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Meta-analysis

The link between COVID-19 and VItamin D (VIVID): A systematic review and meta-analysis

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ABSTRACT

Background: Disease severity and mortality rates due to COVID-19 infection are greater in the elderly and chronically ill patients, populations at high risk for vitamin D deficiency. Vitamin D plays an important role in immune function and inflammation. This systematic review and meta-analysis assesses the impact of vitamin D status and supplementation on COVID-19 related mortality and health outcomes.

Methods: We searched four databases until December 18th 2020, and trial registries until January 20th 2021. Two reviewers screened the studies, collected data, assessed the risk of bias, and graded the evidence for each outcome across studies, independently and in duplicate. Pre-specified outcomes of interest were mortality, ICU admission, invasive and non-invasive ventilation, hospitalization, time of hospital stay, disease severity and SARS-CoV-2 positivity. We only included data from peer-reviewed articles in our primary analyses.

Results: We identified 31 peer-reviewed observational studies. In our primary analysis, there was a positive trend between serum 25(OH)D level <20 ng/ml and an increased risk of mortality, ICU admission, invasive ventilation, non-invasive ventilation or SARS-CoV-2 positivity. However, these associations were not statistically significant. Mean 25(OH)D levels was 5.9 ng/ml (95% CI [−9.5, −2.3]) significantly lower in COVID-19 positive, compared to negative patients. The certainty of the evidence was very low. We identified 32 clinical trial protocols, but only three have published results to-date. The trials administer vitamin D doses of 357 to 60,000 IU/day, from one week to 12 months. Eight megatrials investigate the efficacy of vitamin D in outpatient populations. A pilot trial revealed a significant decrease in ICU admission with calcifediol, compared to placebo (OR = 0.003), but the certainty of the evidence was unclear. Another small trial showed that supplementation with cholecalciferol, 60,000 IU/day, decreased fibrinogen levels, but did not have an effect on D-dimer, procalcitonin and CRP levels, compared to placebo. The third trial did not find any effect of vitamin D supplementation on COVID-19 related health outcomes.

Conclusion: While the available evidence to-date, from largely poor-quality observational studies, may be viewed as showing a trend for an association between low serum 25(OH)D levels and COVID-19 related health outcomes, this relationship was not found to be statistically significant. Calcifediol supplementation may have a protective effect on COVID-19 related ICU admissions. The current use of high doses of vitamin D in COVID-19 patients is not based on solid evidence. It awaits results from ongoing trials to determine the efficacy, desirable doses, and safety, of vitamin D supplementation to prevent and treat COVID-19 related health outcomes.

1. Introduction

The Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), causing COVID-19, was first detected in Wuhan China in December 2019 [1]. Due to its high transmission, the virus has quickly spread to devour all continents, and was declared a global pandemic by the World Health Organization (WHO) in March 11, 2020 [1]. The COVID-19 pandemic is the third outbreak caused by the β-coronavirus family, following Severe Acute Respiratory Syndrome (SARS) in 2002 and Middle East Respiratory Syndrome (MERS) infections in 2012 [2]. However, in contrast to previous outbreaks, COVID-19 has higher transmission rates, and thus incurs more challenges in terms of prevention and treatment [2]. Elderly frail patients are the most susceptible to adverse outcomes from COVID-19, including mortality and other complications [3]. Their risk also increases in the presence of multiple comorbidities

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such as diabetes, cardiovascular disease, respiratory disease, malignancy and obesity [3–6]. Full recovery of elderly patients who survive COVID-19 may take weeks to months. This leads to a fast reduction in muscle mass due to immobilization following hospital discharge, which might lead to an increased risk of frailty, falls, fractures and mortality [7]. These risks are higher in patients who lose weight from COVID-19 associated acute systemic inflammation. The inflammatory process influences several metabolic pathways and leads to weight loss as large as that experienced by cachectic cancer patients [8,9].

Furthermore, this susceptible elderly population is likely to suffer from vitamin D deficiency because of the impaired ability to synthetized vitamin D by the skin, limited sun exposure and malabsorption [7,10,11]. In addition, obesity is also highly associated with vitamin D deficiency due to low vitamin D intake, poor dietary habits and alterations in enzymes responsible for vitamin D supplementation [12]. Several reports have suggested a possible association between vitamin D deficiency (25(OH)D levels <20 ng/ml) and COVID-19 susceptibility [13–15]. Although vitamin D is well known for its action on calcium and bone metabolism, extra-skeletal actions have also been described [16]. Particularly, vitamin D plays a role in cytokine release and inflammation, modulation of innate and adaptive immunity, and may decrease the risk of infections via several mechanisms [17–19]. Vitamin D supplementation decreased the risk of acute respiratory infections including influenza infection by 12% overall. Subgroup analyses revealed that daily or weekly doses of vitamin D decrease the risk of infections by 19% compared to placebo, while bolus regimens do not [20]. The same investigators recently confirmed the above results; vitamin D supplementation decreased the risk of respiratory infections by 11%, and doses of 400–1000 IU/day for at least 12 months were the most protective [21]. The effect of vitamin D on other infections is less clear.

Given the above information, experts suggested the possible protective role of vitamin D in the prevention and treatment of COVID-19 infection [7,13,22]. Experts have also published guidance on vitamin D supplementation for its prevention [23–25]. However, such guidance was not based on a systematic review, nor a rigorous synthesis of the evidence. The NICE guideline recommended vitamin D supplementation at a daily dose of 400 IU during the COVID-19 pandemic [26]. In addition, a joint statement, issued from the Endocrine Society, American Society for Bone and Mineral Research (ASBMR), American Association of Clinical Endocrinologists (AACE), European Calcified Tissue Society (ECTS) and National Osteoporosis Foundation (NOF), recommended a daily dose of 400–1000 IU vitamin D in the COVID-19 pandemic, especially during home isolation for bone protection [27]. These societies do not however recommend supplementation for COVID-19 prevention.

We therefore implemented a systematic review and meta-analysis of observational studies assessing the relationship between serum 25 (OH)D levels and COVID-19 related mortality and other health outcomes, to fill this major knowledge gap. We also systematically collected information on vitamin D randomized trials, including on-going ones, to evaluate the body of the upcoming evidence on the effects of vitamin D supplementation on COVID-19 related outcomes. We hypothesized that vitamin D deficiency is associated with an increased risk of COVID-1 9 related health outcomes and that vitamin D supplementation would decrease these risks.

2. Methods

The protocol of this systematic review and meta-analysis is available online on PROSPERO; registration number CRD42020203960.

2.1. Eligibility criteria

The population of interest consists of adult patients with SARS, MERS or COVID-19 infection. The exposure is vitamin D status in observational studies, and vitamin D in any form and dose, as the intervention in clinical trials.

2.2. Information sources and search strategy

2.2.1. Literature search

We conducted a comprehensive search using four databases: Medline (OVID), Embase.com, CINAHL (EBSCO), and Cochrane until December 18th, 2020, with no limit on language (Appendix A). We used MESH terms and keywords relevant to vitamin D and COVID-19. We planned to include SARS and MERS viruses to detect any indirect evidence in case of scarcity of studies on COVID-19. We have limited the terms related to the Coronaviridae family to the year of the first outbreak (2002–2020). The search strategy was reviewed and verified by our head medical librarian (OEZ). We also added any publications brought to our attention by experts and senior authors, or by manual review of references in the included papers. We searched the grey literature including MedRxiv, the Endocrine Society, ASBMR, Centers for Disease Control and Prevention, International Osteoporosis Foundation, and WHO websites [28,29]. We translated all abstracts written in a language other than English to assess their eligibility.

