Research Paper

Influence of 25-hydroxy-cholecalciferol levels on SARS-CoV-2 infection and COVID-19 severity: A systematic review and meta-analysis

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A R T I C L E   I N F O

A B S T R A C T

Background: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiological agent of coronavirus disease 19 (COVID-19), a respiratory infection that, starting from December 2019, has spread around the world in a few months, becoming a pandemic. The lack of initial knowledge on its management has led to a great effort in developing vaccines and in finding therapeutic weapons capable of improving the clinical outcome of the affected patients. In particular, the possible role of vitamin D status in the management of COVID-19 has been widely analysed, resulting in a great amount of data. This systematic review and meta-analysis aimed to assess whether hypovitaminosis D is a risk factor for developing SARS-CoV-2 infection and whether it affects the worsening of the clinical course of COVID-19.

Methods: Data were extracted through extensive searches in the Pubmed, MEDLINE, Cochrane, Academic One Files, Google Scholar, and Scopus databases from December 2019 to January 2021, using the keywords: "Vitamin D", "25 hydroxy Vitamin D", "25 hydroxycholecalciferol", "cholecalciferol", "COVID 19", "SARS-CoV-2". We included observational cohort, cross-sectional, and case-control studies that evaluated differences in serum levels of 25-hydroxy-cholecalciferol [25(OH)D] in patients who were positive or negative for SARS-CoV-2, in patients with mild or severe forms of COVID-19, and in patients who died or were discharged from the hospital. Finally, studies that evaluated the risk of developing severe illness or death in patients with vitamin D deficiency (VDD), defined as levels of 25(OH)D <20 ng/ml, were also included. We calculated the mean difference (MD) and the 95% confidence intervals (CI) for quantitative variables such as 25(OH)D levels in patients with or without SARS-CoV-2 infection, in those with mild vs. severe COVID-19, or those who have died vs. those who have been discharged. Instead, we calculated odds ratios and 95% CI for qualitative ones, such as the number of patients with severe illness/death in the presence of VDD vs. those with normal serum 25(OH)D levels. A p-value lower than 0.05 was considered statistically significant. The study was registered on PROSPERO (CRD42021241473).

Findings: Out of 662 records, 30 articles met inclusion criteria and, therefore, were included in the meta-analysis. We found that the serum levels of 25(OH)D were significantly lower in patients with SARS-CoV-2 infection than in negative ones [MD -3.99 (-5.34, -2.64); p <0.00001; I^2= 95%]. Furthermore, its levels were significantly lower in patients with severe disease [MD -6.88 (-9.74, -4.03); p <0.00001; I^2=98%] and in those who died of COVID-19 [MD -8.01 (-12.50, -3.51); p = 0.0005; I^2=86%]. Finally, patients with VDD had an increased risk of developing severe disease [OR 4.58 (2.24, 9.35); p <0.0001; I^2=84%] but not a fatal outcome [OR 4.92 (0.83, 29.31); p = 0.08; I^2=94%].

Interpretation: This meta-analysis revealed a large heterogeneity of the studies included due to the different enrolment criteria of patient samples (age, body mass index, ethnicity, comorbidities), the country where they live, all factors influencing serum 25(OH)D levels, and the different criteria used to define the severity of COVID-19. Furthermore, the observational nature of these studies does not allow to establish a cause-effect relationship, even taking into account that 25(OH)D represents a marker of acute inflammation. Treatment with vitamin D might be considered for the primary prevention of SARS-CoV-2 infection and the management of patients with COVID-19. However, further intervention studies are needed to prove this hypothesis.

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Research in context

Evidence before this study

Several studies have recently evaluated the relationship between vitamin D levels and coronavirus disease 19 (COVID 19), indicating the existence of a possible association between vitamin D levels and SARS-CoV2 risk of infection, COVID 19 severity and mortality. To clarify this issue, we performed an extensive research in the Pubmed, MEDLINE, Cochrane, Academic One Files, Google Scholar, and Scopus databases from December 2019 to January 2021.

Added value of this study

To our knowledge, this meta-analysis is the one that included the highest number of studies with patients not treated with vitamin D supplementary therapy.

Implications of all the available evidence

The results of the present meta-analysis would speculate that low vitamin D levels may play a role in the risk of contracting the infection and influencing the severity of the disease. Therefore, supplementation of the nutrient in vitamin D deficiency patients and at risk of severe infection might play a protective role. However, additional evidence is needed to confirm the effects of VDD in the infection and to exclude that the link suggested by the numerous observational studies is not casual.

1. Introduction

The year 2019 witnessed the advent of a new severe acute respiratory syndrome coronavirus (SARS-CoV-2) that causes coronavirus disease 19 (COVID-19). It was originally identified in the Chinese city of Wuhan and, in a few months, it spread to the rest of the world, turning into a global pandemic [1]. To date, there are more than 141 million confirmed cases of COVID-19, including more than 3 million deaths [2]. The virus is transmitted between humans mainly through droplets expelled face-to-face exposure during talking, coughing, or sneezing. However, contact with contaminated surfaces can also be a route of transmission. Following infection, the virus replicates within the host cells after cell membrane penetration mediated by the interaction between the SARS-CoV-2 spike protein and the angiotensin-converting enzyme 2 receptors (ACE2R) [1]. To date, vaccines represent the best therapeutic weapon in the prevention of COVID-19 and the control of the pandemic. However, the rapid development of variants with mutations in the spike protein, the main target of vaccines produced to date, has increased the fear of a possible loss of their effectiveness. Only longitudinal studies conducted in vaccinated people will be able to provide data on the efficacy of the vaccine protection against variants. Thus, the search for therapeutic strategies that can prevent SARS-CoV-2 infection and mitigate the course of COVID-19 is becoming more important, regardless of the infecting viral variant [3].

For this reason, the search for factors predisposing to SARS-CoV-2 infection and capable of influencing COVID-19 severity has been relevant. It quickly became clear that the risk factors for the development of more severe clinical manifestations of the disease were age, male sex, race, and the presence of comorbidities. The latter include hypertension, diabetes mellitus, obesity, kidney failure, and even cigarette smoking [4]. In fact, COVID-19 affects men more than women, due to the higher concentration of ACE2R in the former, which in turn is also related to androgen levels [5]. Furthermore, the disease has been shown to have a more severe course in the elderly than in the young,

possibly due to the increased presence of comorbidities and the decline in immune function associated with advancing age. The latter is related to the development of cytokine storm and hyper-inflammation syndrome, which, in turn, are predictors of disease mortality [6].

In addition to these "classic" risk factors, vitamin D deficiency (VDD) has also attracted considerable interest. Indeed, VDD represents a global health problem, affecting approximately 1 billion people worldwide, within 50% of the population experiencing vitamin D insufficiency (VDI) [7]. According to Endocrine Society guidelines, VDD is diagnosed when serum 25(OH)D levels are below 20 ng/ml and VDI when 25(OH)D ranges from 20 to 30 ng/ml [8].

Important evidence in favor of the role of vitamin D status in COVID-19 is the latitude hypothesis. In fact, vitamin D is produced by the skin from the irradiation of 7-dehydrocholesterol by UVB sunlight. Several studies showed a correlation between latitude and sun exposure and COVID-19. Interestingly, mortality for COVID-19 is higher in mid-latitude countries where VDD has a greater prevalence [9]. Similarly, an American study has shown a negative correlation between SARS-CoV2 infection and the sunlight UV radiation dose [10]. Another study analyzed the data on COVID-19 mortality from 88 countries based on their latitude, showing a statistically significant correlation between the two parameters. Thus, these findings support a possible relationship among 25(OH)D levels, sun exposure, and COVID-19 severity [11]. Moreover, the higher frequency of severe disease in the elderly and dark-skinned individuals may also suggest a correlation with 25(OH)D levels, since vitamin D production is reduced in these individuals [8]. However, great caution and stronger evidence are needed to draw a firm conclusion.

