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The relationship between the severity and mortality of SARS-CoV-2 infection and 25-hydroxyvitamin D concentration — a metaanalysis

Abstract

Introduction: There is increasing scientific interest in the possible association between hypovitaminosis D and the risk of SARS-CoV-2 infection severity and/or mortality.

Objective: To conduct a metanalysis of the association between 25-hydroxyvitamin D (25(OH)D) concentration and SARS-CoV-2 infection severity or mortality.

Material and methods: We searched PubMed, EMBASE, Google scholar and the Cochrane Database of Systematic Reviews for studies published between December 2019 and December 2020. Effect statistics were pooled using random effects models. The quality of included studies was assessed with the Newcastle–Ottawa Scale (NOS). Targeted outcomes: mortality and severity proportions in COVID-19 patients with 25(OH)D deficiency, defined as serum 25(OH)D < 50 nmol/L.

Results: In the 23 studies included (n = 2692), the mean age was 60.8 (SD ± 15.9) years and 53.8% were men. Results suggested that vitamin 25(OH)D deficiency was associated with increased risk of severe SARS-CoV-2 disease (RR 2.00; 95% CI 1.47–2.71, 17 studies) and mortality (RR 2.45; 95% CI 1.24–4.84, 13 studies). Only 7/23 studies reported C-reactive protein values, all of which were > 10 mg/L.

Conclusions 25(OH)D deficiency seems associated with increased SARS-CoV-2 infection severity and mortality. However, findings do not imply causality, and randomized controlled trials are required, and new studies should be designed to determine if decreased 25(OH)D is an epiphenomenon or consequence of the inflammatory process associated with severe forms of SARS-CoV-2. Meanwhile, the concentration of 25(OH)D could be considered as a negative acute phase reactant and a poor prognosis in COVID-19 infection.

Key words: SARS-CoV-2, COVID-19, vitamin D, 25-hydroxyvitamin D, severity

Introduction

Since the COVID-19 pandemic began in December 2019, and whilst waiting for an effective and safe vaccine, there has been increased urgency to achieve drug therapy with new and old drugs. Among the latter candidates is 25-hydroxyvitamin D (25(OH)D), which has been proposed as a potentially modifiable risk factor for COVID-19 outcomes [1].

25(OH)D is a steroid hormone, which comes mainly from the synthesis at the level of the skin, of a precursor that is 7-dehydrocholesterol, which due to the action of ultraviolet light (UVL) B (280–315 nm) exposure is converted into vitamin D3 (cholecalciferol). In some countries at far latitudes, this origin is seasonal only [2]. 25(OH)D can be obtained from diet (e.g., oily fish, eggs, liver) but very few commonly eaten foods contain sufficient amounts, which is why some countries (but only few) have a mandatory vitamin D food fortification policy [3]. The recommended amount of vitamin D intake in the majority of countries is 10 μg or 400 IU of vitamin D daily during winter at least. Vitamin D levels can be affected by obesity, sunscreen, clothing, genetics, gender, smoking and socio-economic status [4]. Vitamin D2 (obtained from dietary intake of mushrooms or some vegetables) and D3 (obtained from sun exposure or diet) are hydroxylated in the liver and kidneys where the active form of this vitamin is generated as, 1,25 Dihydroxycholecalciferol (1,25(OH)2D). Macrophages/dendritic cells and other organ cells also have the ability to convert 25 (OH) D to 1,25(OH)2D via CYP27B1. The biomarker of a patient's VD status is the concentration of total 25(OH)D (25-hydroxyvitamin D) concentration in serum, because vitamin D deficiency correlates better with 25(OH)D than with 1,25(OH)2D [5, 6]. The effects of VD on calcium and phosphate absorption, osteoclast activation, and hence on bone calcification and muscle strength are widely known [7]. The VD receptor (VDR) is very widely expressed, including by all leucocyte classes, and it has been demonstrated that many genes are VD responsive [8], including nearly two hundred genes in monocyte/macrophage cells [9]. Other research has shown that cytokine concentrations and proliferation of immune cells can be modulated by VD [10].

Currently, research designs that have been used to postulate a relationship between hypovitaminosis D and the severity of SARS-CoV-2 infection are either ecological, demographic with risk groups for VD deficiency (Mendelian randomization), or studies on the association of 25(OH)D levels with the risk of having a positive test for the virus [1]. Ecological studies use databases with information on 25(OH)D concentration of populations and countries and relate it to mortality, recovery, severity or susceptibility to SARS-CoV-2 infection. A published metanalysis that included ecological studies in 51 countries found no correlation between 25(OH)D levels and recovery or mortality rates [11]; however, consid-

ering latitude, an inverse relationship was found between mortality and 25(OH)D status in Asia, Middle East and Oceania; and surprisingly, in the USA and South America, the correlation was direct [12–14]. Ilie *et al*. found that the Pearson correlation coefficients between mean 25(OH)D levels and COVID-19 cases, and mean 25(OH)D levels and COVID-19 deaths per million population were negative and statistically significant based on data from 20 European countries [15]; however, this study was re-analyzed by Kumar *et al*., adding to the model the life expectancy factor, and the result was the loss of the significance of 25(OH)D levels as a predictor of mortality from COVID-19 [16].