2.2.2. Trial registries

We searched ClinicalTrials.gov and the WHO primary trial registries, namely the EU Clinical Trials Register (EU-CTR), Australian New Zealand Clinical Trials Registry (ANZCTR), and Iranian Registry of Clinical Trials (IRCT), up until January 20th 2021, for ongoing trials on vitamin D and COVID-19 [30].

2.3. Outcomes

Our primary outcome was mortality rate from COVID-19 infection. The secondary outcomes included SARS-CoV-2 positivity, disease severity, need for hospitalization, hospital stay duration, need for ICU admission, ICU stay duration, need for invasive or non-invasive ventilation, time on respirators, time to symptomatic recovery, time to seronegative conversion, and risk of positive seroconversion of family members. In addition, we evaluated the risk of its complications: acute respiratory distress syndrome, acute respiratory failure, pneumonia, cytokine storm, organ failure, septic shock, disseminated intravascular coagulation, neurological complications, and rhabdomyolysis.

2.4. Study selection and evidence abstraction

All relevant tasks listed below were implemented by 2 independent reviewers (AB, MB), and disagreements were resolved through discussions and/or with input from a content expert (MC, GEHF). Three reviewers (AB, MB, MR) screened ClinicalTrials.gov and WHO primary trial registries for potential ongoing randomized control trials independently.

2.4.1. Study selection

We screened titles and abstracts of all identified records using a priori developed screening sheet. We then screened full texts of potentially eligible articles and recorded the reasons for exclusion.

2.4.2. Data collection and abstraction

We extracted data using standardized data abstraction forms. In case of missing data needed to conduct our meta-analyses, we contacted the authors, with a reminder 2 weeks later. Non-published data obtained from authors by communication are mentioned in the Results section below, as applicable, with permission, and authors who responded are listed in Acknowledgments section.

2.5. Risk of bias assessment

We assessed the quality of all included observational studies by outcomes. We used the New Castle-Ottawa quality scale to evaluate three main domains: selection, comparability, and outcome/exposure [31]. We assessed the quality of the included clinical trials using the Cochrane

Risk of bias tool, version 1 [32]. We also used the Grading of Recommendations Assessment, Development, and Evaluation working group methodology (GRADE) to examine the quality of evidence from included studies for each outcome in our primary analysis [33].

2.6. Data synthesis and analysis

As a primary analysis, we conducted a random-effect meta-analysis, when at least two peer-reviewed studies were available for each predefined outcome using RevMan 5.4. For categorical outcomes, we calculated the relative risk (RR) and its 95% CI in patients with low serum 25(OH)D levels (<20 ng/ml as per the IOM), as compared to those with desirable levels, based on the number of events in each arm. Similarly, we calculated the mean difference (MD) and 95% CI of serum 25(OH)D level in COVID-19 positive compared to COVID-19 negative patients. We assumed that serum 25(OH)D levels are normally distributed due to the large sample size. We therefore considered the mean as the median, and calculated the standard deviation by dividing the interquartile range by 1.35, when not provided [34]. We assessed statistical heterogeneity between studies using the I^2, with significance at p value ≤0.05. We conducted sensitivity analyses using a higher 25(OH)D cutoff (25(OH)D < 30 ng/ml), when such data was available. We did not conduct any subgroup analyses or publication bias assessment because of the limited number of available studies for every outcome of interest.

3. Results

The search strategy identified 6300 citations. Following duplicate removal, we screened a total of 5378 citations, of which 223 articles were potentially eligible. We also screened 19 citations from the grey literature and three articles based on the opinion of experts and senior authors, of which 16 were potentially eligible. From the total of 239 articles we retained 34 articles (Fig. 1), and excluded 205 articles for the reasons outlined in Appendix B. We extracted data from 31 peer-reviewed observational studies, and 3 RCTs, that reported on vitamin D status and COVID-19 health related outcomes. We did not identify any eligible data on MERS or SARS.

3.1. Observational studies

3.1.1. Description of included studies

Table 1 describes the characteristics of the 31 included observational studies ordered by outcome severity [35–65]. These studies reported on mortality from COVID-19 (n = 20), ICU admission (n = 5), length of ICU stay (n = 1), invasive ventilation (n = 7), non-invasive ventilation (n = 4), hospitalization (n = 3), length of hospital stay (n = 5), disease severity (n = 12), pneumonia (n = 1), multi-organ damage (n = 1), acute kidney injury (n = 1), ARDS (n = 5), SARS-CoV-2 positivity (n = 4), and 25(OH)D levels (n = 7). The included studies were conducted in the United States (n = 3), Europe (n = 13), Asia (n = 8) and the Middle East (n = 7). COVID-19 patients were assessed in the inpatient setting in 24 studies [35–38,40–55,57–59,61], in the outpatient setting in 4 studies [62–65] and in both the in and outpatient setting in 3 studies [39,56,60]. The mean age of patients ranged between 42 and 81 years. The percentage of females ranged between 20% and 67%. Participants had multiple comorbidities, most commonly hypertension (25–80%), cardiovascular disease (11–62%) and diabetes (10–44%). Timing for blood withdrawal of serum 25(OH)D was not mentioned in 12 studies. It occurred 10 years prior to study conduct in 2 studies, within 1 year of study conduct in 1 study, within 6 months or 12 weeks prior to admission in 1 study each, or at the time of testing for COVID-19 status or admission to the hospital in COVID-19 positive cases in 10 studies. It was done within 48 h, 7 days of hospital admission, or 7 weeks, or three months, post-discharge, in one study for each time frame. Mean serum 25(OH)D levels ranged between 11 and 35 ng/ml, BMI was between 23.5 and 32 kg/m^2, and the proportion of subjects with a serum 25(OH)D < 20 ng/ml varied between 13 and 82%. Twenty-three studies specified the type of 25(OH)D assay used, and only 3 used liquid chromatography mass spectroscopy. Fourteen studies were cross-sectional [35–37,40,43–46,50–53,59,62], four were case-control [56,60,61,63], eleven were cohort [39,41,42,47–49,54,55,57,64,65], and two were a combination of cross-sectional and case-control designs [38,58]. Appendix C.1 provides the detailed assessment for each of the risk-of-bias domains.

3.1.2. Association of low serum 25(OH)D with COVID-19 related health outcomes

3.1.2.1. Mortality. Twenty studies reported on the association between vitamin D status and mortality [35–54] (Table 1). Of these, 13 were cross sectional studies [35–38,40,43–46,50–53] while 7 were cohort studies [39,41,42,47–49,54]. The sample size ranged from 21 to 984 individuals/study, mean age ranged between 42 and 88 years, and the proportion of female participants varied between 20 and 58%.