For these reasons, several authors have evaluated the difference in 25(OH)D levels between infected patients and healthy ones, as well as the role of VDD in the risk of developing COVID-19 and its complications, but with contrasting results [12−55]. Therefore, this meta-analysis aims to shed light on the numerous data in the literature on this topic to establish whether hypovitaminosis D represents a real risk factor for SARS-CoV-2 infection and, therefore, whether its integration can be an additional weapon in the fight against COVID-19. In detail, we evaluated the influence of 25(OH)D levels on SARS-CoV-2 infection, assessing whether its levels differ in SARS-CoV-2 infected vs. non-infected patients. We also evaluated whether 25(OH)D affects COVID-19 severity by assessing its levels in patients with severe vs. non-severe COVID-19, in patients who died vs. those discharged from the hospital, and by evaluating the odds ratio (OR) of developing severe COVID-19 in patients with VDD vs. those with normal 25(OH)D levels. The analysis was carried out including studies with the following experimental designs: observational cohort, cross-sectional, and case-control.

2. Methods

2.1. Search strategy and selection criteria

This study was performed by applying the Meta-Analysis and Systematic Reviews of Observational Studies (MOOSE) guidelines [56]. It also complies with the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) [57]. The MOOSE and PRISMA checklist have been included as **Supplementary Tables 1** and **2**. The data were extracted through extensive searches in the Pubmed, MEDLINE, Cochrane, Academic One Files, Google Scholar, and Scopus databases from December 2019 to January 2021.

The search strategy included the combination of the following Medical Subjects Headings (MeSH) terms and keywords: "Vitamin D", "25 hydroxy Vitamin D", "25 hydroxycholecalciferol", "cholecalciferol", "COVID 19″, "SARS-CoV-2″. The search was limited to human studies and no language restrictions were applied. Studies were first evaluated for inclusion by reading their abstracts. When the abstract did not reveal whether the study contained data relevant to our

meta-analysis, the full text was read carefully. The identification of eligible studies was carried out independently by two different researchers (A.C. and R.C.). Any disagreements were resolved by a third author (A.E.C.). Other articles were manually extracted by searching the reference lists of the articles selected by the above keywords. Four authors were contacted to resolve our concerns about the data published in their articles.

We considered for inclusion all the observational, cohort, cross-sectional, and case-control studies that evaluated 1) the differences in 25(OH)D levels in patients who were positive or negative for the SARS-CoV-2, 2) the difference in 25(OH)D levels between patients with severe COVID-19 and patients with asymptomatic or pauci-symptomatic disease, and 3) analyzing the correlation between hypo-vitaminosis D and the risk of severe course of COVID-19 and in-hospital mortality. We included those studies that diagnosed COVID-19 by real-time polymerase chain reaction on a nasopharyngeal swab or with at least one positive test for SARS-CoV-2 infection, or with VDD diagnosed when the levels of 25(OH)D were lower than 20 ng/ml according to the Endocrine Society guidelines [8].

All the eligible studies were selected following the PECOS (Population, Exposure, Comparison/Comparator, Outcomes, Study design) model (**Supplementary Table 3**). We excluded from the analysis case reports, comments, letters to the editor, systematic or narrative reviews, and those studies that did not allow extracting of the outcomes of interest, the articles that used vitamin D supplementation, and articles in which serum 25(OH)D levels higher than 20 ng/ml were used to diagnose VDD. Two investigators (A.C. and R.C.) independently evaluated the full text of the studies chosen for eligibility. If any disagreement occurred, a third author (R.A.C. and A.E.C) decided against inclusion or exclusion after discussion.

2.2. Data analysis

Data were extracted from the eligible studies by two independent authors (A.C. and R.C.). Information on first authors, year of publication, country, study design, gender, the total number of patients (including the respective controls) for each outcome, 25(OH)D levels, number of events (severity or death) was collected. Specifically, COVID-19 severity was defined as severe pneumonia, or need for intensive care units, severe pulmonary involvement on CT examination or a composite outcome including respiratory distress, respiratory rate $\geq$30 breaths/min, hypoxemia, oxygen saturation (SpO2) $\leq$93%, respiratory failure requiring mechanical ventilation, shock, or multiple organ dysfunction. The use of different cut-offs for the evaluation of COVID-19 severity and mortality in patients with VDD among studies, allowed us to perform subgroup analysis.

The values of 25(OH)D levels reported in nmol/l were converted in ng/ml. The formula by Wan and colleagues was used to estimate the mean and SD when the data were expressed as the median and interquartile range [58]. The quality of included studies was independently assessed by two authors (A.C. and R.C.) by applying the "Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies" [59] and the "Quality Assessment of Case-Control Studies" scales [60] designed by the National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD, USA. The first requires the answer to 14 items, whereas the second to 12. These two scales allow the assessment of the risk of selection bias, information bias, measurement bias, or confounding bias. Based on the magnitude of the risk of bias, studies can be classified as poor, fair, or good quality. Any disagreement between the two investigators was resolved through discussion with a third investigator (A.E.C. or S.L.V.).

The association between 25(OH)D levels and SARS-CoV-2 infection, disease severity, or mortality was assessed by calculating the mean difference (MD) and the 95% confidence intervals (CI) for the quantitative variables (25(OH)D levels in patients with or without SARS-CoV-2 infection, in those with severe vs. non-severe COVID-19, or death vs. discharged), and calculating the ORs and the 95% CI for qualitative ones [number of severe cases or deaths in patients with VDD vs. those with normal levels of 25(OH)D].

The Cochran-Q and I^2 statistics were used to evaluate the statistical heterogeneity. Specifically, if I^2 resulted lower or equal to 50%, the variation of the studies was considered to be homogenous and the fixed effect model was adopted. If I^2 was higher than 50%, there was significant heterogeneity between studies, and the random-effects model was used. All p values lower than 0.05 were considered statistically significant. Sensitivity analyses were explored using the leave-one-out cross-validation procedure, with consists of the sequential omission of single studies, so to assess the contribution of each study to the pooled estimates, to prove the reliability of the results. The leave-one-out cross-validation procedure was performed through funnel plots. In the case of asymmetric shape, the plots were used to detect putative studies which represented the source of heterogeneity. The analysis was performed using RevMan software v. 5.4 (Cochrane Collaboration, Oxford, UK).

This study was registered in the Internal Prospective Register of Systematic Reviews (PROSPERO) database (https://www.crd.york.ac.uk/PROSPERO), with the registration number CRD42021241473.

2.3. Role of the funding source

3. Results

Using the above-mentioned search strategy, we extracted 662 records. After the exclusion of 310 duplicates, the remaining 352 articles were assessed for inclusion in the meta-analysis. Of these, 82 were judged not pertinent after reading their title and the abstract, 219 were excluded because they were reviews (n = 113), comments (n = 52), systematic review and meta-analyses (n = 5), and letters to the editor (n = 49). The remaining 52 articles were carefully read. Based on the inclusion and exclusion criteria, 12 articles were excluded because of the inability to extract the data required [12−23], whereas 9 were excluded because the patients received vitamin D supplementation [24,61−68], one study was excluded because it considered VDD for values < 30 ng/ml in disagreement with the above-mentioned guidelines [25], and one study was excluded since it did not define a cut-off for VDD [26] (**Supplementary Table 4**). Finally, 29 articles met our inclusion criteria and, therefore, were included in this meta-analysis (**Fig. 1**) [27−55]. **Table 1** shows the main characteristics of the studies selected. All studies were judged to be of fair quality after the quality analysis (**Tables 2 and 3**).

