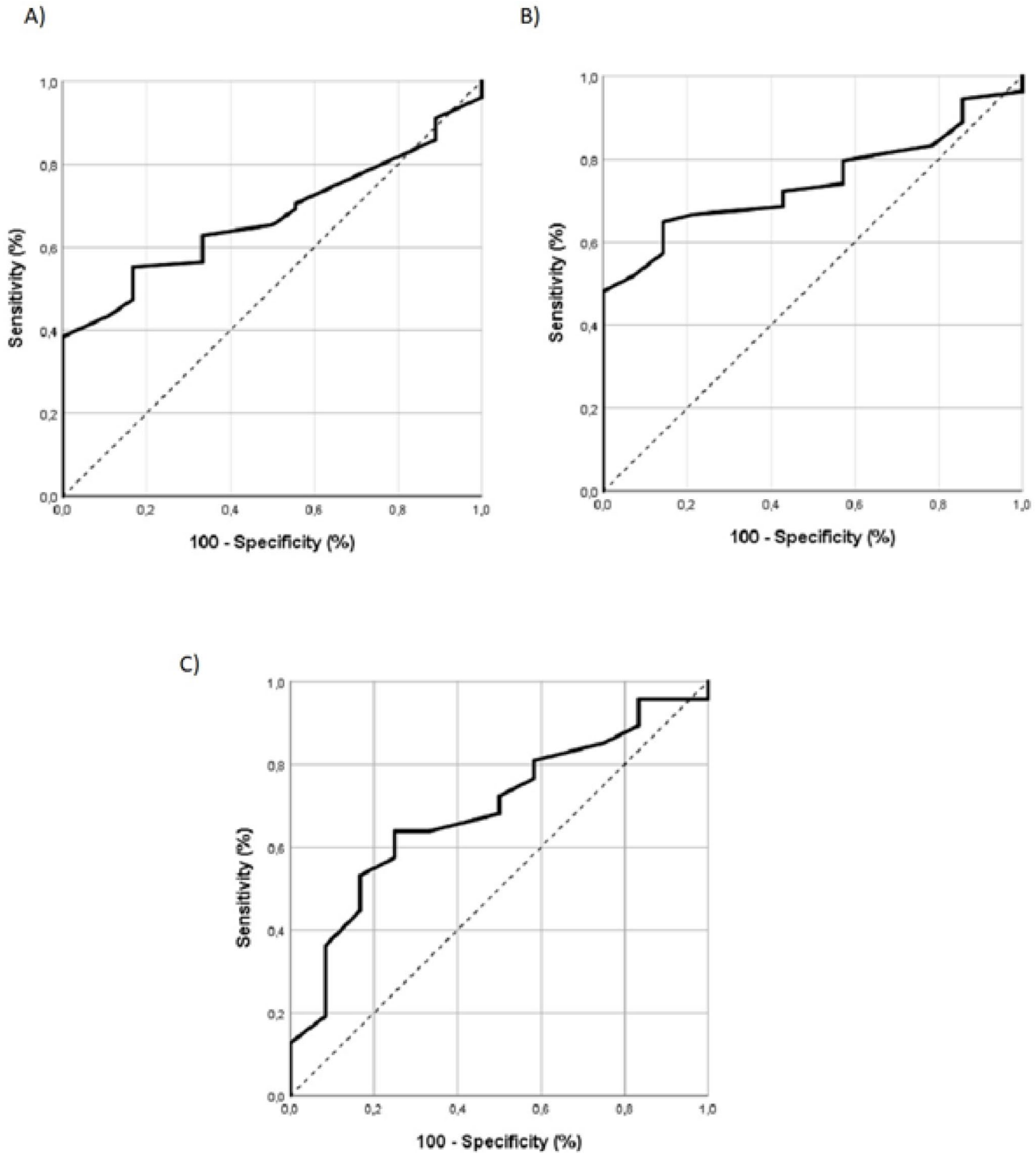

Fig. 2 Correlation and receiver operating characteristic (ROC) analysis and area under the receiver operating characteristic curve (AUROC) of 25(OH) vitamin D concentrations to predict Long COVID. **a**) entire cohort; **b**) matched cohort for cholecalciferol supplementation; **c**) matched cohort for cholecalciferol supplementation ≥ 1000 IU/daily

needs (Table 5). A significant lower occurrence rate of Long COVID was observed in patients supplemented with cholecalciferol (5/43 11.6% vs. 14/43 32.6%, $p=0.036$), especially in those receiving a daily dose ≥ 1000 IU (total n.35) (3/35 8.6% vs. 16/51 31.4%, $p=0.016$), and in those with a daily dose ≥ 750 IU (total n.41) (4/41 9.7% vs. 15/45 33%, $p=0.01$) (Tables 4 and 5). Circulating 25(OH) vitamin D concentrations were available in 14 (14/19 73%) patients