Six studies reported data on different 25(OH)D cutoffs (10, 12, 15.2, 25 and 30 ng/ml) [37,40,41,46,48,54]. Of these, five reported a significant increased risk of mortality in vitamin D deficient COVID-19 patients [40,41,46,48,54]. Six studies did not have numerical data on mortality by vitamin D status [39,45,49–52]. The study of Maghbooli et al. demonstrated a two folds increase in mortality risk in patients with 25(OH)D levels <30 ng/ml. However, we did not include it in the analysis because of the discrepancy between reported results in the text and figures, unavailable original data despite contacting the authors, and the subsequent Editors' statement post-publication [35,66]. Seven studies identified low vitamin D levels as 25(OH)D levels <20 ng/ml and were therefore eligible for the primary analysis [36,38,42–44,47,53]. The individual results of these seven studies are summarized in Table 2.

Combining data from these seven studies (N = 945) revealed a trend between low serum 25(OH)D levels and the risk of mortality (RR = 2.1, 95% CI [0.9–4.8]; I^2 = 76%) (Fig. 2.A). Serum 25(OH)D levels were measured on admission in three studies [36,43,53], within 48 h of admission in one study [42], and within 7 days in one study [38]. The certainty of evidence for this outcome was very low because all studies assessing mortality from COVID-19 were observational of poor quality, the results were inconsistent and imprecise, as shown by the high heterogeneity (I^2 = 76%) and the wide confidence interval (Table 3, Appendix C.1).

Three studies had available data for mortality using a higher serum 25(OH)D cutoff (30 ng/ml) [36,44,54]. Combining their data (N = 373), showed a significant increased risk of mortality in COVID-19 patients with 25(OH)D < 30 ng/ml (RR = 3.1, 95% CI [1.4–6.8]; I^2 = 0%) (Appendix D.1) [36,44,54].

3.1.2.2. ICU admission. Five studies assessed the risk of ICU admission in hospitalized COVID-19 patients with low 25(OH)D levels [35,42,43,47,55] (Table 1). Two studies consisted of cross-sectional designs [35,43], while three were cohorts [42,47,55]. The sample size ranged from 43 to 235 individuals. Mean age was between 46 and 74 years, and the proportion of female participants varied between 38% and 46%.

We included three studies in our main analysis [42,43,47]. Table 2 provides a summary of the results of these individual studies. Pooling data from these studies (N = 480) revealed a trend for an increased risk of ICU admission in COVID-19 patients with 25(OH)D levels<20 ng/ml (RR = 4.9, 95% CI [0.5–44.3], I^2 = 85%) (Fig. 2.B). 25 (OH)D levels were measured during admission in two studies [42,43] and the timing of measurements was not mentioned in the third study [47]. The certainty of evidence of this outcome was very low because all studies were observational, of poor quality, and the results were both very inconsistent and imprecise (Table 3, Appendix C.1).

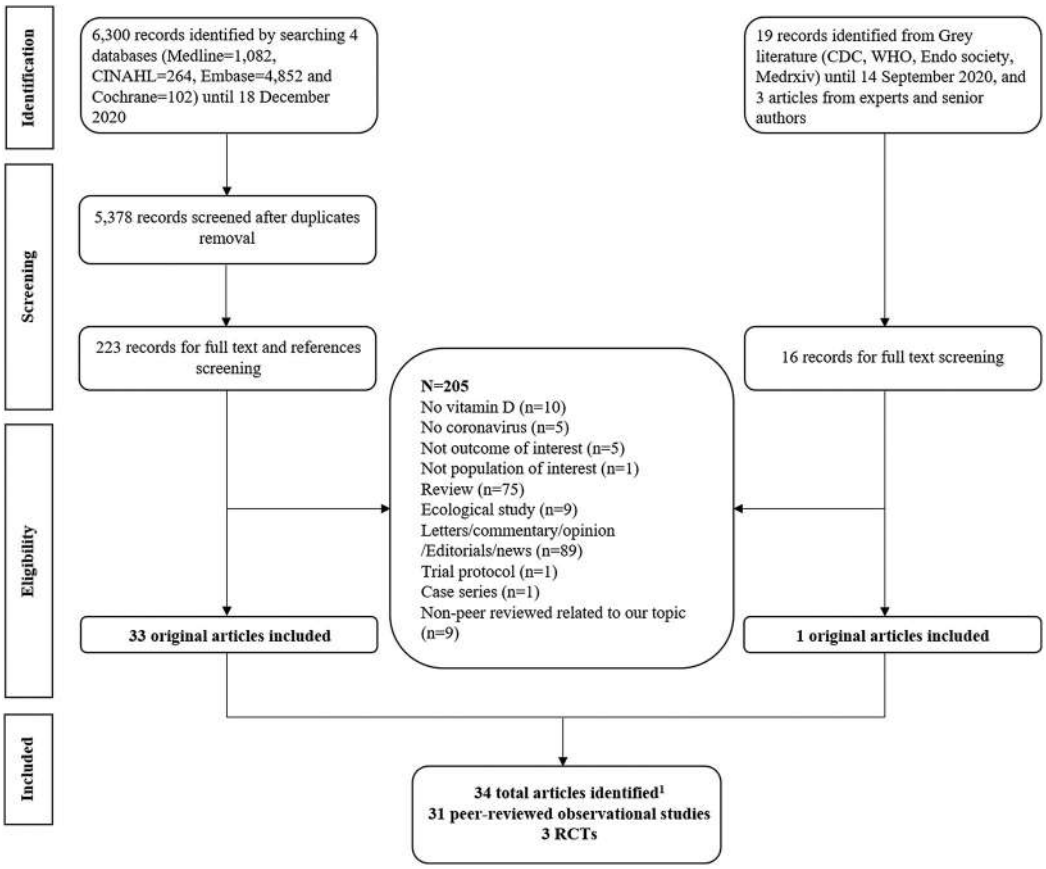

Fig. 1. Flow diagram of articles related to coronaviruses and vitamin D. [1] We did not find any articles related to SARS or MERS and Vitamin D.

One study showed no significant association between 25(OH)D levels <30 ng/ml and the risk of ICU admission (p = 0.3) [35].

Tan et al. conducted a prospective cohort study and showed that there was a decreased risk of ICU admission in 17 COVID-19 patients, who received vitamin D3 at a dose of 1000 IU per day, magnesium and B complex (DMB) for up to 14 days, as compared to 26 COVID-19 patients who received none (unadjusted OR = 0.13, 95% CI [0.03–0.6]) [55].

3.1.2.3. Invasive ventilation. Seven studies assessed the risk of invasive ventilation in hospitalized COVID-19 patients by vitamin D status [35,38,39,43,48,52,54]. Three were cohort studies [39,48,54], and four were of cross-sectional design [35,38,43,52]. The sample size ranged between 30 and 984. The median age varied between 58 and 76 years, and the proportion of female participants was between 20 and 58% (Table 1).

Three studies used different 25(OH)D levels cutoffs (12, 15.2 and 30 ng/ml) [35,39,48]. Only one of the three reported a significant increased risk of invasive mechanical ventilation in COVID-19 patients with 25 (OH)D < 12 ng/ml [39]. Two studies did not report numerical data for invasive mechanical ventilation by 25(OH)D level status [52,54]. Pooling data from the remaining two studies [38,43], showed a trend for an increase in invasive ventilation requirement in patients with 25(OH)D levels <20 ng/ml (RR = 1.3, 95% CI [0.6–2.8]; I^2 = 0%) (Fig. 2.C). Serum 25(OH)D levels were measured on admission [43], and within 7 days of admission [38]. The certainty of evidence of this outcome was very low because both studies were observational of poor quality, and the results were imprecise (Table 3, Appendix C.1).