3.1. 25(OH)D levels in patients SARS-CoV-2 positive or negative

Twelve studies [27−38] evaluated the difference in 25(OH)D levels between virus-negative (n = 374,241) and virus-positive (n = 4879) patients. SARS-CoV-2 positive patients had lower 25(OH)D levels on average than negative patients [MD −3.99 (−5.34, −2.64); p<0.00001] (**Fig. 2A**). The Funnel Plot showed extreme inter-study heterogeneity in the studies included (Chi^2 P<0.00001, I^2=95%) (**Fig. 2B**). The main sources of heterogeneity arose from the studies by Abdollahi and colleagues [27], Chodick and colleagues [29], Hastie and colleagues [31], Luo and colleagues [34], Merzon and colleagues [36], and Rais-Estabragh and colleagues [37]. After their removal, no heterogeneity was found (Chi^2 P = 0.23, I^2=27%), and the analysis still
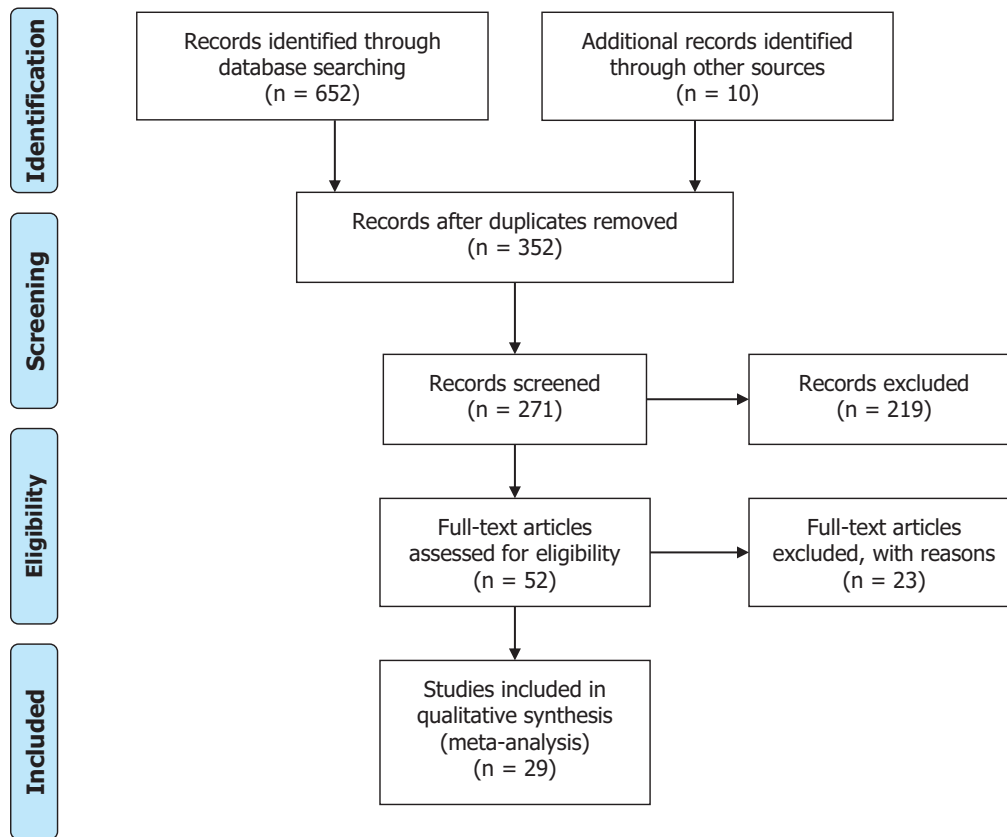
Fig. 1. flowchart of the studies included in the meta-analysis.

showed a statistical significance [MD -7.74 (-8.79, -6.69); p <0.00001]. Since in some studies the 25(OH)D levels were not measured in the same period as the cases [43,45,46,48,50,51], we excluded them from the analysis, but the statistical significance was maintained [MD -7.59 (-10.38, -4.81); $p<0.00001$] **(Supplementary Fig. 1A)**. The study by Abdollahi and colleagues also represented a source of heterogeneity (Chi^2 $P = 0.26$, I^2=24%) **(Supplementary Fig. 1B)** and, after its exclusion, significance was still present [MD -8.46 (-9.99, -6.92); $p< 0.00001$]**.**

3.2. 25(OH)D levels in patients with severe or non-severe COVID-19

We found 12 studies [34,38$-$48] assessing differences in 25(OH)D levels between patients with severe or mild COVID-19. The study by Alipio and colleagues was considered twice as it compared 25(OH)D levels between patients with mild vs. severe or critical forms of COVID-19 [39]. Specifically, 25(OH)D levels were clearly lower in the 609 patients with severe disease compared to the 922 patients with a non-severe course of the disease [MD -6.88 (-9.74, -4.03); p <0.00001] **(Fig. 3A)**. Also, in this case, inter-study heterogeneity was found (Chi^2 $P<0.00001$, I^2=98%) **(Fig. 3B)**. After the removal of the studies by Luo and colleagues [34], Alipio and colleagues [39], and Jain and colleagues [42], identified as a source of heterogeneity at the Funnel Plot, the analysis showed homogeneity of the remaining studies (Chi^2 $P = 0.83$, I^2=0%) maintaining the statistical significance [MD -4.89 (-6.36, -3.43); $p < 0.00001$].

3.3. 25(OH)D levels in COVID-19 patients who died compared to those discharged

The data on the difference in 25(OH)D levels between patients with COVID-19 who died compared to those who were discharged could be extracted from 8 studies [33,34,39,42,43,48$-$50]. The statistical analysis showed that patients experiencing death had significantly lower 25(OH)D levels than discharged ones [MD -8.01 (-12.50, -3.51); $p = 0.0005$] **(Fig. 4A)**. Inter-study heterogeneity was observed also in this analysis (Chi^2 $P<0.00001$, I^2=86%) **(Fig. 4B)**. The studies by Kerget and colleagues [44], Mardani and colleagues [35], and Abrishami and colleagues [49], were identified as the source of heterogeneity. When data from these articles were excluded, the heterogeneity became non-significant (Chi^2 $P = 0.15$, I^2=41%) and the difference in 25(OH)D levels between dead and discharged patients retained its statistical significance [MD: -3.61 (-5.51, -1.70); $p = 0.0002$].

3.4. Risk of severe COVID-19 in patients with vitamin D deficiency

The evaluation of the risk of severe COVID-19 in VDD patients was performed in data extracted from 11 studies [32,34,39,40,42,52,53]. The study by Cereda and colleagues [52] was considered twice since they evaluated both the percentage of patients with severe pneumonia and that of patients admitted to the intensive care units as an outcome of severity. Again, Alipio's [39] study was considered twice for the reasons reported above. Finally, the study by Jain and colleagues [42] was also considered twice since they assessed the risk of infection severity both in patients with 25(OH)D <20 ng/ml and then in patients with levels below 10 ng/ml. Specifically, the statistical analysis showed that patients with VDD had a higher risk of a severe disease course than patients without deficiency [OR 4.58 (2.24, 9.35); p <0.0001], regardless of the cut-off values considered to establish the efficiency **(Fig. 5A)**. The Funnel plot showed that the heterogeneity found (Chi^2 $P<0.00001$, I^2=84%) was attributable to three studies [32,39,42] **(Fig. 5B)**. Once the data from these studies were excluded, heterogeneity was no longer observed (Chi^2 $P = 0.53$, I^2=0%) and the risk of developing a severe course of the disease in VDD patients remained significant [OR 2.47 (1.80, 3.37); $p < 0.00001$].

Table 1

Main characteristics of the studies included in this meta-analysis.