Table 3 Multiple logistic regression analysis of possible risk factors to predict Long COVID presence in the entire cohort

a) Model 1 analysis			
	OR	95% CI	*P* value
Male gender	0.61	0.24–1.56	0.3
Obesity	2.9	1.15–7.33	**0.024**
Age	0.99	0.95–1.04	0.94
Arterial hypertension	1.55	0.57–4.2	0.39
Cardiovascular disease	1.6	0.32–7.8	0.56
Diabetes mellitus	1.14	0.23–5.6	0.87
COPD	2.25	0.46–10.1	0.32
Hospitalization for acute COVID-19	0.82	0.27–2.44	0.71
Cholecalciferol supplementation	0.31	0.1–0.94	**0.038**
b) Model 2 analysis			
	OR	**95% CI**	**P value**
Male gender	0.28	0.074-1.1	0.07
Obesity	3.5	1.05–8.5	**0.039**
Age	1.02	0.96–1.09	0.38
Arterial hypertension	2.4	0.66–8.4	0.18
Cardiovascular disease	0.84	0.04–7.5	0.91
Diabetes mellitus	0.32	0.03–4.5	0.39
COPD	2.9	0.22–8.1	0.41
Hospitalization for acute COVID-19	1.31	0.26–3.54	0.74
25(OH) vitamin D concentrations	0.88	0.8–0.97	**0.009**

OR, odds ratio; CI, confidence interval; COPD, chronic obstructive pulmonary disease

P values reported in bold are those statistically significant

with Long COVID and in 54 (54/67 81%) without. Patients presenting with Long COVID were characterized by statistically significant lower 25(OH) vitamin D concentrations as compared to those without, and by a non-significant trend toward higher prevalence of vitamin D deficiency (Table 5).

As resulted with the match 1:1, no statistically significant differences were observed between patients with and without cholecalciferol supplementation regarding age, gender, BMI, comorbidities and hospitalization needs (Table 4). ROC analyses showed that the global performances of 25(OH) vitamin D concentrations to predict Long COVID was significant with the best youden index of 21.5 ng/mL (67% sensitivity, 79% specificity and AUROC 74.3%, $p=0.005$, CI 0.63–0.86) (Fig. 2b). Multiple regression analyses including demographic characteristics, comorbidities, and cholecalciferol supplementation (analysis model 1) or 25(OH) vitamin D concentrations (analysis model 2) were performed to estimate the predictor risk factors for Long COVID occurrence in this matched cohort. In model 1 analysis, cholecalciferol supplementation resulted as the only variable significantly and independently associated with the Long COVID (Table 6a). In model 2 analysis, lower 25(OH) vitamin D concentrations resulted as the only variable significantly and independently associated with the Long COVID occurrence (Table 6b).

Table 4 Comparisons of demographics, clinical characteristics and COVID-19 outcomes between patients treated or not with cholecalciferol in the matched cohort for vitamin D supplementation

	Matched cohort	Vitamin D group	Non-Vitamin D group	*p* value*
N. of patients	86	43	43	
Age, years	56 (50–67)	58 (53–68)	56 (46–64)	0.09
Male sex, n. (%)	26 (30.2%)	13 (30.2%)	13 (30.2%)	>0.99
BMI, kg/m^2	26.3 (23.9–32)	27 (23.8–31.8)	26.3 (24–32)	0.61
Obesity, n. (%)	28 (32.6%)	14 (32.6%)	14 (32.6%)	>0.99
Arterial hypertension, n. (%)	32 (37.2%)	16 (37.2%)	16 (37.2%)	>0.99
Cardiovascular Disease, n. (%)	8 (9.3%)	4 (9.3%)	4 (9.3%)	>0.99
Diabetes mellitus, n. (%)	9 (10.5%)	4 (9.3%)	5 (11.6%)	>0.99
COPD, n. (%)	6 (7.1%)	3 (7.1%)	3 (7.1%)	>0.99
25(OH) vitamin D concentrations, ng/mL	24 (15–29)	28 (25–31)	15 (12-19.7)	**<0.001**
Vitamin D deficiency, n. (%)	24/68 (35.3%)	3/40 (7.5%)	21/28 (75%)	**<0.001**
Hospitalization for acute COVID-19, n. (%)	56 (65.1%)	25 (58%)	31 (73%)	0.25
Long COVID, n. (%)	19 (22.1%)	5 (11.6%)	14 (32.6%)	**0.036**

n. number, BMI body mass index, COPD chronic obstructive pulmonary disease * p values are referred to analysis of differences between patients supplemented or not with cholecalciferol