3.1.2.4. Non-invasive ventilation. Four studies assessed the risk of non-invasive ventilation, using high flow oxygen masks or nasal cannula, in hospitalized COVID-19 patients by vitamin D status [37–39,43] (Table 1). Three were cross-sectional studies [37,38,43] and one was a cohort design [39]. The sample size ranged between 50 and 197. The mean age varied between 58 and 81 years, and the proportion of women was between 38 and 58% (Table 1). Two studies reported a significant increased risk of non-invasive ventilation in COVID-19 patients with vitamin D 25(OH)D level < 12 ng/ml. We excluded them from our primary analysis due to the lower 25(OH)D cutoff [37,39]. Pooling data from the remaining two studies [38,43], showed no association between non-invasive ventilation requirement and 25(OH)D levels <20 ng/ml (RR = 1.1, 95% CI [0.3–3.8]; I^2 = 23%) (Fig. 2.D). The certainty of evidence of this outcome was very low due to the poor quality of both observational studies and the imprecise results (Table 3, Appendix C.1).

3.1.2.5. Hospitalization. Three studies assessed the risk of hospitalization from COVID-19 disease in patients with low 25(OH)D levels [39,56,57]. One was a case-control study [56] and two were cohort studies [39,57]. The sample size ranged between 80 and 7807. The median age was 60 years in 1 study [39], and the percentage of women varied between 49 and 60%. We did not conduct a meta-analysis for this outcome because of heterogeneity in the cutoffs used for serum 25(OH)D levels. Radujkovic et al. showed a significantly higher proportion of patients with 25(OH)D levels <12 ng/ml in hospitalized patients compared to the outpatients (p = 0.004) [39]. Merzon et al. revealed a significant association between low 25(OH)D levels and increased risk of hospitalization due to COVID-19 (OR = 2.1, 95% CI [1.01–4.3]). However, this association was not significant in adjusted analysis (OR = 1.95, 95% CI [0.98–4.86]) [56]. Macaya et al. showed that COVID-19 patients with serum 25(OH)D levels <20 ng/ml were more likely to be admitted to the hospital as compared to those with desirable vitamin D status, but this difference was not statistically significant (p = 0.051) [57].

Table 1
Characteristics of the included observational studies.

Author Country Year [reference]	Study design	Sampling method Period	Sample size	Age Mean ± SD or median [IQR] (years)	% female	BMI Mean ± SD or median [IQR] (kg/m^2)	25(OH)D levels cutoff (ng/ml)	25OHD assay Timing of 25(OH)D measurement	Outcomes	Overall quality assessment[a]
Maghbooli [35] Iran 2020	Cross-sectional	Hospitalized COVID-19 patients Until May 1, 2020	N = 235	58.7 ± 15.2	38.7%	27.4 ± 4.6	VDD: <20 VDI: 20–29	Electro-chemiluminescence On admission or during hospitalization	1. Mortality 2. ICU admission 3. IMV 4. Disease severity (as per CDC) 5. ARDS 6. AKI 7. Multi-organ damage 8. Length of hospital stay	Poor
Karonova [36] Russia 2020	Cross-sectional	Hospitalized COVID-19 patients April 1 to May 15, 2020	N = 80 VDD: 57 VDI: 16 VDR: 7	53.2 ± 15.7	46.2%	NA	VDD: <20 VDI: 20–29	CLIA During hospitalization	1. Mortality 2. Disease severity	Poor
Baktash [37][c,d,e] UK 2020	Cross-sectional	Hospitalized COVID-19 patients March 1 to April 30, 2020	N = 70 VDD: 39 VDR: 31	VDD: 79.5 ± 9.5 VDR: 81.2 ± 7.2	40% VDD: 38.5% VDR: 41.9%	VDD: 25 (23–32) VDR: 24 (20–27)	VDD: ≤12	NA During acute illness	1. Mortality 2. NIV requirement 3. CXR changes 4. Mean difference of serum 25(OH)D between COVID-19 positive and COVID-19 negative patients	Poor
Im [38][b] South Korea 2020	Case-control for mean difference of 25(OH)D level Cross-sectional for all other outcomes	Hospitalized COVID-19 patients February to June 2020	N = 50 VDD: a. 37 b. 12 VDR: 13	57.5 [34.5–68.0]	58%	NA	VDD: a. ≤20 b. ≤10	LC-MS Within 7 days of admission	1. ECMO or death 2. IMV requirement 3. HFNO requirement 4. Oxygen requirement 5. Pneumonia	Good for mean difference of 25(OH)D levels Poor for all other outcomes
Radujkovic [39][d] Germany 2020	Cohort	COVID-19 patients (inpatient and outpatient) Median follow-up: 66 days (range 2–92 days) March 18 to June 18, 2020	N = 185 VDD: 41 VDI: 118 VDR: 67	60 [49–70] VDD: 66 [53–78] VDR: 58 [47–67]	49% VDD: 44% VDR: 50%	NA	VDD: <12 VDI: <20	ADVIA Centaur vitamin D total assay On admission	1. Mortality 2. IMV requirement 3. HFNO requirement 4. NC requirement 5. Hospitalization	Poor
Carpagnano [40] Italy 2020	Cross-sectional	Records of COVID-19 hospitalized adults March 11, to April 30, 2020	N = 42 VDD: 34 a. 11 b. 13 c. 10 VDR: 8	65 ± 13 VDD: a. 64 ± 13 b. 60 ± 6.9 c. 74 ± 11 VDR: 64 ± 18	28.6% VDD: a. 36.4% b. 7.7% c. 20% VDR: 62.5%	28.5 ± 5 VDD: a. 28 ± 4.1 b. 31 ± 6 c. 29 ± 4.8 VDR: 27 ± 4	VDD: a. 20–29 b. 10–19 c. <10	CLIA NA	1. Mortality in 25OHD <10 vs ≥10 2. Disease Severity: mild ARDS (PaO2/-FiO2 200–300), moderate ARDS (PaO2/FiO2 100–200) and severe ARDS (PaO2/FiO2<100) 3. ARDS (PaO2/FiO2<300)	Poor
Abrishami [41] Iran 2020	Cohort	Hospitalized COVID-19 patients February 28 to April 19, 2020	N = 73	55.2 ± 15	35.6%	NA	VDD: <25	Roche Diagnostics "Vitamin D Total" cobas e411 immunoassay analyzer At admission or up to 3 days from CT scan imaging	1. Mortality 2. Disease severity by CT lung stage	Good for mortality Fair for disease severity

(continued on next page)

Table 1 (*continued*)