First Author	Year	Country	Study design	Sample size	Mean Age		GenderMale/Female		Ethnicity		Outcome evaluated	Time at 25(OH)D levels assessment
Abdollahi	2020	Iran	Case-control study	402	SARS-CoV-2 + SARS-CoV-2 -	48.0 ± 16.5 46.34±13.5	SARS-CoV-2 + SARS-CoV-2 -	66/135 66/135	NR		Difference in mean 25(OH)D levels between COVID-19 positive and controls	NR
Abrishami	2020	Iran	Retrospective study	73	SARS-CoV-2 + SARS-CoV-2 -	55.2 ± 15.0 /	SARS-CoV-2 + SARS-CoV-2 -	47/26 /	NR		Difference in 25(OH)D levels between dead and discharged	Generally performed within 3 days of hospital admission
Alipio	2020	Southern Asian	Retrospective multicenter study	212	NR		NR		NR		Difference in 25(OH)D levels between mild and severe cases and assessment of the risk for severe COVID-19 in patients with VDD	25(OH)D tested on average 12−13 days before hospitalization, at the time of hospitalization and every 7 days after hospitalization. For analysis they used those on admission to the hospital
Arvinte	2020	USA	Pilot study	21	SARS-CoV-2 + SARS-CoV-2 -	60.2 ± 17.4 /	SARS-CoV-2 + SARS-CoV-2 -	15/6 /	SARS-CoV-2 + SARS-CoV-2 -	Caucasian: 4 Hispanic: 17 /	Difference in 25(OH)D levels between patients who died or were discharged from the hospital	Admission to hospital
Baktash	2020	UK	Prospective Cohort Study	105	SARS-CoV-2 + SARS-CoV-2 -	81 (SD NR) 83.4 ± 8.1	SARS-CoV-2 + SARS-CoV-2 -	42/28 15/20	SARS-CoV-2 + SARS-CoV-2 -	Caucasian: 50 South Asian: 18 East Asian: 2 Afro-Caribbean: 1 Caucasian: 30 South Asian: 3 East Asian: 0 Afro-Caribbean: 3	Difference in mean 25(OH)D levels between COVID-19 patients and controls. Assessment of the risk for COVID-19 related mortality in patients with VDD	Admission to hospital
Carpagnano	2020	Italy	Retrospective, observational single-center study	42	SARS-CoV-2 + SARS-CoV-2 -	65.0 ± 13.0 /	SARS-CoV-2 + SARS-CoV-2 -	30/12 /	NR		Assessment of the risk for mortality by COVID-19 in patients with VDD	Performed within 12 h of admission to RICU
Cereda	2020	Italy	Single-center cohort study	129	SARS-CoV-2 + SARS-CoV-2 -	73.6 ± 13.9 /	SARS-CoV-2 + SARS-CoV-2 -	70/59 /	SARS-CoV-2 + SARS-CoV-2 -	/ /	Assessment of the risk for COVID-19 severity and related mortality in patients with VDD	Performed within 48 h of admission to hospital
Chodick	2020	Israel	Cross-sectional study	14,520	SARS-CoV-2 + SARS-CoV-2 -	40.6 (19.1) 37.0 (19.1)	SARS-CoV-2 + SARS-CoV-2 -	788/529 6092/7111	NR		Difference in mean 25(OH)D levels between COVID-19 patients and controls	NR
D'Avolio	2020	Swiss	Retrospective Cohort Study	107	SARS-CoV-2 + SARS-CoV-2 -	73.3 ± 12.5 72.0 ± 15.9	SARS-CoV-2 + SARS-CoV-2 -	19/8 39/41	NR		Difference in mean 25(OH)D levels between COVID-19 patients and controls	Generally performed within 3 days of molecular testing for diagnosis of SARS-CoV-2 infection
De Smet	2020	Belgium	Retrospective observational study	186	SARS-CoV-2 + SARS-CoV-2 -	67.0 ± 20.9 /	SARS-CoV-2 + SARS-CoV-2 -	109/77 /	NR		Difference in 25(OH)D levels between mild and severe cases and between dead or discharged patients. Assessment of the risk for COVID-19 severe forms in patients with VDD	Admission to hospital

Table 1 (*Continued*)

First Author	Year	Country	Study design	Sample size	Mean Age		GenderMale/Female		Ethnicity		Outcome evaluated	Time at 25(OH)D levels assessment
Faul	2020	Ireland	Observational study	33	SARS-CoV-2 + SARS-CoV-2 -	NR /	SARS-CoV-2 + SARS-CoV-2 -	33/0 /	SARS-CoV-2 + SARS-CoV-2 -	Caucasian: 33 /	Difference in 25(OH)D levels between mild and severe COVID-19 patients	Admission to hospital
Hastie-Mackay	2020	UK	Retrospective cohort study	348,598	SARS-CoV-2 +	NR	SARS-CoV-2 +	265/184	SARS-CoV-2 +	White: 385 Black: 32 South Asian:19 Other: 13	Difference in mean 25(OH)D levels between COVID-19 patients and controls	Pre-hospedalization (at least 10 years old dosages)
					SARS-CoV-2 -	NR	SARS-CoV-2 -	168,391/179,758	SARS-CoV-2 -	White: 331,464 Black: 5022 South Asian:5917 Other: 5746		
Hernandez	2020	Spain	Case-control Study	394	SARS-CoV-2 + SARS-CoV-2 -	59.5 ± 16.8 61.0 ± 7.47	SARS-CoV-2 + SARS-CoV-2 -	123/74 123/74	NR		Difference in mean 25(OH)D levels between COVID-19 patients and controls. Assessment of the risk for COVID-19 severity and related mortality in patients with VDD	Admission to hospital
Im	2020	South Korea	Case-control study	200	SARS-CoV-2 + SARS-CoV-2 -	52.2 ± 20.7 52.4 ± 20.2	SARS-CoV-2 + SARS-CoV-2 -	21/29 NR	NR		Difference in mean 25(OH)D levels between COVID-19 patients and controls	Dosing performed on average within 2 days of hospital admission and no later than 7 days
Jain	2020	India	Prospective observational study	154	SARS-CoV-2 + SARS-CoV-2 -	NR /	SARS-CoV-2 + SARS-CoV-2 -	95/69 /	NR		Difference in 25(OH)D levels between mild and severe cases. Assessment of the risk for COVID-19 severe forms or mortality in patients with VDD	Admission to hospital
Karonova	2020	Russia	Observational cohort study	80	SARS-CoV-2 + SARS-CoV-2 -	53.2 ± 15.7 /	SARS-CoV-2 + SARS-CoV-2 -	43/37 /	NR		Difference in 25(OH)D levels between mild and severe COVID-19 forms and between dead or discharged patients	NE
Kerget	2020	Turkey	Case-control Study	88	SARS-CoV-2 + SARS-CoV-2 -	49 ± 21.1 35.2 ± 6.9	SARS-CoV-2 + SARS-CoV-2 -	41/47 8/12	NR		Difference in 25(OH)D levels between mild and severe COVID-19 forms and between dead or discharged patients	Admission to hospital
Lau	2020	UK	Retrospective observational cohort study	20	SARS-CoV-2 + SARS-CoV-2 -	65.2 ± 16.2 /	SARS-CoV-2 + SARS-CoV-2 -	9/11 /	SARS-CoV-2 + SARS-CoV-2 -	African/American: 15 /	Difference in 25(OH)D levels between mild and severe COVID-19 patients	NR
Luo	2020	China	Retrospective cross-sectional study	895	SARS-CoV-2 + SARS-CoV-2 - SARS-CoV-2 -	54.3 ± 15.6 54.7 ± 8.2 /	SARS-CoV-2 + SARS-CoV-2 - SARS-CoV-2 -	148/187 257/303 /	NR		Difference in 25(OH)D levels between COVID-19 patients and controls. Difference in 25(OH)D levels between mild and severe COVID-19 forms and between dead or discharged patients. Assessment of the risk for COVID-19 severity and related mortality in patients with VDD	Admission to hospital

Table 1 (*Continued*)