P values reported in bold are those statically significant

Vitamin D supplementation with cholecalciferol at daily doses ≥ 1000 IU matched analyses and Long COVID

Patients supplemented with cholecalciferol with median daily dose ≥ 1000 IU were matched on a 1:1 ratio for age, sex, concomitant comorbidities, and acute COVID-19 severity with those without supplementation. A total of 70 patients were included in the analyses and characteristics of patients' cohort are summarized in Table 7. Long COVID was diagnosed in 15 patients (15/70 21.4%). A biochemical assessment of serum circulating 25(OH) vitamin D concentrations was available in 59 patients (35 supplemented with cholecalciferol and 24 without) and median values were 25 [16–29] ng/mL. In this matched cohort as well, prevalence of vitamin D deficient status was significantly higher in patients without supplementation as compared to those supplemented, and serum 25(OH) vitamin D concentrations were significantly higher in patients receiving cholecalciferol as compared to those without supplementation (Table 7). In this matched cohort, no statistically significant

Table 5 Demographics, clinical characteristics and cholecalciferol supplementations of patients with and without Long COVID in the matched cohort for vitamin D supplementation

	Long COVID (*n*.19)	Non-Long COVID (*n*.67)	*P* value
Age, years	60 (52–70)	56 (50–67)	0.54
Male gender, n. (%)	6 (31.6%)	20 (29.8%)	>0.99
BMI, kg/m^2	26.8 (25.4–33.7)	26 (23.8–31.9)	0.31
Obesity, n. (%)	8 (42%)	20 (29.8%)	0.4
Arterial hypertension, n. (%)	10 (52%)	35 (33%)	0.17
Cardiovascular disease, n. (%)	3 (16%)	5 (7.5%)	0.68
Diabetes mellitus, n. (%)	3 (16%)	6 (8.9%)	0.41
COPD, n. (%)	3 (16%)	3 (4.3%)	0.12
Hospitalization for acute COVID-19, n. (%)	14 (73%)	42 (63%)	0.43
25(OH) vitamin D concentrations, ng/mL	18 (14.7–21)	26 (17–30)	**0.005**
Vitamin D deficiency, n. (%)	8/14 (57%)	16/54 (30%)	0.066
Cholecalciferol supplementation, n. (%)	5 (26%)	38 (57%)	**0.036**
Cholecalciferol supplementation≥750 IU, n. (%)	4 (21%)	37 (55%)	**0.01**
Cholecalciferol supplementation≥1000 IU, n. (%)	3 (16%)	32 (48%)	**0.016**

n. number, BMI body mass index, COPD chronic obstructive pulmonary disease, IU international units

P values reported in bold are those statistically significant

Table 6 Multiple logistic regression analysis of possible risk factors to predict Long COVID presence in the matched cohort for vitamin D supplementation

a) Model 1 analysis

	OR	95% CI	*P* value
Male gender	0.44	0.1–1.95	0.28
Obesity	1.9	0.58–6.48	0.27
Age	1.02	0.97–1.05	0.38
Arterial hypertension	2.4	0.66–8.7	0.18
Cardiovascular disease	1.5	0.23–9.2	0.68
Diabetes mellitus	1.02	0.15–6.5	0.98
COPD	3.5	0.43–10.5	0.24
Hospitalization for acute COVID-19	0.76	0.18–3.14	0.7
Cholecalciferol supplementation	0.22	0.06–0.76	**0.017**

b) Model 2 analysis

	OR	**95% CI**	**P value**
Male gender	0.22	0.033-1.4	0.11
Obesity	3.8	0.75–9.2	0.1
Age	1.04	0.96–1.12	0.22
Hypertension	3.6	0.68–7.4	0.13
Cardiovascular disease	0.31	0.004-8.5	0.58
Diabetes mellitus	0.31	0.02–5.8	0.43
COPD	7.3	0.19–9.1	0.22
Hospitalization for acute COVID-19	0.71	0.1–4.54	0.72
25(OH) vitamin D concentrations	0.86	0.76–0.96	**0.01**

OR, odds ratio; CI, confidence interval; COPD, chronic obstructive pulmonary disease;

P values reported in bold are those statistically significant

differences were observed between patients with and without Long COVID regarding age, gender, BMI, comorbidities and hospitalization needs (Table 8). A significant lower occurrence rate of Long COVID was observed in patients supplemented with cholecalciferol at daily doses≥1000 IU (3/35 8.5% vs. 12/35 34.2%, $p=0.018$). Circulating 25(OH) vitamin D concentrations were available in 12 (12/15 80%) patients with Long COVID and in 47 (47/55 85%) without. Patients presenting with Long COVID were characterized by statistically significant lower 25(OH) vitamin D concentrations as compared to those without (Table 8).