Author Country Year [reference]	Study design	Sampling method Period	Sample size	Age Mean ± SD or median [IQR] (years)	% female	BMI Mean ± SD or median [IQR] (kg/m^2)	25(OH)D levels cutoff (ng/ml)	25OHD assay Timing of 25(OH)D measurement	Outcomes	Overall quality assessment[a]
Cereda [42]Italy 2020	Cohort	Hospitalized COVID-19 patients March–April 2020	N = 129 VDD: 99 VDI/R: 30	73.6 ± 13.9 VDD: 77 [64–85] VDI/R: 77.5 [65–86]	45.7%	24.7 [22.5–27.6]	Severe VDD: <10 Moderate VDD: 10–20 VDI: 20–30	CLIA Within 48 h of hospital admission	1. Mortality 2. ICU admission 3. Disease severity (biochemical markers)	Poor
Hernandez [43][b,d] Spain 2021	Cross-sectional	Hospitalized COVID-19 patients March 10 to March 31, 2020	N = 197 VDD: 162 VDR: 35	61 [47.5–70] VDD: 62 [48–70.3] VDR: 58 [45–69]	37.6% VDD: 34.6% VDR: 51.4%	29.2 ± 4.7 VDD: 29 ± 4.9 VDR: 29.8 ± 4.1	VDD: <20	CLIA On admission	1. Mortality 2. ICU admission 3. IMV 4. NIV 5. Length of hospital stay 6. ARDS	Poor
Karahan [44] Turkey 2020	Cross-sectional	Hospitalized COVID-19 patients April 1 to May 20, 2020	N = 149 VDD: 103 VDI: 34 VDR: 12	63.5 ± 15.3	45.6%	NA	VDD: <20 VDI: 20–29	ECLIA NA	1. Mortality 2. Disease severity (as per the Chinese guidelines)	Poor
Luo [45] China 2020	Cross-sectional	Hospitalized COVID-19 patients February 27 to March 21, 2020	N = 335 VDD: 235 VDR: 117	56 [43–64]	55.8%	23.5 ± 3.13	VDD: <10	CLIA On admission	1. Mortality 2. Disease severity (as per the Chinese guidelines) 3. Length of hospital stay	Poor
Anjum [46] Pakistan 2020	Cross-sectional	Hospitalized COVID-19 patients March to July 2020	N = 140 Severe VDD: 60	42.5 ± 14.7	41.4%	23.5 ± 3.6	Severe VDD: <10	NA NA	Mortality	Poor
Jain [47] India 2020	Cohort	Hospitalized COVID-19 patients From June 5, 2020 (6 weeks)	N = 154 VDD: 90 VDR: 64	46.1	44.8%	27.1	VDD: <20	Automated immunoassays NA	1. Mortality 2. ICU admission	Poor
Vassiliou [48] Greece 2020	Cohort	COVID-19 patients admitted to ICU March 22 to August 3, 2020	N = 30 VDD: 15 VDR: 15	65 ± 11 VDD: 67 ± 13 VDR: 63 ± 9	20% VDD: 27% VDR: 13%	VDD: 26.4 ± 1.9 VDR: 27.6 ± 1.9	VDD: <15.2	ECLIA On ICU admission	1. Mortality 2. Length of ICU stay 3. IMV 4. Disease severity: mild ARDS (PaO2/-FiO2 200–300), moderate ARDS (PaO2/FiO2 100–200) and severe ARDS (PaO2/FiO2 < 100) 5. ARDS	Poor
Annweiler [49][d] France 2020	Cohort	Hospitalized COVID-19 patients March to April 2020	N = 77 G1[f]: 29 G2: 16 G3: 32	88 [85–92] G1: 88 [87–93] G2: 85 [84–89] G3: 88 [84–92]	49.4% G1: 69% G2: 31.3% G3: 40.6%	NA	NA	NA NA	Mortality	Good
Arvinte [50][e] USA 2020	Cross-sectional	Critically ill COVID-19 patients May 2020	N = 21	60.2 ± 17.4	28.6%	31.6 ± 7.3	NA	NA NA	Mortality	Poor
Hamza [51] Pakistan 2020	Cross-sectional	Hospitalized COVID-19 patients March 1 to April 30, 2020	N = 168	42.3 ± 13.7	44%	NA	VDD: <20 VDI: 20–30	NA NA	1. Mortality 2. Disease severity	Poor
Ling [52][d,e] UK 2020	Cross-sectional	Hospitalized COVID-19 patients January 25 to August 5, 2020	Hospital 1: 444 Hospital 2: 231 Hospital 3: 309	Hospital 1: 74 [63, 83] Hospital 2: 76 [61–84] Hospital 3: 70 [56–84]	A	NA	VDD: <10 VDI: 10–20	Hospital 1: UniCel Dxl 800 Access Immunoassay System Hospital 2: cobas e 801 analytical unit Hospital 3: ADVIA Centaur XPT Immunoassay System	1. Mortality 2. IMV 3. Length of hospital stay	Fair for mortality Poor for all other outcomes

								Up to 12 weeks prior to admission		
De Smet [53][c] Belgium 2020	Cross-sectional	Records of hospitalized COVID-19 patients March 1, to April 7, 2020	N = 186 VDD: 109 VDR: 77	69 [52–80]	41.4% VDD: 33% VDR: 53.2%	NA	VDD: <20	LC-MS/MS On admission and within 24 h from chest CT staging	1. Mortality 2. Disease severity by CT lung stage	Poor
Angelidi [54][e] 2021 USA	Cohort	Hospitalized COVID-19 patients February 1 to May 15, 2020	N = 144 VDD: 79 VDR: 65	66 [55–74] VDD: 60 [48–72] VDR: 68 [63.5–76]	55.6% VDD: 48.1% VDR: 64.5%	29 [25.2–33.3] VDD: 30 [26.3–34.7] VDR: 28 [24.6–32.3]	VDD: <30	ECLIA During admission or within 6 months	1. Mortality 2. IMV 3. Length of hospital stay	Good
Tan [55][d] Singapore 2020	Cohort	Hospitalized COVID-19 patients January 15, to April 15, 2020	N = 43 DMB: 17 VDR: 26	DMB: 58.4 ± 7 VDR: 64.1 ± 7.9	DMB: 35.3% VDR: 42.3%	NA	NA	NA NA	Deterioration post-DMB administration leading to: 1. Any form of O2 therapy and/or intensive care support 2. O2 therapy (but not ICU support) 3. ICU support	Poor
Merzon [56] Israel 2020	Case-Control	14,022 Records of individuals tested for COVID-19 25OHD data available for 7807 individuals February 1, to April 30, 2020	N = 7807 VDD: 1020 VDI: 5648 VDR: 1139	NA	59.6%	NA	VDD: <20 VDI: 20–29	DiaSorin Chemi-luminescence assay NA	1. Hospitalization 2. SARS-CoV-2 positivity	Good
Macaya [57][d] Spain 2020	Cohort	COVID-19 patients admitted to ER March 5 to March 31, 2020	N = 80 VDD: 45 VDR: 35	NA	56.3%	NA	VDD: <20	CLIA On admission or within the 3 previous months.	1. Hospitalization 2. Disease severity	Poor for hospitalization Fair for disease severity
Ye [58] China 2020	Case-Control for SARS-CoV-2 positivity and mean difference of serum 25(OH)D Cross-sectional for disease severity	Cases: Hospitalized patients COVID-19 patients Controls: Healthy February 16 to March 16, 2020	N = 142 VDD: 41 VDR: 101 Cases: 62 VDD: 26 VDR: 36	Cases: 43 [32–59] Controls: 42 [31–52]	61.3% Cases: 63% Controls: 60%	NA	VDD: <20 VDI: 20–30	ECLIA NA	1. Disease severity (as per the Chinese guidelines) 2. SARS-CoV-2 positivity 3. Mean difference of serum 25(OH)D between COVID-19 positive and COVID-19 negative patients	Poor
Kerget [59] Turkey 2020	Cross-sectional	Hospitalized COVID-19 positive patients March 24 to May 15, 2020	N = 88	49.1 ± 21.1	53.4%	NA	NA	ELISA On admission	ARDS	Poor
Meltzer [60][d,e] USA 2020	Case-Control	4314 patients tested for COVID-19 499 patients had a 25OHD level in the year before testing. March 3, to April 10, 2020	N = 499 VDD: 178 VDR: 321	45.7 VDD: 45.6 VDR: 50.7	65% VDD: 78% VDR: 73%	29.8 VDD: 30.5 VDR: 29.4	VDD: <20	NA Within 1 year before COVID-19 testing	SARS-CoV-2 positivity	Good
Abdollahi [61][c]Iran 2020	Case-Control	Hospitalized patients February 20 to April 20, 2020	N = 402 VDD: 2 VDI: 292 VDR: 108	47.2	67.2%	NA	VDD: <10 VDI: 10–30 VDR: 30–100	ELISA NA	1. SARS-CoV-2 positivity 2. Mean difference of serum 25(OH)D between COVID-19 positive and COVID-19 negative patients	Poor
Mardani [62][c] Iran 2020	Cross-sectional	Individuals referred to outpatient clinic for COVID-19	N = 123	NA	52.8%	NA	VDD: <10 VDI: 10–30	ELISA NA	Mean difference of serum 25(OH)D between COVID-19 positive and COVID-19 negative patients	Poor