First Author	Year	Country	Study design	Sample size	Mean Age		GenderMale/Female		Ethnicity		Outcome evaluated	Time at 25(OH)D levels assessment
Mardani	2020	Iran	Case-control study	123	SARS-CoV-2 + SARS-CoV-2 -	43.3 ± 14.5 40.8 ± 15.8	SARS-CoV-2 + SARS-CoV-2 -	35/28 30/30	NR		Difference in mean 25(OH)D levels between COVID-19 patients and controls and between dead or discharged patients	Admission to hospital
Merzon	2020	Israel	Population based study	7807	SARS-CoV-2 + SARS-CoV-2 -	35.6 ± 15.6 47.4 ± 21.0	SARS-CoV-2 + SARS-CoV-2 -	385/397 2849/4176	NR		Difference in mean 25(OH)D levels between COVID-19 patients and controls	Pre-hospedalization (not specified when)
Panagiotou	2020	UK	Retrospective study	134	SARS-CoV-2 + SARS-CoV-2 -	NR /	SARS-CoV-2 + SARS-CoV-2 -	73/61 /	SARS-CoV-2 + SARS-CoV-2 -	Caucasian: 128 Asian: 4 Afro-Caribbean: 1 Other: 1 /	Difference in 25(OH)D levels between mild and severe COVID-19 forms. Assessment of the risk for severe COVID-19 forms in patients with VDD	Admission to hospital
Pizzini	2020	Austria	Prospective Multicentre Observational Study	109	SARS-CoV-2 + SARS-CoV-2 -	58.0 ± 14.0 /	SARS-CoV-2 + SARS-CoV-2 -	65/44 /	NR		Difference in 25(OH)D levels between mild and severe COVID-19 forms	25(OH)D assays performed 8 weeks after disease onset
Radujkovic	2020	Germany	Prospective Observational Study	185	SARS-CoV-2 + SARS-CoV-2 -	50.7 ± 15.7 /	SARS-CoV-2 + SARS-CoV-2 -	95/90 /	NR		Difference in 25(OH)D levels between mild and severe COVID-19 forms	Admission to hospital
Raharusuna	2020	Indonesia	Retrospective cohort study	780	SARS-CoV-2 + SARS-CoV-2 -	54.5 (SD NR) /	SARS-CoV-2 + SARS-CoV-2 -	380/400 /	NR		Assessment of the risk for COVID-19 mortality in patients with VDD	Pre-hospedalization (not reported the time of the last dosage)
Raisi-Estabragh	2020	UK	Prospective cohort study	4510	SARS-CoV-2 + SARS-CoV-2 -	68.1 ± 9.2 68.91±8.72	SARS-CoV-2 + SARS-CoV-2 -	696/630 1505/1679	SARS-CoV-2 + SARS-CoV-2 -	White: 1.141 Black: 76 Asian: 60 Chinese: 6 Mixed: 9 Other: 34 White: 2927 Black: 91 Asian: 78 Chinese: 3 Mixed: 24 Other: 61	Difference in mean 25(OH)D levels between COVID-19 patients and controls	Pre-hospedalization (at least 10 years old dosages)
Szeto	2020	USA	Retrospective cohort study	93	SARS-CoV-2 + SARS-CoV-2 -	NR /	SARS-CoV-2 + SARS-CoV-2 -	44/49 /	SARS-CoV-2 + SARS-CoV-2 -	Black: 27 /	Assessment of the risk for COVID-19 severity and related mortality in patients with VDD	Prehospitalization (25(OH)D levels measured within the previous year and on average 136 days prior to hospital admission)
Vassiliou	2020	Greek	Prospective observational cohort study	30	SARS-CoV-2 + SARS-CoV-2 -	65.0 ± 11.0 /	SARS-CoV-2 + SARS-CoV-2 -	24/6 /	NR		Difference in 25(OH)D levels between dead and discharged COVID-19 patients and assessment of the risk for COVID-19 mortality in patients with VDD	Admission to ICU

Table 1 (Continued)

First Author	Year	Country	Study design	Sample size	Mean Age	Gender Male/Female	Ethnicity	Outcome evaluated	Time at 25(OH)D levels assessment
Ye	2020	China	Case-control study	142	SARS-CoV-2 + 41.7 ± 15.9 SARS-CoV-2 - 44.7 ± 20.5	SARS-CoV-2 + 32/48 SARS-CoV-2 - 23/39	NR	Difference in mean 25 (OH)D levels between COVID-19 patients and controls, and between patients with severe or non-severe forms of COVID-19. Assessment of the risk for severe COVID-19 forms in patients with VDD	Admission to hospital

Abbreviation: 25(OH)D, 25-hydroxy-cholecalciferol; VDD, vitamin D deficiency; COVID-19, coronavirus disease 19; NR, Not Reported; SARS-CoV-2 +, patients positive for severe acute respiratory syndrome coronavirus 2 infection; SARS-CoV-2 -, patients negative for severe acute respiratory syndrome coronavirus 2 infection; SD, standard deviation; NE, Not evaluated; ICU, Intensive Care Unit; RICU, Respiratory Intermediate Care Unit.

3.5. Mortality risk in patients with vitamin D deficiency

Finally, the mortality risk in VDD patients was assessed by analysis of data from 9 studies [32,34,40,42,51–55]. In detail, no significant increased risk of mortality was found in VDD patients compared to patients with adequate 25(OH)D levels [OR 4.92 (0.83, 29.31); $p = 0.08$] regardless of the cut-off values considered for the deficiency (**Supplementary Fig. 2A**). An inter-study heterogeneity was found (Chi^2 $P<0.00001$, I^2=94%), and its source was due to studies by Jain and colleagues [42] and Raharusuna and colleagues [55] (**Supplementary Fig. 2B**). When these studies were excluded from the analysis, the funnel plot showed homogeneity of the remaining studies (Chi^2 $P = 0.15$, I^2=36%), and the increased mortality risk for COVID-19 in presence of VDD was confirmed to be non-significant [OR 1.30 (0.83, 2.03); $p = 0.23$].

4. Discussion

The great interest in the protective role of adequate 25(OH)D levels in SARS-CoV-2 infection arises from the fact that vitamin D has pleiotropic effects due to the systemic expression of vitamin D receptors (VDRs). Approximately 11,031 possible VDR-target genes have so far been identified. They are involved in metabolism (43%), cell and tissue morphology (19%), cell junction and adhesion (10%), differentiation and development (10%), angiogenesis (9%), and epithelial to mesenchymal transition (5%) [69]. Moreover, a recent meta-analysis including 19 studies showed a significant and non-linear correlation between 25(OH)D levels and risk and severity of acute respiratory tract infection (ARTI) with an increased risk for levels below 15 ng/ml [70]. The results of the present meta-analysis support this evidence and suggest a role for vitamin D in the fight against COVID-19. We found that not only 25(OH)D levels are lower in SARS-CoV-2 infected patients than in healthy people, but also that 25(OH)D levels are lower in patients with a severe course of the disease and in those who die for COVID-19. Furthermore, we reported that VDD is a factor that may increase the risk to develop more severe disease, although it does not appear to increase the risk of mortality by COVID-19.