As resulted with the match 1:1, no statistically significant differences were observed between patients with and without cholecalciferol supplementation regarding age, gender, BMI, comorbidities and hospitalization needs (Table 7). ROC analyses showed that the global performances of 25(OH) vitamin D concentrations to predict Long COVID was significant with the best youden index of 23 ng/mL (64% sensitivity, 75% specificity and AUROC 68.9%, $p=0.045$, CI 0.53–0.84) (Fig. 2c). A multiple regression analysis including demographic characteristics, comorbidities, and cholecalciferol supplementation was performed to estimate the predictor risk factors for Long COVID occurrence in this matched cohort. Cholecalciferol supplementation with a daily dose≥1000 IU resulted as the only variable significantly and independently associated with the Long COVID occurrence (Table 9).

Discussion

To our knowledge, this is the first study to evaluate the impact of chronic cholecalciferol supplementation on post-acute COVID-19 recovery, and we observed a potential protective role of vitamin D supplementation in reducing the occurrence of Long COVID syndrome through the increase in serum 25(OH) vitamin D concentrations.

The role of vitamin D in regulating both the innate and adaptive immune systems, as well as in modulating several extra-skeletal processes, is well established. A large number of genes and immune cells are directly or indirectly regulated by active vitamin D through the activation of vitamin D receptors (VDRs). Within the innate immune system, active vitamin D enhances the function of monocytes and macrophages, stimulating antibacterial mechanisms such as phagocytosis and bactericidal activity [33]. Conversely, in the adaptive immune system, active vitamin D has been shown to downregulate T-helper type 1–mediated

Table 7 Comparisons of demographics, clinical characteristics and COVID-19 outcomes between patients treated or not with cholecalciferol in the matched cohort for vitamin D supplementation at daily doses ≥ 1000 IU

	Matched cohort	Vitamin D group	Non-Vitamin D group	*p* value*
N. of patients	70	35	35	
Age, years	61 (53–68)	65 (53–70)	61 (51–67)	0.36
Male sex, n. (%)	26 (37.1%)	13 (37.1%)	13 (37.1%)	>0.99
BMI, kg/m^2	27 (24-32.9)	27 (23.8–34)	26.8 (25-32.7)	0.74
Obesity, n. (%)	25 (35.7%)	12 (34.3%)	13 (37.1%)	>0.99
Hypertension, n. (%)	29 (41.4%)	13 (37.1%)	16 (45.7%)	0.63
Cardiovascular Disease, n. (%)	8 (11.4%)	4 (11.4%)	4 (11.4%)	>0.99
Diabetes mellitus, n. (%)	9 (12.9%)	4 (11.4%)	5 (14.2%)	>0.99
COPD, n. (%)	6 (8.6%)	3 (8.6%)	3 (8.6%)	>0.99
25(OH) vitamin D concentrations, ng/mL	25 (16–29)	29 (26–33)	15 (12-19.7)	**<0.001**
Vitamin D deficiency, n. (%)	20/59 (33.8%)	2/35 (5.7%)	18/24 (75%)	**<0.001**
Hospitalization for acute COVID-19, n. (%)	52 (74.3%)	23 (65.7%)	29 (82.8%)	0.17
Long COVID, n. (%)	15 (21.4%)	3 (8.5%)	12 (34.2%)	**0.018**

n. number, BMI body mass index, COPD chronic obstructive pulmonary disease, IU international units * p values are referred to analysis of differences between patients supplemented or not with cholecalciferol

P values reported in bold are those statically significant

Table 8 Demographics, clinical characteristics and cholecalciferol supplementations of patients with and without Long COVID in the matched cohort for vitamin D supplementation at daily doses ≥ 1000 IU