(continued on next page)

Table 1 (*continued*)

Author Country Year [reference]	Study design	Sampling method Period	Sample size	Age Mean ± SD or median [IQR] (years)	% female	BMI Mean ± SD or median [IQR] (kg/m^2)	25(OH)D levels cutoff (ng/ml)	25OHD assay Timing of 25(OH)D measurement	Outcomes	Overall quality assessment[a]
		testing During March 2020								
D'Avolio [63][c] Switzerland 2020	Case-Control	Individuals who underwent PCR and a 25(OH)D measurement March 1 to April 14, 2020	N = 107	73 [63–81]	45.8%	NA	NA	LC-MS/MS Within 7 weeks of the PCR result	Mean difference of serum 25(OH)D between COVID-19 positive and COVID-19 negative patients	Poor
Raisi-Estabragh [64][c,e] UK 2020	Cohort	Individuals tested for COVID-19 March 16 to May 18, 2020	N = 4510	NA	51.2%	NA	NA	NA Between 2006 and 2010	Mean difference of serum 25(OH)D between COVID-19 positive and COVID-19 negative patients	Good
Hastie [65][c] UK 2020	Cohort	Individuals tested for COVID-19 March 16 to April 14, 2020	N = 1474	NA	NA	NA	VDD: <10 VDI: 10–20	CLIA Between 2006 and 2010	Mean difference of serum 25(OH)D between COVID-19 positive and COVID-19 negative patients	Good

ARDS: acute respiratory distress syndrome, AKI: acute kidney injury, CXR: chest X-ray, CLIA: chemi-luminescence immunoassay, DMB: vitamin D, magnesium and vitamin B12 supplementation, ECLIA: electro-chemi-luminescence immunoassay, ECMO: extra-corporeal membrane oxygenation, ELISA: enzyme-linked immunosorbent assay, HFNO: high flow nasal oxygen, ICU: intensive care unit, IMV: invasive mechanical ventilation, IQR: interquartile range, ITU: intensive therapy unit, LC-MS: liquid chromatography-mass spectrometry, NA: not available, NC: nasal cannula, NIV: non-invasive ventilation, SE: standard error; OD: once daily, VDD: vitamin D deficient, VDI: vitamin D insufficient, VDR: vitamin D replete.

[a] Detailed quality assessment is provided in Appendix C.1 using the New Castle-Ottawa quality scale.

[b] Only two studies reported on the mean 25(OH)D levels in patients with deficiency and those with desirable levels.

[c] Studies with data on 25(OH)D levels by SARS-CoV-2 positivity status.

[d] In these studies, a proportion of participants were supplemented with vitamin D.

[e] Studies with data on ethnicities. The majority of participants in Baktash 2020 and Ling 2020 were Caucasians, in Angelidi 2021 the majority were non-Hispanic blacks, in Meltzer 2020 the majority were non-Hispanics, in Raisi-Estabragh 2020 and in Arvinte 2020 the majority were Hispanics.

[f] Group 1: COVID-19 patients who had received oral boluses of vitamin D supplements (50,000 IU vitamin D3 per month, or the doses of 80,000 IU or 100,000 IU vitamin D3 every 2–3 months) over the preceding year. Group 2: COVID-19 patients usually not supplemented but who received an oral supplement of 80,000 IU vitamin D3 within a few hours of the diagnosis of COVID-19. Group 3: all COVID-19 patients who had received no vitamin D supplements.

Table 2
Summary of results of the included studies in the primary analysis per outcome.