These results suggest the hypothesis that vitamin D intake may be a useful therapeutic weapon for the prevention of infection and the treatment of patients with COVID-19. Already on other acute respiratory diseases, a previous meta-analysis of RCTs concluded that vitamin D supplementation is safe and protective. Furthermore, the protective effects of its administration are greater when the levels of 25(OH)D are low and in particular when they are below 10 ng/ml [71]. Instead to date, there is still little, albeit promising, evidence on the effect of Vitamin D supplementation on COVID-19. The available data are based on studies involving a small number of patients. The SHADE study conducted on 40 patients, 24 of whom treated with a daily administration of 60,000 IU of cholecalciferol for more than 7 days until reaching a concentration of 25(OH)D >50 ng/ml, showed that supplementation for a short time with vitamin D is associated with a higher virus clearance rate than in untreated patients [67]. In another study, carried out on 66 elderly nursing-home patients who contracted COVID-19, Annweiler and colleagues showed that the 57 patients who received a bolus of 80,000 IU of vitamin D3 shortly before or after the diagnosis had less severe disease and a better survival rate than the 9 untreated patients [61]. Similarly, Castillo and colleagues found that administration of high doses of calcifediol (0.266 mg on day 1, 3, and 7 of hospitalization and then weekly until discharge) in 50 patients with SARS-CoV-2 infection was associated with less severe COVID-19 and, therefore, to a lesser need for hospitalization in intensive care unit than the 26 untreated patients [64]. High-dose cholecalciferol booster therapy has been correlated with a lower risk of mortality from COVID-19 [65]. In contrast, another study showed that 25(OH)D at a dose of 25,000 IU in the 3 months before infection was not only not associated with an improvement in

Table 2
Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies.

Author	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Abrishami et al. 2020	+	+	+	+	–	NR	+	–	+	–	+	NA	+	+
Alipio et al. 2020	+	+	+	–	–	+	NR	+	+	+	+	NA	+	+
Arvinte et al. 2020	+	+	+	–	–	NR	–	–	+	–	+	NA	+	–
Baktash et al. 2020	+	+	+	–	–	–	–	+	+	–	+	NA	+	–
Carpagnano et al. 2020	+	+	+	–	–	NR	–	+	+	–	+	NA	+	+
Cereda et al. 2020	+	+	+	–	–	+	–	+	+	–	+	NA	+	+
Chodick et al.2020	+	+	+	–	–	NR	–	–	+	–	+	NA	+	+
D'Avolio et al. 2020	+	+	+	+	–	–	–	–	+	–	+	NA	+	–
De Smet et al.2020	+	+	+	–	–	–	–	+	+	–	+	NA	+	–
Faul et al. 2020	+	+	+	–	–	NR	NR	–	NR	NR	+	NA	+	–
Hastie-Mackay et al. 2020	+	+	+	+	–	+	+	+	+	NR	+	NA	+	+
Jain et al. 2020	+	+	+	+	+	NR	–	+	+	–	+	NA	+	+
Karonova et al. 2020	not assessable because in Russian language													
Lau et al. 2020	+	+	+	–	–	+	–	–	+	–	+	NA	+	–
Luo et al. 2020	+	+	+	–	–	+	–	+	+	–	+	NA	+	+
Merzon et al. 2020	+	+	+	+	–	+	NA	+	+	NR	+	NA	+	+
Panagiotou et al. 2020	+	+	+	–	–	NR	–	+	+	–	+	NA	+	–
Pizzini et al. 2020	+	+	+	+	–	+	–	+	+	–	+	NA	+	–
Radujkovic et al. 2020	+	+	+	–	–	+	–	+	+	–	+	NA	+	+
Raharusuna et al. 2020	+	+	+	–	–	+	–	+	+	–	+	NA	+	+
Raisi-Estabragh et al. 2020	+	+	+	+	–	+	+	–	+	–	+	NA	+	+
Szeto et al. 2020	+	+	+	–	–	+	NR	+	+	+	+	NA	+	+
Vassiliou et al. 2020	+	+	+	+	–	+	–	+	+	–	+	NA	+	–

1. Was the research question or objective in this paper clearly stated?.
2. Was the study population clearly specified and defined?.
3. Was the participation rate of eligible persons at least 50%?.
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study pre-specified and applied uniformly to all participants?.
5. Was a sample size justification, power description, or variance and effect estimates provided?.
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?.
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?.
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?.
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?.
10. Was the exposure(s) assessed more than once over time?.
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?.
12. Were the outcome assessors blinded to the exposure status of participants?.
13. Was loss to follow-up after baseline 20% or less?.
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?.

Table 3
Quality Assessment of Case-Control Studies.

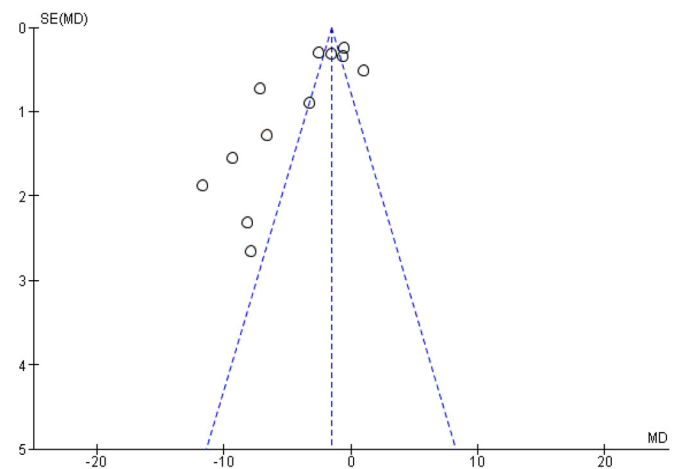| Author	1	2	3	4	5	6	7	8	9	10	11	12
Abdollahi et al. 2020	+	+	–	+	+	+	NA	+	–	+	NA	–
Hernandez et al.	+	+	–	+	+	+	NA	–	–	+	NA	+
Im et al.	+	+	–	+	–	+	NA	+	–	+	NA	–
Kerget et al. 2020	+	+	–	–	–	+	NA	+	–	+	NA	–
Mardani et al. 2020	+	+	–	+	+	+	NA	+	–	+	NA	–
Ye et al.	+	+	–	+	–	+	NA	+	–	+	NA	+

1. Was the research question or objective in this paper clearly stated and appropriate?.
2. Was the study population clearly specified and defined?.
3. Did the authors include a sample size justification?.
4. Were controls selected or recruited from the same or similar population that gave rise to the cases (including the same timeframe)?.
5. Were the definitions, inclusion and exclusion criteria, algorithms or processes used to identify or select cases and controls valid, reliable, and implemented consistently across all study participants?.
6. Were the cases clearly defined and differentiated from controls?.
7. If less than 100 percent of eligible cases and/or controls were selected for the study, were the cases and/or controls randomly selected from those eligible?.
8. Was there use of concurrent controls?.
9. Were the investigators able to confirm that the exposure/risk occurred prior to the development of the condition or event that defined a participant as a case?.
10. Were the measures of exposure/risk clearly defined, valid, reliable, and implemented consistently (including the same time period) across all study participants?.
11. Were the assessors of exposure/risk blinded to the case or control status of participants?.
12. Were key potential confounding variables measured and adjusted statistically in the analyses? If matching was used, did the investigators account for matching during study analysis?.
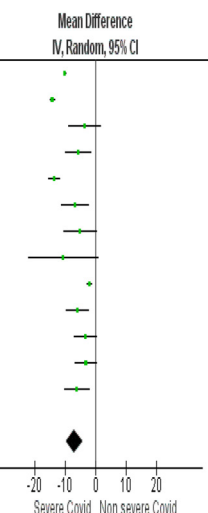
Fig. 2. Panel A. Forest plot of studies that assessed 25(OH)D levels as a continuous variable in SARS-CoV-2 positive and negative patients. **Panel B.** Funnel plot showing the source of heterogeneity of studies that evaluated 25(OH)D levels as a continuous variable in SARS-CoV-2 positive and negative patients.
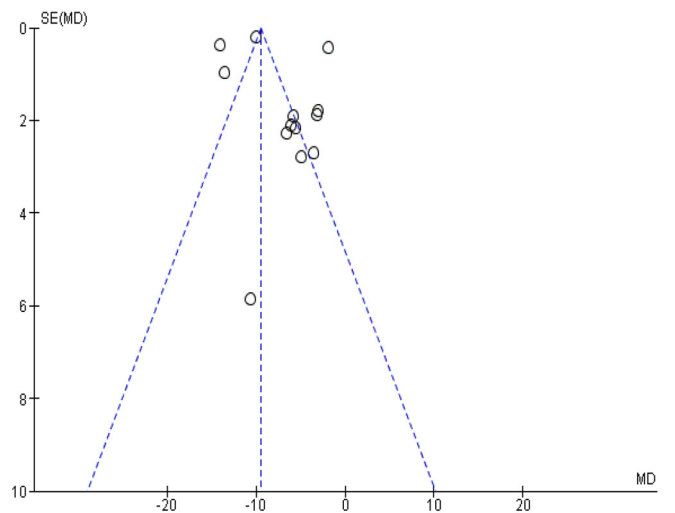Serum 25(OH)D levels are expressed in ng/ml.