	Long COVID (*n*.15)	Non-Long COVID (*n*.55)	*P* value
Age, years	63 (53–77)	61 (53–68)	0.35
Male gender, n. (%)	6 (40%)	20 (36.3%)	>0.99
BMI, kg/m^2	26.8 (25.4–34)	27 (24–32)	0.48
Obesity, n. (%)	6 (40%)	19 (54.3%)	0.76
Arterial hypertension, n. (%)	9 (60%)	35 (36.3%)	0.14
Cardiovascular disease, n. (%)	3 (20%)	5 (9.1%)	0.35
Diabetes mellitus, n. (%)	3 (20%)	6 (10.9%)	0.39
COPD, n. (%)	3 (20%)	3 (5.5%)	0.11
Hospitalization for acute COVID-19, n. (%)	13 (87%)	42 (71%)	0.32
25(OH) vitamin D concentrations, ng/mL	19.5 (14.2–24)	26 (18–30)	**0.045**
Vitamin D deficiency, n. (%)	6/12 (50%)	14/47 (30%)	0.3
Cholecalciferol supplementation, n. (%)	3 (20%)	32 (58%)	**0.018**

n. number, BMI body mass index, COPD chronic obstructive pulmonary disease, IU international units

P values reported in bold are those statistically significant

Table 9 Multiple logistic regression analysis of possible risk factors to predict Long COVID presence in the matched cohort for vitamin D supplementation at daily doses ≥ 1000 IU

	OR	95% CI	*P* value
Male gender	0.56	0.12–2.6	0.46
Obesity	1.36	0.35–5.35	0.66
Age	1.36	0.96–1.11	0.35
Arterial hypertension	2.2	0.53–9.1	0.27
Cardiovascular disease	1.46	0.23–9.5	0.69
Diabetes mellitus	1.18	0.18–7.7	0.86
COPD	3.96	0.46–10.8	0.2
Hospitalization for acute COVID-19	1.55	0.27–9.1	0.65
Cholecalciferol supplementation	0.16	0.035-0.7	**0.016**

OR, odds ratio; CI, confidence interval; COPD, chronic obstructive pulmonary disease, IU international units

P values reported in bold are those statistically significant

inflammatory responses, inhibit the secretion of proinflammatory cytokines, and promote the maturation of regulatory T lymphocytes with anti-inflammatory properties [34]. Consequently, active vitamin D contributes to a shift from a proinflammatory state toward adaptive immune tolerance. Beyond its immunomodulatory effects, vitamin D may also influence other clinical domains typically affected in the context of Long COVID. Emerging evidence suggests that vitamin D plays a crucial role in brain health, with deficiency consistently associated with cognitive decline and an increased risk of neurodegenerative and neurological disorders [35]. Although the underlying mechanisms remain not fully understood and are still under investigation, the widespread expression of VDRs in brain regions critical for neuronal function suggests that vitamin D may exert its neuroprotective effects through both genomic and non-genomic pathways. These include the modulation of neuroinflammation, enhancement of amyloid-β clearance, and reduction of oxidative stress, ultimately promoting synaptic plasticity, neuronal survival, and neurotransmitter synthesis [35–37]. Several recent large meta-analyses strongly support that vitamin D deficiency predisposes individuals to the development of various neurological and neurodegenerative disorders, while vitamin D supplementation has been shown to potentially attenuate disease progression and mitigate cognitive decline [38–41]. In the context of Long COVID, vitamin D deficiency has been consistently associated with

the persistence of neurological symptoms in several studies. In our previous work conducted on a cohort of adult patients evaluated six months after hospital discharge for acute COVID-19, we observed significantly lower 25(OH) vitamin D concentrations in those with persistent neurocognitive symptoms compared with those without (14.6 vs. 20.6 ng/mL) [17]. Similarly, in a study assessing children three months after hospitalization for COVID-19, Long COVID was more frequently observed among those with vitamin D deficiency [19]. Moreover, vitamin D–deficient children more often exhibited neurological symptoms than those without deficiency (80% vs. 41.9%) [19]. Finally, in a study specifically including patients with Long COVID, individuals with vitamin D deficiency presented significantly higher scores for memory impairment, mental fatigue, and depressive symptoms compared with those with normal vitamin D status [20].

Beyond its role in neurocognitive function, vitamin D may also play a relevant role in modulating musculoskeletal health in the context of Long COVID, given the typical detrimental impact of the syndrome on this system. VDRs have been shown to participate in intracellular signaling pathways related to calcium metabolism and in the regulation of myoblast proliferation and differentiation, processes essential for maintaining adequate muscle mass and strength [42, 43]. Clinical evidence supports the beneficial effects of vitamin D supplementation on the preservation of muscle mass and strength, particularly in individuals with vitamin D deficiency [44, 45]. Conversely, several studies investigating Long COVID have reported that patients experiencing physical fatigue, musculoskeletal pain, or reduced physical performance exhibited lower 25(OH) vitamin D concentrations compared with those without musculoskeletal impairments [19–21].