Outcome (N studies)	Studies [reference]	% events (VDD v/s VDR)	RR or OR (VDD v/s VDR)	25(OH)D levels per outcome status	Overall quality[a]
Mortality (N = 7)	Cereda et al. [42]	24.2% v/s 33.3% (p = 0.22)	OR_{adj} = 0.28, 95% CI [0.09–0.99][c]	NA	Poor
	De Smet et al. [53]	18.3% v/s 9.1%[b]	OR_{adj} = 3.87, 95% CI [1.30–11.55][d]	Mortality: 15.2 ng/ml No mortality: 18.9 ng/ml (p = 0.02)	Poor
	Hernandez et al. [43]	10.2% v/s 11.4% (p = 0.765)	NA	NA	Poor
	Im et al. [38]	8.1% v/s 7.7%[b]	NA	NA	Poor
	Jain et al. [47]	21% v/s 3.1%[b]	NA	NA	Poor
	Karahan et al. [44]	62.1% v/s 10.9%[b]	25(OH)D levels as predictor: OR = 0.90, 95% CI [0.86–0.94] OR_{adj} = 0.93, 95% CI [0.88–0.98][e]	Mortality: 10.4 ± 6.4 ng/ml No mortality: 19.3 ± 11.2 ng/ml (p < 0.001)	Poor
	Karonova et al. [36]	21.1% v/s 4.3%[b]	OR_{adj} = 9.1, 95% CI [2.5–33.6][f]	Mortality: 10.8 ± 6.1 ng/ml No mortality: 17.8 ± 13.4 ng/ml (p = 0.02)	Poor
ICU admission (N = 3)	Cereda et al. [42]	5.1% v/s 0% (p = 0.26)	NA	NA	Poor
	Hernandez et al. [43]	27.2% v/s 17.1% (p = 0.217)	NA	NA	Poor
	Jain et al. [47]	67.8% v/s 3.1%[b]	NA	ICU: 14.35 ± 5.79 ng/ml No ICU: 27.89 ± 6.21 ng/ml (p = 0.0001)	Poor
IMV requirement (N = 2)	Hernandez et al. [43]	22.8% v/s 17.1% (p = 0.576)	NA	NA	Poor
	Im et al. [38]	10.8% v/s 7.7%[b]	NA	NA	Poor
NIV requirement (N = 2)	Hernandez et al. [43]	7.4% v/s 2.9% (p = 0.471)	NA	NA	Poor
	Im et al. [38]	16.2% v/s 23.1%[b]	NA	NA	Poor
SARS-CoV-2 positivity (N = 3)	Meltzer et al. [60]	18% v/s 11% (p = 0.11)	RR = 1.77[g] (p < 0.02)	NA	Good
	Merzon et al. [56]	10.3% v/s 10.0%[b]	OR = 1.58, 95% CI [1.13–2.09] OR_{adj} = 1.5, 95% CI [1.13–1.98][h]	NA	Good
	Ye et al. [58]	63.4% v/s 35.6%[b]	NA	NA	Poor
Mean 25(OH)D levels (N = 5)	Abdollahi et al. [61]	NA	NA	Median (IQR) COVID: 24 (19–29) ng/ml No COVID: 26 (21–35) (p = 0.001)	Poor
	Baktash et al. [37]	NA	NA	Median (IQR) COVID: 27 (20–47) nmol/l No COVID: 52 (31.5–71.5) nmol/l (p = 0.0008)	Poor
	D'Avolio et al. [63]	NA	NA	Median (IQR) COVID: 11.1 (8.2–21.0) ng/ml No COVID: 24.6 (8.9–30.5) ng/ml (p = 0.004)	Poor
	Raisi-Estabragh et al. [64]	NA	25(OH)D levels as predictor: OR_{adj} = 1.0, 95% CI [1.0–1.0][i]	COVID: 33.88 ± 27.01 nmol/l No COVID: 35.45 ± 26.78 nmol/l	Good
	Ye et al. [58]	63.4% v/s 35.6%	NA	Median (IQR) COVID: 55.6 (41.9–66.1) nmol/l No COVID: 71.8 (57.6–83.7) nmol/l (p < 0.05)	Poor

ICU: intensive care unit, IMV: invasive mechanical ventilation, NIV: non-invasive ventilation, NA: not available, OR: odds ratio, OR_{adj}: adjusted odds ratio, RR: risk ratio, VDD: vitamin D deficiency, VDR: vitamin D replete.

[a] Detailed quality assessment is provided in Appendix C.1 using the New Castle-Ottawa quality scale.
[b] p-value not reported.
[c] Adjusted for age, sex, C-reactive protein, ischemic heart disease and severe pneumonia.
[d] Adjusted for age, sex and comorbidities.
[e] Adjusted for WBC, lymphocyte and albumin.
[f] Adjusted for obesity.
[g] Adjusted for age, sex, BMI, ethnicity, employment, race and comorbidities.
[h] Adjusted for age, sex, BMI, socioeconomic status, smoking and comorbidities.
[i] Adjusted for age, sex and ethnicity.

3.1.2.6. Length of hospital stay. Five studies assessed the length of hospital stay in COVID-19 patients with low 25(OH)D levels [35,43,45,52,54]. One was a cohort study [54], and four were cross-sectional [35,43,45,52]. Two studies did not report the length of hospital stay by 25(OH)D level status [45,52]. Hernandez et al. reported a significantly higher length of hospital stay in COVID-19 patients with serum 25(OH)D <20 ng/ml (median = 12 days (IQR 8–17)) as compared to those with desirable vitamin D status (median = 8 days (IQR 6–14)) (p = 0.013) [43]. Two studies had available data for length of hospital stay using a higher serum 25(OH)D cutoff (30 ng/ml) [35,54]. Combining their data (N = 379), showed no significant difference in length of hospital stay between COVID-19 patients with 25(OH)D < 30 ng/ml compared to those with more desirable levels (MD = 0, 95% CI [−0.97, 0.97]; I^2 = 0%) (Appendix D.2) [35,54].

3.1.2.7. Disease severity. Twelve studies assessed the risk of severe COVID-19 disease by vitamin D status [35,36,40–42,44,45,48,51,53,57,58]. Eight studies were of cross-sectional design, and 4 were cohort studies (Table 1). The sample size ranged between 30 and 335, age ranged between 42 and 74 years, and 20–61% was women.

The definition of severity and the cutoff for 25(OH)D levels varied between studies; we therefore could not pool results from all studies (Table 1). Two studies defined disease severity as SaO2 < 93% or respiratory distress or lung infiltrates more than 50% within 24 to 48 h or respiratory failure or organ failure [35,44]. Pooling data from these two studies (N = 384), as a sensitivity analysis, showed a trend for an increased COVID-19 disease severity in patients with serum 25(OH)D < 30 ng/ml (RR = 3.0, 95% CI [0.19–48.2]; I^2 = 77%) (Appendix D.3). Serum 25(OH)D was measured on admission in one study [35], and timing of measurement was not specified in the other [44].