Fig. 3. Panel A. Forest plot of studies that assessed 25(OH)D levels as a continuous variable in patients with severe course of COVID-19 than those with mild course. **Panel B.** Funnel plot showing the source of heterogeneity of studies that evaluated 25(OH)D levels as a continuous variable in patients with severe course of COVID-19 than those with mild course. Serum 25(OH)D levels are expressed in ng/ml.

hospitalization rate, but also appeared to be a risk factor for increased hospital mortality [63]. Another recent RCT showed that a single high dose of 200,000 IU of vitamin D3 did not significantly reduce the length of hospitalization, in-hospital mortality, admission to intensive care unit, or the need for mechanical ventilation in treated patients compared to those who received placebo [72]. Certainly, more randomized clinical trials with larger samples, well-standardized 25(OH)D dosages, and different vitamin D formulations are needed to better clarify the role of vitamin D supplementation in the treatment of COVID-19.

The present study is a meta-analysis performed to date on the largest number of studies on this topic. The only other study that extensively assessed the evidence gathered up to December 2020 is the National Institutes of Health and Care Excellence (NICE) report

[73], which concluded that there is no enough evidence to recommend vitamin D administration to prevent and/or treat COVID-19. However, unlike the NICE report, our meta-analysis included studies that were not included in the NICE systematic review. Furthermore, unlike the latter, we excluded studies in which vitamin D was given to patients. This choice, in our opinion, allows us to evaluate the effect of vitamin D deficiency without incurring the bias dictated by its supplementation. We certainly agree with the NICE report that stronger evidence is needed to establish a clear correlation between serum 25(OH)D levels and COVID-19.
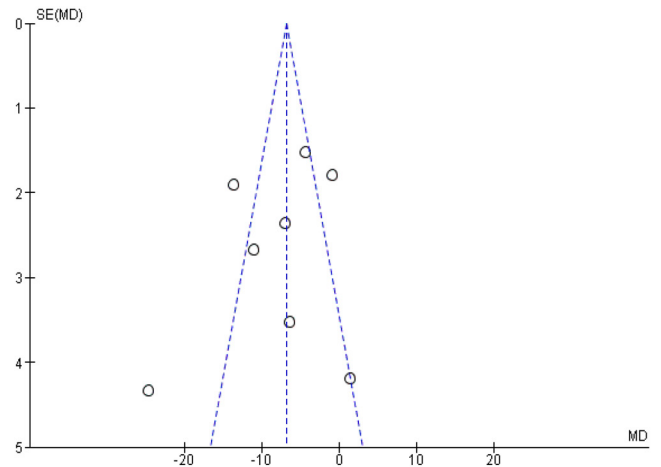
Another large meta-analysis, including 31 studies, has recently been published [74]. It concluded that 25(OH)D levels are on average lower in patients infected with SARS-CoV-2 than in negative patients. However, differently from the present meta-analysis, the authors did
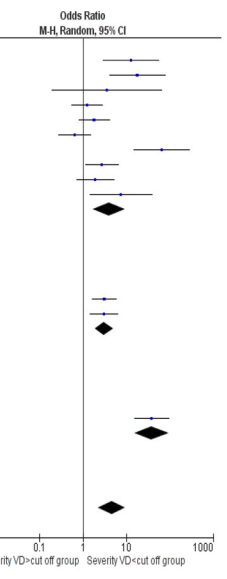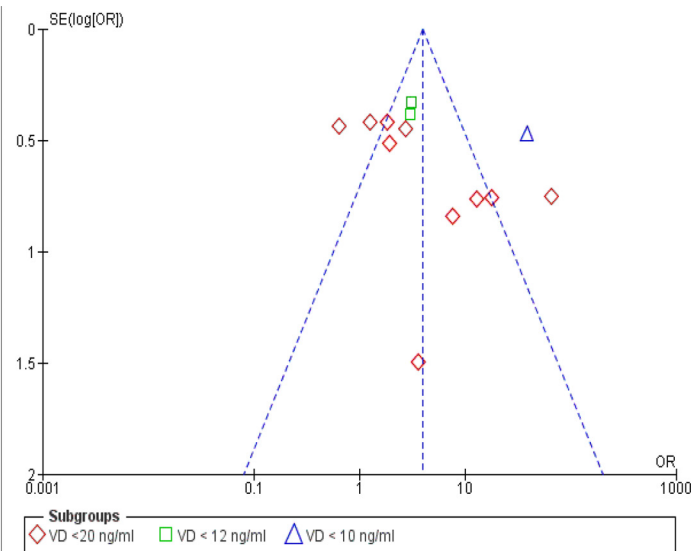
Fig. 4. **Panel A.** Forest plot of studies that assessed 25(OH)D levels as a continuous variable in patients with COVID-19 who died than discharged ones. **Panel B.** Funnel plot showing the source of heterogeneity of studies that evaluated 25(OH)D levels as a continuous variable in patients with COVID-19 who died than discharged ones. Serum 25(OH)D levels are expressed in ng/ml.

Fig. 5. **Panel A.** Forest plot of studies that assessed the risk of a severe course of disease in subjects with 25(OH)D values below or above a specified cut-off. The different cut-offs used by the studies allowed for subgroup analysis. Studies using cut-off values higher than those established by the Endocrine Society for the diagnosis of Vitamin D Deficiency (<20 ng/ml) were not included. **Panel B.** Funnel plot showing the source of heterogeneity of studies that evaluated the risk of a severe course of disease in subjects with 25(OH)D below or above a specified cut-off.

not find any statistically significant correlation between VDD and COVID-19 related health outcomes, but only a trend toward. This discrepancy may relate, also in this case, to the different studies included [29,39,41,45−47,53,55]. Furthermore, Bassatne and colleagues included studies in which patients received supplemental therapy, whereas we excluded intervention studies because, as above-mentioned, this represents a bias for the interpretation of the results [24,61,65,68]. Another reason that could explain the different outcome is that we excluded studies which did not clearly establish the cut-off of VDD [26] or those that used cut-offs higher than 20 ng/ml for the definition of VDD [25]. This difference in the eligibility and exclusion criteria could explain the different conclusions between the two meta-analyses.

All the studies included in this meta-analysis were judged as of fair quality at the quality analysis. Nevertheless, some limitations should be considered. The main limitation is that all the studies included in this meta-analysis are observational. As such, it is not possible to establish a cause-effect relationship between serum 25 (OH)D levels and SARS-CoV2 infection and severity of COVID-19. Moreover, the analysis revealed a large heterogeneity of the studies included. This mostly relies on the different criteria for patient enrollment. Indeed, patients differed for age, ethnicity, and body mass index (BMI), and all these factors, in turn, influence serum 25(OH)D levels. Moreover, the comorbidity exclusion criteria used to select the patient samples are not well-defined in many of these studies. Another reason for heterogeneity is represented by the different 25

(OH)D cut-off used for the diagnosis of VDD by the various authors. There is not an established cut-off value below which systemic effects of VDD appear. Indeed, although literature suggests that skeletal effects occur for 25(OH)D levels below 12 ng/ml [75], clear threshold values for the onset of extra-skeletal effects have not been established. However, it is generally accepted that to avoid these effects values of at least 20 ng/ml or even higher than 30 ng/ml are necessary [76]. In support of this, our meta-analysis showed a correlation between 25(OH)D levels and severity of COVID-19 independently of stratified analysis for different subgroups, thus suggesting that values <20 ng/ml may already be sufficient to predispose to the development of increased disease severity.