Mendelian randomization studies use genetic variants as markers to evaluate a causal relationship in observational data [17], and have been used in studies of the association between 25(OH)D and severity of COVID-19 infection, based on the fact that the polymorphism of the VD receptor has an impact on the response to 25(OH)D. Mendelian randomization studies use the genetic variant as a surrogate variable for 25(OH)D deficiency, to infer the causal effect of an exposure [25(OH)D concentration] to an outcome (COVID-19 susceptibility, severity or mortality) [18]. Currently, 3 studies have been published using Mendelian randomization on the association of 25(OH)D concentration with the risk or severity of COVID-19; one found a relationship [19]which impedes good immune function, is common during winter and spring in regions of high latitude. There is good evidence that vitamin D deficiency contributes to the seasonal increase of virus infections of the respiratory tract, from the common cold to influenza, and now possibly also COVID-19. This communication explores key factors that make it more likely, particularly in combination, that individuals are vitamin D deficient. These factors include old age, obesity, dark skin tone and common genetic variants that impede vitamin D status. Precision nutrition is an approach that aims to consider known personal risk factors and health circumstances to provide more effective nutrition guidance in health and disease. In regard to avoiding vitamin D deficiency, people with excess body fat, a dark skin tone or older age usually need to use a moderately dosed daily vitamin D supplement, particularly those living in a high-latitude region, getting little ultraviolet B exposure due to air pollution or staying mostly indoors. Carriers of the GC (group-specific component, but the other two did not [18, 20]. The limitations of this type of study is that it

uses a surrogate for 25(OH)D deficiency, and in severe cases of COVID-19, it is necessary to have the serum 25(OH)D concentration at the time of hospitalization for COVID-19; on the other hand, the polymorphism with which the individual was born does not predict numerous other factors that could have affected 25(OH)D status.

The objective of the present study was to perform a systematic review and meta-analytic study on severity or mortality of SARS-CoV-2 infection and 25-hydroxyvitamin D concentration based in observational studies and randomized clinical trials.

Material and methods

This study was conducted following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) [21].

Search strategy

Two independent investigators performed a systematic search in PubMed, EMBASE, Google Scholar, preprint servers (medRxiv, bioRxiv and Research Square) and the Cochrane Database of Systematic Reviews for studies published between December 2019 and December, 2020. In addition, we conducted a secondary search based on the references lists of the retrieved articles. The PubMed search strategy is detailed in the Supplementary file.

Eligibility criteria

We searched for randomized controlled trials (RCTs) or observational studies reporting data on serum 25(OH)D concentration and SARS-CoV-2 infection severity or mortality. We included studies in English or other (Russian and Spanish) language (all ages) meeting the following criteria: a) COVID-19 patients were diagnosed according to the interim guidance of the World Health Organization [22]; b) inclusion of the mean and standard deviation for laboratory test values of 25(OH)D, and sample size with demographics, comorbidities, and complications; c) the study presented data on hazard ratios (HRs), relative risks (RRs), or odds ratios (ORs) with confidence intervals (CIs) or offered enough data to allow these to be calculated (including via email correspondence with original authors if necessary); and d) SARS-CoV-2 infection severity criteria were described (generally defined as admission to intensive care unit, acute respiratory distress syndrome and/or need for mechanical ventilation).

The following exclusion criteria were applied: reviews, abstracts, discussion summaries, and insufficient reported data including absence of vitamin D measurement.

Quality assessment

The quality of observational studies (cohort and case-control studies) and RCTs were assessed according to the Newcastle-Ottawa Quality Assessment Scale (NOS) [23] and the Cochrane Risk of Bias Assessment Tool [24], respectively. Two investigators evaluated the quality of the studies independently. Conflicting results were resolved by discussion and involvement of a third reviewer if necessary.

Data extraction

The following data were extracted from each study: authors, study location, year of publication, study design, number of participants, sex, age at baseline, serum VD level, outcome definition, and effect estimates with 95% confidence intervals (CIs). Targeted outcomes: COVID-19 mortality and/or severity proportions. Even though some studies have considered other 25(OH)D cut-off values [25], in this study we defined Vitamin D deficiency as serum hydroxyvitamin D level <50 nmol/L (< 20 ng/mL) [26]. In a sub-analysis, more severe deficiency was defined as < 30 nmol/L (< 12 ng/mL) [27].

Statistical analyses

Primary analyses evaluated the association (HRs, RRs or ORs) between 25-hydroxyvitamin D concentration and SARS-CoV-2 infection severity or mortality. In the metaanalysis, in order to calculate the effect size of 25(OH)D concentration and gender, the relative risk or odds ratio published by the authors of the included studies were used. We applied random effects with an inverse variance method to calculate the pooled RRs and 95% CIs according to the heterogeneity between the studies [28]. The overall estimates in the pooled analysis were obtained using Stata 13 software (Stata Corp LP, College Station, TX).