Based on the potential role of vitamin D in modulating post-acute recovery after COVID-19, several seminal studies have reported promising benefits of its supplementation, particularly in the musculoskeletal and neurocognitive domains, in line with the findings discussed above. A pilot double-blind trial demonstrated that treatment with cholecalciferol (2000 IU/day) for six weeks following COVID-19 was associated with reduced serum creatine kinase levels and slight, although non-significant, improvements in physical performance as assessed by the six-minute walk test compared with placebo [23]. In another double-blind, randomized, placebo-controlled trial, patients presenting with post-COVID fatigue or neuropsychiatric symptoms were assigned to receive either 60000 IU of vitamin D weekly or placebo for eight weeks. Significant improvements were observed in the vitamin D group in the Chalder Fatigue Scale, the 21-item Depression, Anxiety, and Stress Scale, and the Addenbrooke's Cognitive Examination III, reflecting reductions in fatigue and anxiety, as well as improvements in cognitive performance [26]. Interestingly, differences in efficacy have also been observed among the various vitamin D supplementation strategies adopted. In a prospective interventional study involving patients diagnosed with post-COVID syndrome, most of whom presented with vitamin D deficiency, oral cholecalciferol was administered for two months using different regimens, either daily dosing or weekly bolus administration [24]. Both bolus and daily supplementation were significantly associated with enhanced antioxidant defense activity and reduced inflammatory parameters; however, only the daily regimen was additionally linked to a significant reduction in anxiety and depression scores. Conversely, some studies have reported null effects of vitamin D supplementation in this context. In an interventional clinical trial, patients received a single oral bolus of 200,000 IU vitamin D_3 or placebo at the time of hospital admission for acute COVID-19 and were re-evaluated by telephone interviews at six months and one year after discharge. No significant improvements in post-COVID symptoms were observed in the vitamin D–treated group compared with placebo [28]. Importantly, no data on 25(OH) vitamin D concentrations after supplementation were reported, and at baseline most participants were not vitamin D deficient, which may have limited the effectiveness of the intervention.

Indeed, as widely recognized for skeletal outcomes [46], the effects of vitamin D supplementation on extra-skeletal conditions also appear to be markedly influenced by several key factors, as demonstrated in multiple meta-analyses and post-hoc investigations of major randomized controlled trials evaluating the extra-skeletal role of vitamin D [2, 29, 47]. Firstly, since the potential benefits of vitamin D supplementation may be limited when administered to individuals who are not vitamin D deficient, the assessment of baseline 25(OH) vitamin D concentrations is essential to appropriately determine dosing regimens initiating supplementation only when deficiency is confirmed, and avoiding unnecessary treatment in those with sufficient status. Secondly, most of the beneficial effects of vitamin D supplementation are achieved when deficiency is properly corrected and maintained. Therefore, it is recommended to re-evaluate 25(OH) vitamin D concentrations after supplementation to assess the individual's response and adjust the dosage if necessary, particularly considering the difficulty some populations have in sustaining adequate vitamin D status over time. Thirdly, given the genomic and non-genomic mechanisms through which vitamin D exerts its biological effects, especially those implicated in extra-skeletal outcomes, it is reasonable to hypothesize that such effects cannot be achieved through short-term supplementation alone. These findings collectively suggest that the effectiveness of vitamin D

supplementation in post-COVID recovery may depend on baseline vitamin D status, supplementation strategy, and duration of treatment.

In light of these considerations, the present study aimed, for the first time, to specifically investigate the role of chronic cholecalciferol supplementation, prescribed for the management of vitamin D deficiency, in influencing the occurrence of Long COVID in a cohort of patients who had previously experienced acute COVID-19. We observed that patients treated with cholecalciferol for at least 24 months exhibited a reduced risk of developing Long COVID compared with a matched control population not receiving vitamin D supplementation. Although matching was performed on age and other major covariates, minor residual differences in age were observed between groups; however, age was consistently included in multivariable models, where it did not emerge as an independent predictor of Long COVID.

Notably, the potential protective effect of vitamin D was most evident when doses equivalent to at least 750–1000 IU/day were used. These dosages correspond to the minimum regimens currently recommended by several international and national guidelines for the effective correction of vitamin D deficiency in adult populations [2, 29–31]. This finding is further supported by the adequate median 25(OH) vitamin D concentrations achieved in our supplemented cohort, reinforcing the potential benefits of vitamin D when administered at appropriate doses and over sufficient duration. In addition, we found that vitamin D deficiency and lower pre-infection 25(OH) vitamin D concentrations were significantly associated with an increased risk of subsequent Long COVID development. Expanding our previous report, which demonstrated an association between the presence of Long COVID and lower 25(OH) vitamin D concentrations measured at 6-month follow-up [17], these novel findings further suggest a potential prospective and predictive role of vitamin D status in influencing the risk of Long COVID.