Furthermore, the country where the patients live must also be taken into account. In fact, VDD is more frequent in Western Europe (especially in the UK) than in Northern Europe and even more in Southern and Eastern Europe [77]. Moreover, the highest prevalence of VDD occurs in Middle Eastern countries (Turkey, Iran, Israel), China, and India [76,77]. This difference in the prevalence of VDD across countries may explain why the mean serum 25(OH)D levels are profoundly different among studies.

Additionally, a different definition of disease severity occurs among studies that evaluated the correlation between VDD and COVID-19 severity. Some of them considered the development of severe pneumonia, others the need for treatment in the intensive care unit, others the severity of lung involvement on imaging scans, and some relied on a composite index. Furthermore, in most of the studies, the condition of VDD was established only at diagnosis without any indication on the duration of the state of hypovitaminosis D. This represents another major bias since evidence suggests that 25(OH)D may be a marker of acute inflammation. In particular, in conditions of acute inflammation, 25(OH)D decrease because of the lower levels of vitamin D binding protein (VDBP) that binds approximately 85% of circulating 25(OH)D levels. This decrease generally occurs already in the first 24 h after the onset of the acute event and seems to be related to VDBP reduced half-life, decreased hepatic synthesis due to alteration of metabolic processes that occur in acute inflammatory states, and an increased capillary permeability resulting, in turn, in protein leakage [78]. As further evidence of this mechanism, 25(OH)D levels appear to decrease as PCR levels, a known marker of acute inflammation, increase [79].

However, a recent study on the child population showed that severe or non-severe pneumonia or inflammation does not affect 25(OH)D levels [80]. Similarly, Alipio and colleagues reported that pre-hospitalization 25(OH)D levels were not different from those measured during the hospitalization period for COVID-19 [39]. Moreover, Bertoldo and colleagues studying the correlation between 25(OH)D levels and acute phase response after infusion of nitrogen-containing bisphosphonates showed that low 25(OH)D levels before bisphosphonate administration are more frequently associated with the onset of an acute-phase response (even after adjustment for confounding factors). In turn, more severe acute phase responses, characterized by more elevated PCR levels, are significantly associated with a reduction in 25 (OH) D levels [81]. On this basis we could hypothesize that even in COVID-19 lower levels of 25(OH)D base can be associated with a more severe course and that, in turn, the latter can further influence 25(OH)D levels.

The only studies included in the meta-analysis that used prehospitalization 25(OH)D levels had mixed results. In fact, Szeto and colleagues [53], measuring serum 25(OH)D levels on average 136 days earlier, showed no correlation between prehospitalization 25(OH)D levels and the severity of COVID-19. However, it must be considered that although 25(OH)D levels seem to maintain certain stability over time (especially after adjustment for the season), in this study 25(OH)D levels on average 4 months old were used for statistical comparison. Considering a period of enrollment of patients from February to May, 25(OH)D values of some patients could be altered already by

several months of restrictions due to the pandemic. Therefore, pre-hospitalization 25(OH)D levels measured a long time before might not correlate adequately with those at the time when the infection was contracted. Similarly, studies by Hastie and colleagues [31] and Raise-Estebragh and colleagues [37] showed no correlation between 25(OH)D levels and the risk of infection after adjustment for confounding factors. However, both studies used a sample extracted from the same database where the last measurement of 25(OH)D values was at least 10 years old.

Conversely, Merzon and colleagues [36] studied patients of the Leumit Health Services database who tested positive for SARS-CoV-2 infection between February and April 2020 and had at least one previous 25(OH)D assay. This study showed significantly lower levels of 25(OH)D in SARS-CoV-2-positive patients compared with virus-negative patients, with a significant association between low 25(OH)D levels and an increased likelihood of SARS-CoV-2 infection that remained significant after adjustment for confounding factors. Furthermore, 25(OH)D levels were significantly lower in hospitalized than in non-hospitalized patients, but with a non-significant association between low 25(OH)D values and the risk of hospitalization after adjustment for confounding factors. However, in this case, there were no data on when 25(OH)D was measured. The study by Kaufman and colleagues [19] on 191,779 patients, showed a positive association between lower SARS-CoV-2 positivity rate and higher circulating 25(OH) D levels that remained significant in a multivariable logistic model.

Given the conflicting results of the studies and their several limitations, there is certainly a need for longitudinal trials that evaluate the relationship correlation between 25(OH)D levels before infection (but close to it) and the outcomes of COVID-19. Moreover, at the same time, these studies should analyze the variation of 25(OH)D levels during the infection to establish whether the abrupt lowering of metabolite levels is secondary to a more severe outcome of the disease or may itself represent a worsening factor for the disease. Alternatively, studies analyzing free 25(OH)D values might allow us to overcome the bias secondary to the theory of 25(OH)D as a negative acute-phase marker.

Finally, the statistical analysis of several studies lacks adjusted analysis for possible confounding factors (BMI, age, ethnicity, comorbidities), thus preventing the establishment of a certain correlation between 25(OH)D levels and the outcomes analyzed.

We found that the correlation of VDD with the risk of in-hospital mortality was not statistically significant although, in patients who died, the levels of 25(OH)D were significantly lower than in patients who were discharged from the hospital. This apparent discrepancy between these results is partly due to the lack of studies carried out on a large number of patients. Moreover, some studies used a cut-off to distinguish patients with VDD from those with adequate levels of 25(OH)D considered low according to Endocrine Society guidelines. Therefore, among patients considered to have normal 25(OH)D levels, some patients had VDD or VDI. The only two studies that make a clear distinction are that of Raharusuna and colleagues and Alipio and colleagues. The results of these studies showed that the risk of mortality in patients with VDD was much higher than in patients with normal 25(OH)D levels [39,53]. Finally, because the studies consider in-hospital mortality without specifying which complication led to the patient death, we are not certain whether the fatal event resulted from the infection or whether the disease severity was an aggravating condition of an already compromised baseline situation. In this sense, more specific studies analyzing the mortality associated with complications of SARS-CoV-2 infection are needed.

In addition to all the above-mentioned limits, intervention studies aimed at evaluating the possible benefits of vitamin D administration in the management of COVID-19 reported a reduction in infection and hospitalization rates [62−65,67]. These findings can probably only be explained if a cause-and-effect association is established.

Therefore, further intervention studies on larger series should be encouraged to evaluate the effects of vitamin D in patients with COVID-19, to clarify the association between VDD and SARS-CoV2 infection or COVID-19 severity.

In conclusion, this meta-analysis, despite the several limitations present in the various studies considered, may allow hypothesizing a role for low 25(OH)D levels in the risk of contracting SARS-CoV-2 infection and in developing more severe forms of COVID-19. However, additional evidence is needed to confirm the effects of VDD in the infection and to exclude that the link suggested by the numerous observational studies is not casual, as already observed for cardiovascular diseases, adiposity, glucose metabolism, mood disorders, muscular function, and others [82]. Furthermore, the effects of vitamin D supplementation need to be clearly studied. Therefore, we can only speculate that vitamin D supplementation could be considered in the therapeutic repertoire of patients with COVID-19.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Contributors

A.C. Conceptualization, Formal analysis, Data curation, Methodology, Writing-original draft; R.C. Data curation, Formal Analysis, Writing-review & editing; R.A.C. L.M.M. and F.B. Visualization, Validation; A.A. and S.L.V. Supervision, Writing-review & editing; A.E.C. Conceptualization, Project administration, Writing-review & editing. All authors have approved the final version of the paper.

Data sharing statement

Data are available upon request by sending an email to aldo.calogero@unict.it.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2021.100967.

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