Results

After screening 745 citations, 23 studies (5 cohort, 11 cases and controls, 7 cross sectional observational studies) were included (Figure 1), combining to a total sample of 2692 participants. The characteristics of the included studies are summarized in Table 1. The studies were from Belgium [29], China [30, 31], Germany [32], India
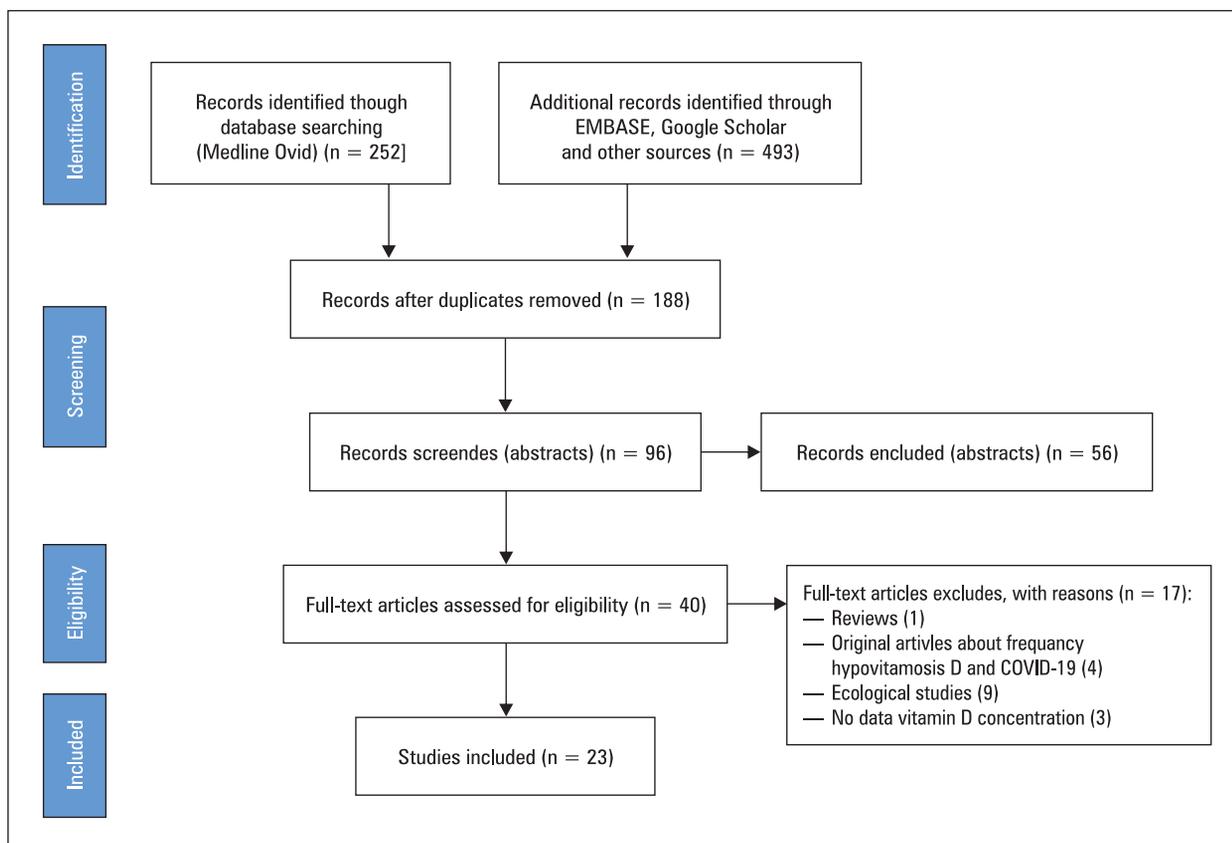
Figure 1. Flowchart of the included studies

[33], Iran [34, 35], Italy [36–38], Philippines [39], Spain [40, 41], Switzerland [42], South Korea [43], Turkey [44, 45], Russia [46], The Netherlands [47], UK [48, 49] and USA [50,51]. Overall, mean age was 60.8 (SD 15.9) years and 53.8% were men. The mean NOS score of the included studies was 8.1 (range: 7–9). The outcomes reported in the included papers are presented in Table 1.

As shown in Figure 2, the metaanalysis suggested that 25(OH)D < 50 nmol/L (< 20 ng/mL) was associated with an increased risk of severe disease (RR 2.00; 95% CI 1.47–2.71, 17 studies) and mortality (RR 2.45; 95% CI 1.24–4.84, 13 studies). Only 7/23 papers reported C-reactive protein values, all of which were> 10 mg/L [31, 38, 41, 44, 52–54].

Subgroup analyses were conducted to assess the effects of age, sex, and the alternative 25(OH)D cut-off value (< 30 nmol/L) separately (Table 2). We found that the severity risk seemed higher in people < 60 years of age (p = 0.040, 4 studies). The severity risk seemed to increase as the cut-off point for 25-hydroxyvitamin D concentration decreased (p = 0.025, 4 studies). Male sex (p < 0.001, 7 studies) also had higher risk of severity and/or mortality.

Two studies analyzed receiver operating characteristic (ROC) curve analyses to find the 25(OH)D cut-off point with the highest sensitivity and specificity for the prediction of severity and/or mortality. Abrishami *et al.* (2020) found the cut-off point of < 62.5 nmol/L (< 25 ng/mL) to have a sensitivity of 75% and a specificity 72%, for differentiating deceased and discharged patients [35]. Ye *et al.* (2020) observed that a cut-off point of 41.19 nmol/L had a sensitivity of 87% and a specificity of 70% for predicting illness severity [30].