The role of vitamin D deficiency as merely a biochemical epiphenomenon of COVID-19 or as a potential pre-existing risk factor for adverse disease outcomes has long been debated. In most studies, 25(OH) vitamin D concentrations were assessed after the onset of the acute phase of the disease, raising the possibility that vitamin D deficiency could represent part of the so-called "acute phase reaction," in which systemic inflammation leads to a transient reduction in circulating 25(OH) vitamin D concentrations [48, 49]. However, in a previous work, lower 25(OH) vitamin D concentrations were found to predict worse COVID-19 outcomes even among patients with non-severe disease, thereby minimizing the potential confounding effect of acute systemic inflammation on vitamin D status [7]. Furthermore, in the present study, 25(OH) vitamin D concentrations were measured prior to COVID-19 infection, definitively excluding the influence of the acute phase response on vitamin D status and reinforcing the hypothesis that pre-existing vitamin D deficiency may exert a detrimental impact on health outcomes. Given the relatively mild changes in vitamin D status in the absence of major therapeutic or lifestyle modifications, and considering that chronic supplementation was maintained over time in treated individuals, 25(OH) vitamin D concentrations assessed up to 12 months prior to infection were considered representative of chronic pre-infectious vitamin D status. The 12-month time window also reflected the practical real-word restriction policies in healthcare access during the pandemic period, when non-urgent routine laboratory testing was not always readily available. In addition, although seasonal variability cannot be entirely excluded, the median intervals between hospital visits, 25(OH) vitamin D measurements and SARS-CoV-2 infection were relatively short, and most supplemented patients were on stable long-term regimens, further supporting that the assessed 25(OH) vitamin D concentrations reflected baseline status rather than transient fluctuations. Consistent with previous findings, our study also confirmed that obesity represents a significant risk factor for the development of Long COVID syndrome [50, 51]. Within the context of our work, this observation deserves particular attention given the well-established relationship between vitamin D metabolism and obesity [6, 11, 52]. Indeed, obese individuals are typically characterized by lower circulating 25(OH) vitamin D concentrations and a higher prevalence of vitamin D deficiency compared with normal-weight counterparts. Vitamin D is a fat-soluble steroid and its volumetric dilution into adipose tissue mass when increased supports the relationship between lower vitamin D concentrations and obesity. Moreover, several additional pathophysiological mechanisms, such as reduced time spent outdoors and consequently lower sunlight exposure, decreased dietary intake of vitamin D, and impaired hepatic and adipose tissue hydroxylation, have been proposed to further account for the association between obesity and vitamin D deficiency [11, 52]. Although no significant differences in cholecalciferol use were observed between obese and non-obese participants in our cohort, a potential influence of obesity on modulating the effects of vitamin D supplementation on Long COVID risk cannot be excluded. Importantly, however, the protective effect of chronic cholecalciferol supplementation remained statistically significant even after matching for BMI and obesity prevalence.

Another noteworthy finding of our study is the high prevalence of individuals with vitamin D deficiency who did not receive specific supplementation within our community healthcare setting. This observation is not surprising, as in our country the Italian Medicines Agency (AIFA), the national regulatory authority that defines the therapeutic indications

of drugs eligible for reimbursement by the Italian National Health Service (INHS), has, in recent years, restricted the reimbursement of vitamin D formulations (cholecalciferol and calcifediol) only to adult patients with osteoporosis or to those with severe vitamin D deficiency (< 12 ng/mL) [31]. This policy was introduced given the increase in vitamin D prescription in the general population, which raised concerns about the potential for inappropriate supplementation. However, the potential extra-skeletal effects of vitamin D were not taken into account and, although, in the months following the introduction of these regulatory restrictions, a 20–40% reduction in reimbursable vitamin D prescriptions was indeed reported, an alarming rate of supplementation discontinuation was observed particularly among patients with osteometabolic disorders and high-risk fragile individuals, precisely those who would benefit from it the most [53]. Thus, these findings suggest that restrictive reimbursement policies may have unintentionally limited vitamin D supplementation among individuals who would benefit most, and a more balanced approach that considers not only healthcare costs but also the potential extra-skeletal health consequences of vitamin D deficiency, as highlighted by the current literature, should therefore be recommended to mitigate the potential negative impact of the widespread vitamin D deficiency affecting the general population.