Discussion

The main finding of the present paper is that according to the included observational studies, 25(OH)D deficiency (serum 25-hydroxyvitamin D concentration < 50 nmol/L) was associated with an increased risk of severe disease and mortality from SARS-CoV-2 infection. Our findings do not imply causation because they only summarize the conclusions of observational studies. For example, it is not possible to extrapolate that in acute patients with COVID-19 who have hypovitaminosis D, the immediate replacement of vitamin

Table 1. Characteristics of the 23 studies included in the metanalysis

Author	Country	Study design	Total sample	Sex male [%]	Mean age	Outcome	25(OH)D measurement method	Cut point 25-hydroxyvitamin D [nmol/L]	Comorbidities	C reactive protein [mg/L]	NOS
Carpagnano *et al.* 2020	Italy	CC	42	71	65	Mortality	Chemiluminescence immunoassay method	25	Hypertension (62%), cardiovascular disease (38%), chronic kidney disease (38%), diabetes type II (26%), malignancy (12%), COPD (12%).	Mean (SD): VD (50–25nmol/L) = 101 (79.93); VD (< 25 nmol/L) = 102 (79.98)	9
Panagiotou *et al.* 2020	UK	CS	134	29.7	68.5	Severity (admission to ICU)	NR	50	Hypertension (42%), diabetes (28%), malignancy (11%), respiratory (31%), cardiovascular disease (15%), kidney and liver diseases (14%)	NR	7
Alipio *et al.* 2020	Philippines	CS	212	NR	NR	Severity (mild/ordinary vs Severe/critical; CT scan chest y pO2)	NR	50	NR	NR	8
De Smet *et al.* 2020	Belgium	CC	186	58.6	69	Severity (Chest CT)	Elecsys® vitamin D total II (Roche, Switzerland)	50	Chronic lung disease (15%), coronary artery disease (59%), diabetes (14%)	NR	8
Lau *et al.* 2020	USA	CS	20	45	65.2	Severity (ICU vs floor)	UniCel Dxl 600 Access Immunoassay System (Beckman Coulter)	50	Hypertension (75%), diabetes (35%)	NR	8
Radujkovic *et al.* 2020	Germany	CC	185	51	60	Severity (Invasive mechanical ventilation)	immunoassay (ADVIA Centaur Vitamin D Total Assay)	30	Cardiovascular disease (31%), diabetes (10%), chronic kidney disease (4%), chronic lung disease (8%), malignancy (9%).	NR	9
Baktash *et al.* 2020	UK	C	70	60	81.3	Mortality and Severity (Ventilation requirement)	NR	30	Hypertension (57%), diabetes mellitus (43%), ischaemic heart disease (25%), chronic respiratory disease (22%), heart failure (20%).	Median: VD (≤ 30 nmol/L) = 191 (108–274); VD (> 30 nmol/L) = 155 (96–252) p = 0.32	9
Mardani *et al.* 2020	Iran	O	63	55.6	43.3	Mortality	ELISA method (Monobind, USA),	50 and 30	NR	NR	8
Pepkowitz *et al.* 2020	USA	CC	37	65.57	71.5	Severity (admission ICU)	NR	50	NR	NR	8

Table 1. cont. Characteristics of the 23 studies included in the metanalysis

Author	Country	Study design	Total sample	Sex male [%]	Mean age	Outcome	25(OH)D measurement method	Cut point 25-hydroxyvitamin D [nmol/L]	Comorbidities	C reactive protein [mg/L]	NOS
Macaya et al. 2020	Spain	CC	80	43.75	69	Severity (death, ICU admission or requirement of high flow oxygen)	chemiluminescent immunoassay, Abbott Diagnostics	50	Hypertension (63%), diabetes mellitus (40%), cardiac disease (24%), advanced chronic kidney disease (33%), chronic respiratory disease (15%)	NR	8
Hars et al. 2020	Switzerland	O	160	40.63	85.9	Mortality	NR	50	NR	NR	7
Karahan et al. 2020	Turkey	C	149	54.4	63.5	Mortality and severity (Chinese Clinical Guideline for classification)	NR	50	Hypertension (57%), coronary artery disease (22%), diabetes mellitus (41%), chronic kidney disease (20%), congestive heart failure (12%)	Mean (SD): surviving = 62.4 ± 71.2; deceased = 108.7 ± 78.3 (p < 0.001)	8
Ye et al. 2020	China	CC	60	37	43	Severity (National Health Commission of China classification)	NR	50	Diabetes (8%), hypertension (10%), renal failure (27%), COPD (2%).	NR	9
Yilmaz et al. 2020	Turkey	CC	40	47.5	8.48	Severity (experts' consensus statement)	Shimatzu device by high performance liquid chromatography method	50	NR	Median VD (< 50 nmol/L) = 1 (0.2–160); VD (≥ 50 noml/L) = 0.7 (0.2–10.8)	8
Im et al. 2020	South Korea	CC	50	42	52.2	Severity (Pneumonias with or without an oxygen supply, and death)	liquid chromatography–tandem mass spectrometry method	50	NR	NR	8
Hernández et al. 2020	Spain	CC	197	62.4	61	Mortality and Severity (mechanical ventilation)	automated electrochemiluminescence system	50	Hypertension (39%), diabetes (17%), cardiovascular disease (11%), COPD (8%), active cancer (4%), immunosuppression (8%)	Median overall = 56 (26.3–118.5) VD (< 50 nmol/L) = 61 (31–136); VD (≥ 50 nmol/L) = 32 (23–87); p = 0.064	9
Campi et al. 2020	Italy	C	103	68	66.12	Mortality and Severity (Admission to ICU)	LIASON 25-OH Vitamin D Total Assay	50	At least 1 comorbidity (61%), CV diseases (12%)	NR	9
Abrishami et al. 2020	Iran	CC	73	64.4	55.18	Mortality	Roche Diagnostics "Vitamin D Total" cobas e411immunoassay analyzer	50	Hypertension (25%), chronic kidney disease (22%), ischemic heart disease (18%), diabetes mellitus (15%), asthma/COPD (10%)	NR	8