Our study has some limitations. The retrospective design did not allow for an exhaustive evaluation of the direct causal influence of vitamin D supplementation on Long COVID, and the underlying biological mechanisms warrant further investigation. The number of patients included was relatively limited due to the strict inclusion and exclusion criteria adopted. However, this approach allowed detailed analyses with appropriate statistical adjustments for multiple potential confounders. Another limitation is the lack of circulating vitamin D measurements in the entire cohort, which would have been useful to more comprehensively assess the overall effects of vitamin D status on Long COVID risk. Nevertheless, we were able to confirm that the supplementation regimens adopted were effective in correcting vitamin D deficiency, and that vitamin D deficiency resulted as a potential predictive risk factor for Long COVID. An additional limitation derived from the observational and retrospective design of our study is the possible presence of residual confounding related to variables not systematically assessed in our cohort. Individuals receiving chronic vitamin D supplementation may potentially differ from non-supplemented subjects in healthy behavior, dietary habits, physical activity, sun exposure, or socioeconomic status, all of which could potentially influence both vitamin D status and the risk of Long COVID. However, although residual confounding cannot be fully excluded, our cohort was derived from a single tertiary center within the same geographical and temporal setting, and matching was performed for major demographic and clinical variables, and, importantly, the primary exposure of interest in our study was chronic supplementation rather than vitamin D status per se, which may partially reduce the influence of short-term behavioral or environmental variability on our findings. In addition, since the present cohort reflects patients evaluated during the early phase of the pandemic, prior to widespread vaccination and in the context of earlier SARS-CoV-2 variants, the external validity of our results to contemporary clinical settings needs to be further investigated. Indeed, vaccination has been consistently associated with reduced severity of acute COVID-19 and a lower incidence of Long COVID, and newer virus variants may differ in their inflammatory burden and systemic impact. Therefore, these factors could potentially modify the associations observed in our study. Finally, to ensure homogeneity we specifically evaluated the effects of only cholecalciferol, excluding other forms of vitamin D supplementation available in clinical practice. Despite these limitations, this is the first study to investigate the role of chronic vitamin D supplementation in influencing post-acute recovery following COVID-19, providing novel insights into its potential protective role against Long COVID.

In conclusion, we report that chronic vitamin D supplementation may act as a potential protective factor against the development of Long COVID through normalization of vitamin D status. This association remained significant after adjustment for multiple potential confounders and was particularly evident with daily doses of at least 750–1000 IU. Unlike previous studies, we observed this protective effect in a cohort chronically treated with vitamin D, rather than following short-term supplementation. Furthermore, the medium-to-long-term follow-up after acute infection adopted in our study likely allowed a more accurate identification and characterization of patients with post-infectious recovery impairment who may benefit from closer clinical monitoring and preventive or therapeutic interventions, potentially including safe and low-cost strategies such as vitamin D supplementation. Overall, these findings reinforce the clinical relevance of assessing and maintaining adequate vitamin D status as a modifiable pathophysiological factor in the context of Long COVID, particularly given the widespread prevalence of vitamin D deficiency in the general population worldwide. Although the single-center nature of our study does not allow for definitive conclusions regarding causality, future large-scale investigations are warranted to elucidate the underlying mechanisms linking vitamin D status to Long COVID syndrome. Preventive and therapeutic strategies, particularly in populations at higher risk of both hypovitaminosis D and adverse post-COVID outcomes, such as older adults and individuals with obesity

or comorbid conditions, should include the assessment of circulating vitamin D status and, when appropriate, correction through supplementation.

Authors' contributions All authors contributed equally.

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Data availability The data supporting the findings of this study are owned jointly by the sponsor and the investigator and are not publicly available. Data are available from the corresponding author upon reasonable request and subject to approval by the sponsor.

Declarations

Conflict of interest Andrea Giustina has occasionally consulted for Abiogen. Luigi di Filippo received research grant to Institution from Abiogen and his research activities are partly supported by Glucocorticoid induced Osteoporosis Skeletal (GIOSEG). All other authors have no conflict of interest to declare. The Work submitted for publication is original and has not been published in any language or format and has not been submitted elsewhere for print or electronic publication consideration.

Ethical approval The study-protocol complies with the Declaration of Helsinki and was approved by the hospital ethics committee (protocol no. CET 178–2023 ABIO/NC/06 ClinicalTrials.gov ID NCT06452082).

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