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Table 1. cont. Characteristics of the 23 studies included in the metanalysis

Author	Country	Study design	Total sample	Sex male [%]	Mean age	Outcome	25(OH)D measurement method	Cut point25-hydroxyvitamin D [nmol/L]	Comorbidities	C reactive protein [mg/L]	NOS
Walk et al. 2020	The Netherlands	C	133	69	68.2	Severity (the need for invasive ventilation and/or death)	liquid chromatography-tandem mass spectrometry	50 and 25	Hypertension (36%), diabetes mellitus (22%), cardiac or cardiovascular disease (28%)	NR	8
Karonova et al. 2020	Russia	CS	80	53.8	53.2	Mortality	NR	50	Diabetes (15%), hypertension (46%),	NR	7
Luo et al. 2020	China	CS	335	44.2	56	Severity (respiratory failure requiring mechanical ventilation, shock, multiple organ dysfunction)	chemiluminescence immunoassay (DiaSorin, Inc.).	30	NR	Median: severe group = 64.5 (12.3–422); non–severe group = 12 (5.5–30)	8
Cereda et al. 2020	Italy	C	129	54.3	77	Mortality and Severity (Severe pneumonia, ICU admission)	chemiluminescence immunoassay	50	COPD (13%), diabetes (31%), hypertension (70%), ischemic heart disease, (41%), cancer (21%), chronic kidney disease (19%)	Median: overall = 10.38 (5.19–16.46); VD (≥ 50 nmol/L) = 6.81 (4.0–14.39); VD (< 50 nmol/L) = 11.15 (5.56–17.14)	9
Jain et al. 2020	India	CC	154	66.66	54.42	Mortality and Severity (requiring ICU admission)	automated immunoassays -Architecti1000sr Make 2015	50	NR	NR	9

25(OH)D — 25-Hydroxyvitamin D; C — cohort; CC — case control; CI — confidence interval; COPD — *chronic obstructive pulmonary disease*; CS — cross-sectional; CV — cardiovascular disease; ICU — *intensive care unit*; NOS — Newcastle-Ottawa Scale; NR — not reported; SD — standard deviation; VD — vitamin D

A

B
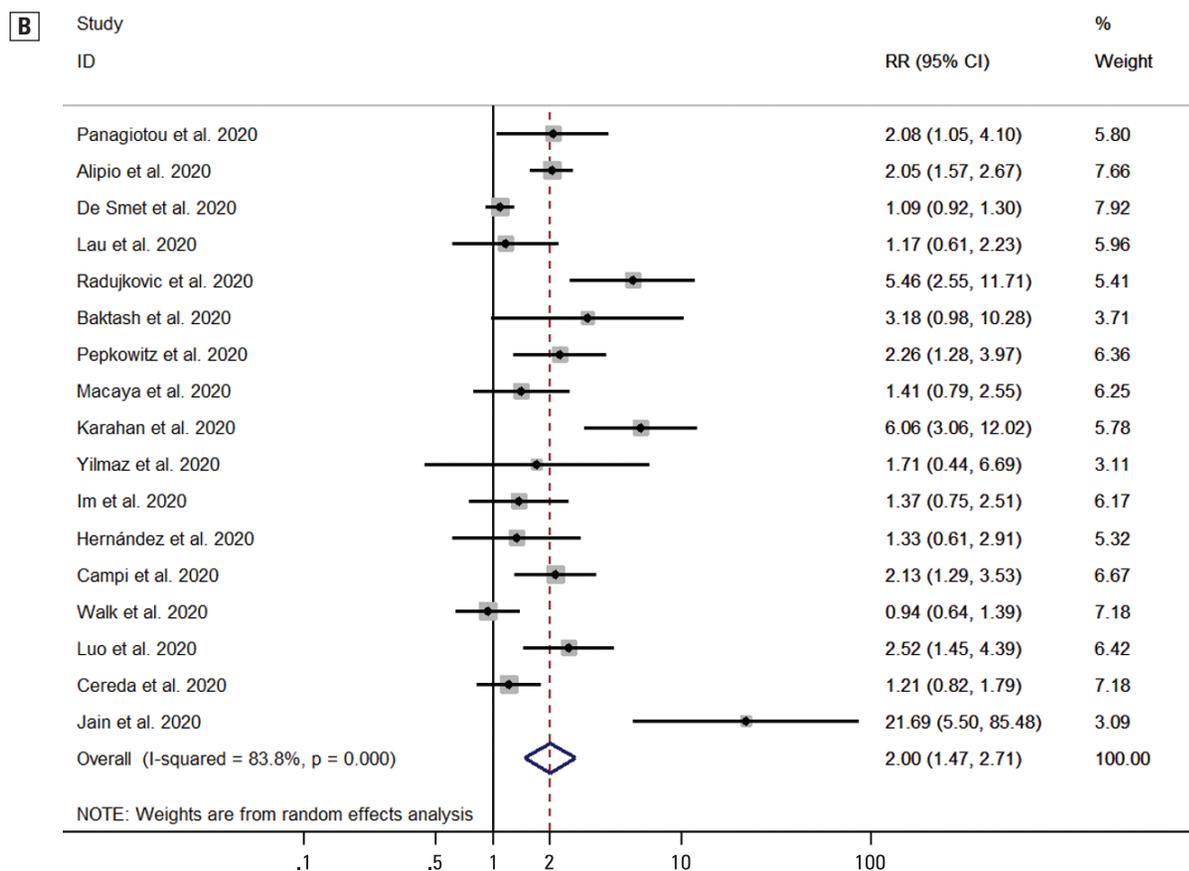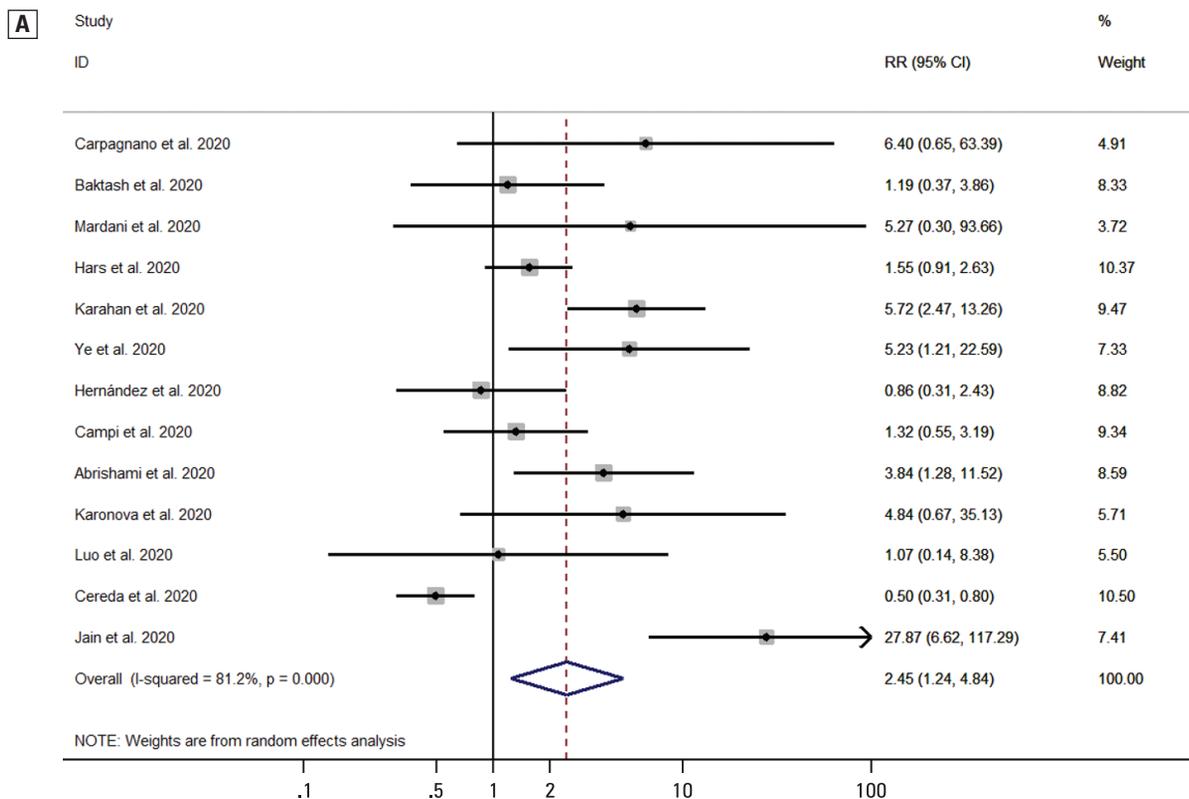
Figure 2. A. Forest plot of the metaanalysis of the association between mortality from SARS-CoV-2 infection and 25-hydroxyvitamin D concentration (< 50 nmol/L); **B.** Forest plot of the metaanalysis of the association between severity of SARS-CoV-2 infection and 25-hydroxyvitamin D concentration (< 50 nmol/L). Analysis model: random effects. CI — confidence interval; RR — relative risk

Table 2. Association between severity of SARS-CoV-2 infection and serum 25-hydroxyvitamin D concentration: summary of subgroup analyses

Subgroup	Studies (n)	RR (95% CI)	P
Severity			
Cut point 25-hydroxyvitamin D concentration [nmol/L]			
< 30	4	2.45 (1.12–5.37)	0.025
< 50	14	1.79 (1.30–2.46)	< 0.001
Mortality			
Cut point 25-hydroxyvitamin D concentration [nmol/L]			
< 30	4	1.85 (0.79–4.37)	0.160
< 50	10	2.67 (1.20–5.94)	0.016
Severity			
Mean age [years]*			
< 60	4	3.05 (1.05–8.86)	0.040
≥ 60	12	1.83 (1.29–2.58)	0.001
Mortality			
Mean age [years]*			
< 60	6	5.47 (2.31–12.95)	< 0.001
≥ 60	7	1.48 (0.71–3.09)	0.298
Gender (severity/mortality)			
Male	7	2.62 (1.77–3.87)	< 0.001
Female	3	1.28 (0.49–3.32)	0.613

*One study (Alipio *et al.* 2020) did not report mean age data

D could improve the prognosis [55]. Another important finding is that there is the possibility that the low concentrations of 25(OH)D reported are an epiphenomenon of the inflammatory process associated with severe SARS-CoV-2 infection, although only 7/23 studies reported C-reactive protein values, all of which were > 10 mg/L.

We found that the severity risk seemed higher in people younger than 60 years of age; however, this finding related to only 4 studies and had a marginal P value. Our conclusion that the severity risk seemed to increase as the cut-off point for 25(OH)D concentration decreased adds biological plausibility to the findings. In addition, higher male risk profile, as clinically expected, is given the known epidemiology of COVID-19 infection [56]. It has to be noted that a tradeoff between our two outcomes exists, as very severe cases who died were counted as mortality and not severity for the purpose of association with 25(OH)D status.

The findings of the present study can be compared with similar meta-analytical studies and others published prior to the pandemic, on risk associated with severity of acute respiratory infections. Ghasemian *et al.* (2020) and Chen *et al.* (2020) published two meta-analytic studies where they found an association between 25(OH)D deficiency and insufficiency with mortality from SARS-CoV-2 infection; however, ecological studies have the limitation that the reported

mortality could have varied in each of the countries, as the pandemic evolved [12, 57]. Another meta-analytic study (5 articles included) found a mean 25(OH)D concentration of 18 ng/mL in severe COVID-19 cases (95% CI: 1–35) and 26 ng/mL in non-severe cases (95% CI: 23.9–28.7) [11]. Pereira *et al.* (2020) noted that a 25(OH)D concentration of < 50 nmol/L was associated with an increased risk of hospitalization (3 studies) and mortality from COVID-19 (5 studies) [58]. In a systematic study (7 papers), Munshi *et al.* (2020) found that patients with poor prognosis had significantly lower serum concentrations of 25(OH)D compared to those with good prognosis, representing an adjusted standardized mean difference of -5.12 (95% Cl = -9.14 to -1.10, p = 0.012) [59]. Pham *et al.* (2020) published in 2019 a meta-analytic study where they found that a 25(OH)D concentration of <50 nmol/L was inversely associated with risk and severity of acute respiratory tract infection [60]. Zhou *et al.* published a meta-analytical study in 2019 where they documented an increased risk of community-acquired pneumonia in patients with 25(OH)D deficiency (< 50 nmol/L) [61]. Martineau *et al.* published in 2017 a meta-analytic study with 25 randomized clinical trials, finding that vitamin D supplementation reduced the frequency of acute respiratory infection; however, the benefit was greater in those who were receiving daily or weekly 25(OH)D, and protective effects were

stronger in those with baseline 25-hydroxyvitamin D concentrations < 25 nmol/L; and the benefit was not significant in those who received bolus doses [62].

The possible mechanisms that could explain the inverse relationship between the concentration of 25(OH)D and the frequency of presentation of severe forms of SARS, would be its angiotensin-converting enzyme 2 (ACE2) down-regulation, regulation of IL-6 and prevention of hypocalcemia. Mok *et al.* in *in vitro* studies with Vero E6 cells (African green monkey kidney cells) and hNECs (human nasal epithelial cells) found that calcitriol, the active form of vitamin D, has potent activity against SARS-CoV-2; the hypothesis of the possible mechanism of antiviral action would be in the post-entry phase of viral replication [63]. The mechanism of entry into human cells of SARS-CoV-2 is through ACE2, which is part of the renin-angiotensin system (RAS). It has been postulated that 25(OH)D would have a possible mechanism of protection against acute lung injury (ALI) / acute respiratory distress syndrome (ARDS), through a negative endocrine RAS modulator which inhibits renin expression and generation. This mechanism would be carried out by its inducing action of ACE2 / Ang- (1-7) / MasR axis activity and inhibits renin and the ACE / Ang II / AT1R axis, thereby increasing expression and concentration of ACE2, MasR and Ang-(1–7) [64]. Recently, high levels of ACE have been found in patients with severe COVID-19 with low 25(OH)D concentrations [34]; these findings are compatible with the harmful effect of high levels of ACE in Ang II generation and promote the detrimental effects of the AT1R classical axis (inducing vasoconstriction, inflammation, oxidative stress, and cell proliferation).

Recently, a systematic review found a correlation between premorbid levels of IL-6 and mortality from COVID-19; additionally, the reviewed studies reported concomitant decrease in 25(OH)D concentrations [65]. On the other hand, one of the known actions of concentrations is to modulate the activity of IL-6; therefore, potentially, the control of hypovitaminosis D could reduce the risk of presentation of severe forms of COVID-19 [65]. McGregor *et al.* (2020) found that CD4+ T cells in the bronchoalveolar lavage fluid (BALF) of patients with COVID-19 are Th1-skewed and that VDR is among the top regulators of genes induced by SARS-CoV-2 [66]. Part of the pathophysiology associated with cytokine storm is due to suppression of Th1 cooperative responses, which favors the Th2 type with excessive release of tumor necrosis alpha (TNF-alpha) and interleukins. 25(OH)D causes epigenetic re-modelling, induces and recruits a set of TFs (transcription factor), including STAT3 (signal transducer and activator of transcription 3), c-JUN and BACH2 (BTB Domain And CNC Homolog 2) that collectively repress Th1 and Th17 programs and induces IL-10 via IL-6-STAT3 signaling [66]. Recently, a significant increase in inflammation markers (IL-6, TNFα and serum ferritin levels) has been reported in critically ill COVID-19 patients deficient in 25(OH)D (< 50 nmol/L) [33]. Medical College the current study was undertaken as continuous prospective observational study of 6 weeks. Participants were COVID-19 patients of age group 30–60 years admitted during the study period of 6 weeks. Study included either asymptomatic COVID-19 patients (Group A. Sun *et al.* (2020) found that 74% of patients admitted for severe COVID-19 had hypocalcemia and low concentrations of 25(OH)D and hypoproteinemia, and for these reasons they propose hypocalcemia as a biomarker of clinical severity and prognosis [67]. Actually, the studies on the relationship between the concentration of 25(OH)D and SARS-CoV-2 infection are showing that there is a disturbed parathyroid-vitamin-D axis, which would last up to 8 weeks after the discharge of a patient with SARS-CoV-2 infection with hypovitaminosis D [68]we aimed to investigate associations of VITD status to disease presentation within the CovILD registry. This prospective, multicenter, observational study on long-term sequelae includes patients with COVID-19 after hospitalization or outpatients with persistent symptoms. Eight weeks after PCR confirmed diagnosis, a detailed questionnaire, a clinical examination, and laboratory testing, including VITD status, were evaluated. Furthermore, available laboratory specimens close to hospital admission were used to retrospectively analyze 25-hydroxyvitamin D levels at disease onset. A total of 109 patients were included in the analysis (60% males, 40% females.

It is important to discuss whether low concentrations of 25(OH)D in patients with severe COVID-19 infection is a cause or consequence of severe COVID-19 infection, for three main reasons: absence of baseline 25(OH)D measurement before infection, prior knowledge that the concentration of 25(OH)D decreases as a consequence of an inflammatory process, and most of the studies described on this association did not report the concentration of C-reactive protein (CRP) together with that of 25(OH)D. Before the

COVID-19 pandemic, it was known that 25(OH)D concentration decreases as a consequence of an inflammatory state, that is, it is considered a negative acute phase reactant [69–71] . Additionally, it has been described that this decrease in 25(OH)D during these inflammatory processes can persist for up to 3 months [70]. It has been recommended that a reliable clinical interpretation of the 25(OH)D concentration can be made only if the C-reactive protein (CRP) is < 10 mg/L [71], because it has been described that 25(OH)D concentrations are inversely correlated with CRP concentrations [72]. The mechanism involved in the decrease in serum 25(OH)D during an acute inflammatory state would be associated with the decrease in vitamin D binding protein (VDBP) and increased urinary loss of VDBP that occurs in a systemic inflammatory response (SIR) [69, 70]. In the present paper, only 7/23 studies reported the concentration of C-reactive protein (CRP), and in all of which it was > 10 mg / L. [31, 38, 41, 44, 52–54], therefore, there would be the possibility that one of the causes of the reported decrease in 25(OH)D is an epiphenomenon of the inflammatory process of SARS-CoV-2. Regardless of whether it is its cause or effect, measurement of 25(OH)D concentration should be considered a marker of inflammation, in addition to markers for inflammation and tissue damage in prognostic models for COVID-19 [29].

The present metaanalysis has its limitations, the main one being that it is based on observational studies and not on interventional studies such as randomized controlled clinical trials. Additionally, the studies used different methodologies to assess 25(OH)D status (e.g. LC-MS/MS, ELISA). It is important to emphasize that no causality can be inferred from our results. However, evidence from observational studies is better than that based on the ecological ones. A limitation is that many studies did not report if the 25(OH)D concentration was measured before or during COVID-19 infection, but in the future there may be scope for analyzing vitamin D in hair to solve this issue [73]the number of requests for vitamin D measurement keeps dramatically increasing year-on-year. Currently, the recognised best marker of vitamin D status is the concentration of the 25-hydroxyvitamin D (25(OH. Indeed, it is important to investigate whether SARS-CoV-2 infection, especially the severe forms, cause a decrease in the concentration of 25(OH)D, in patients who previously had them at normal levels. On the other hand, there is a possible 'healthy user effect' confounder, that is, higher concentrations of 25(OH)D could be seen in people who eat well, have healthy lifestyles and spend more time outdoors exposed to sunlight, which in turn makes them more generally resilient in the face of any acute illness [74]. In other words, 25(OH)D status could be epiphenomenally associated with SARS-CoV-2 outcomes.

In conclusion, at present the evidence available supports the hypothesis of increased SARS-CoV-2 risk of infection severity and mortality in patients with 25(OH)D deficiency (< 50 nmol/L). Our findings do not imply causality but support further research in this area, including the conduct of robustly designed randomized controlled trials. On the other hand, new studies should be designed to determine if decreased 25(OH)D is an epiphenomenon or consequence of the inflammatory process associated with severe forms of SARS-CoV-2. Meanwhile, the concentration of 25(OH)D could be considered as a negative acute phase reactant and a poor prognosis in COVID-19 infection.

Conflict of interest

None declared.

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