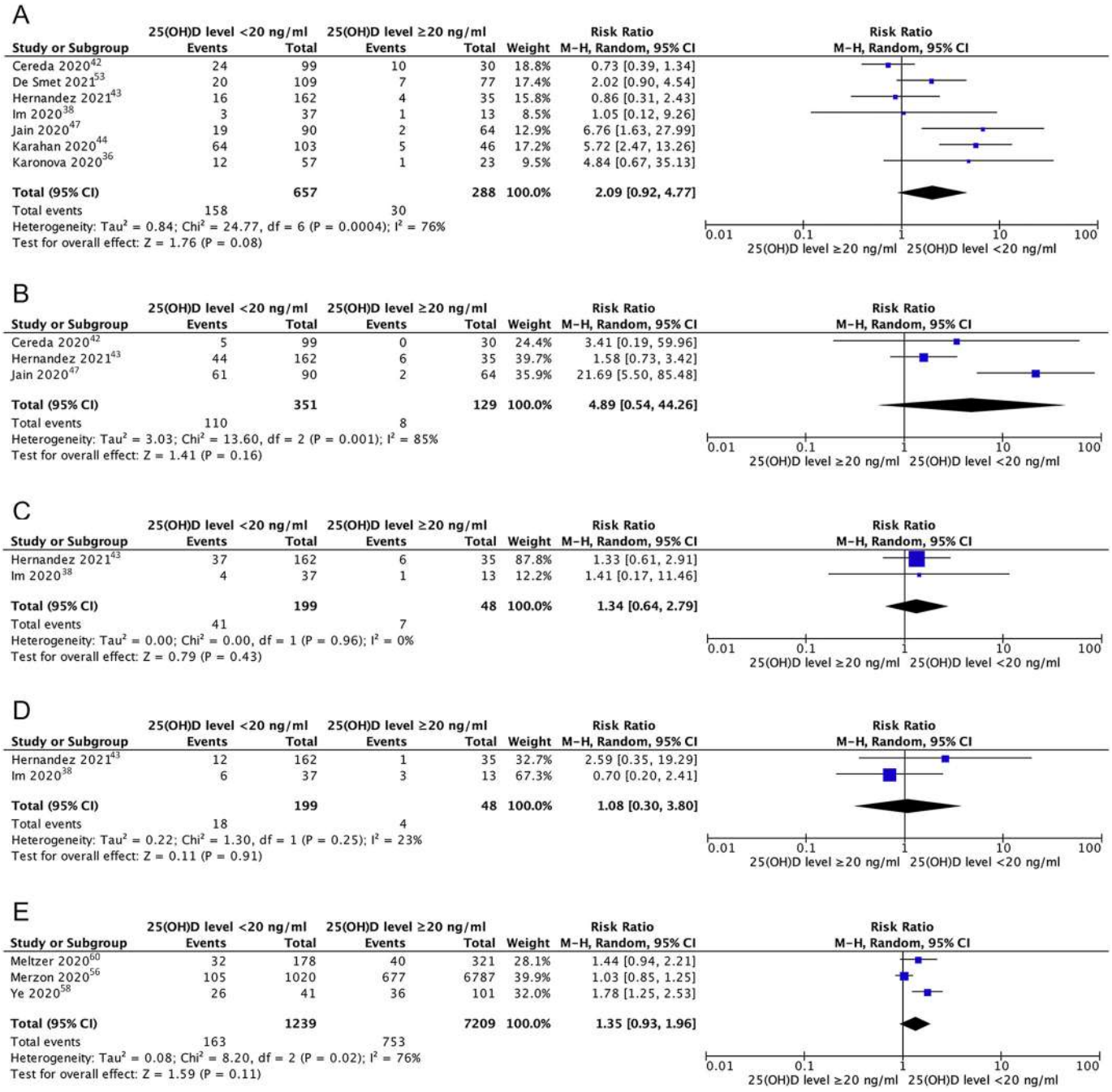

Fig. 2. Forest plots of the association between serum 25(OHD) levels <20 ng/ml and COVID-19 outcomes. A. COVID-19 mortality. B. ICU admission. C. Invasive mechanical ventilation requirement. D. Non-invasive ventilation requirement. E. SARS-CoV-2 positivity status.

3.1.2.8. Acute respiratory distress syndrome. Four cross-sectional studies and one cohort study assessed the association between acute respiratory distress syndrome (ARDS) and vitamin D status in patients with COVID-19 infection [35,40,43,48,59]. Sample size ranged between 30 and 235 individuals, mean age ranged between 49 and 72 years and percent female participants ranged between 20 and 53% (Table 1). None of these studies had data for 25(OH)D levels <20 ng/ml, therefore a main analysis was not conducted for this outcome.

Two studies had available data for ARDS in patients with 25(OH)D levels < 30 ng/ml [35,40]. Combining their data revealed no significant increase in the risk of ARDS in COVID-19 patients with low 25(OH)D levels (<30 ng/ml) (Appendix D.4). Vitamin D levels were measured during hospitalization in one study [35], while the timing of measurements was not mentioned in the other [40].

3.1.2.9. SARS-CoV-2 positivity. Four case-control studies assessed the risk of being SARS-CoV-2 positive by PCR in patients with low 25(OH)D levels and presenting for potential symptoms of COVID-19 [56,58,60,61]. The mean age of participants was 46 and 47 years in 2 studies [60,61]. The percentage of women participants varied between 60 and 67%.

Three studies defined serum 25(OH)D < 20 ng/ml and were therefore included them in a meta-analysis [52,56,60]. The individual results of these studies are summarized in Table 2. The pooled effect (N = 8448) showed a higher risk of SARS-CoV-2 positivity in patients with low 25(OH)D levels (RR = 1.4, 95% CI [0.9–2], I^2 = 76%) (Fig. 2.E). But while timing of blood draw was specified as within a year before COVID testing in one [60], it was not mentioned in the remaining two studies [56,58]. The certainty of evidence of

Table 3
Evidence profile for COVID-19 related health outcomes based on the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) working group methodology.

Certainty assessment							No of patients		Effect		Certainty	Importance
No studies	Study design	Risk of bias	Inconsistency[1]	Indirectness[2]	Imprecision[3]	Other considerations[4]	25(OH)D < 20 ng/ml	25(OH)D ≥ 20 ng/ml	Relative (95% CI)	Absolute (95% CI)		
Mortality												
7	Observational studies	Very serious[a]	Very serious[b]	Not serious	Serious[c]	None	158/657 (24.0%)	30/288 (10.4%)	RR 2.09 (0.92 to 4.77)	114 more per 1000 (from 8 fewer to 393 more)	⊕◯◯◯ VERY LOW	CRITICAL
ICU admission												
3	Observational studies	Very serious[a]	Not serious	Not serious	Very serious[d]	None	110/351 (31.3%)	8/129 (6.2%)	RR 4.89 (0.54 to 44.26)	241 more per 1000 (from 20 fewer to 1000 more)	⊕◯◯◯ VERY LOW	CRITICAL
IMV requirement												
2	Observational studies	Very serious[a]	Not serious	Not serious	Serious[c]	None	41/199 (20.6%)	7/48 (14.6%)	RR 1.34 (0.64 to 2.79)	50 more per 1000 (from 53 fewer to 261 more)	⊕◯◯◯ VERY LOW	CRITICAL
NIV requirement												
2	Observational studies	Very serious[a]	Not serious	Not serious	Serious[c]	None	18/199 (9.0%)	4/48 (8.3%)	RR 1.08 (0.30 to 3.80)	7 more per 1000 (from 58 fewer to 233 more)	⊕◯◯◯ VERY LOW	CRITICAL
SARS-CoV-2 positivity												
3	Observational studies	Serious[e]	Not serious	Not serious	Serious[c]	None	163/1239 (13.2%)	753/7209 (10.4%)	RR 1.35 (0.93 to 1.96)	37 more per 1000 (from 7 fewer to 100 more)	⊕◯◯◯ VERY LOW	IMPORTANT

ICU: intensive care unit, IMV: invasive mechanical ventilation, NIV: non-invasive ventilation.
CI: confidence interval; RR: risk ratio.
[1] Inconsistency refers to an unexplained heterogeneity of results.
[2] Direct evidence consists of research that directly compares the interventions which we are interested in, delivered to the populations in which we are interested, and measures the outcomes important to patients.
[3] Results are imprecise when studies include relatively few patients and few events and thus have a wide confidence interval (CI) around the estimate of the effect.
[4] Other considerations include publication bias, large effect, plausible confounding and dose-response gradient.
[a] All studies were of poor quality.
[b] High unexplained heterogeneity.
[c] Wide confidence interval.
[d] Very wide confidence interval.
[e] One study was of poor quality.

this outcome was low (Table 3). These studies consisted of two case-control studies of good quality [56,60] and one case-control study of poor quality [58]. The results were both imprecise and very inconsistent (Table 3).

Two studies had data on 25(OH)D < 30 ng/ml [56,61]. Pooling data from both studies (N = 8209) in a sensitivity analysis showed that 25 (OH)D < 30 ng/ml was associated with a 1.5 higher risk of COVID-19 positivity compared to vitamin D sufficient patients (RR = 1.5, 95% CI [1.3, 1.8], I^2 = 0%) (Appendix D.5).

3.1.2.10. Other health related outcomes. Im et al. did not report a significant difference in serum 25(OH) levels, measured within 7 days of admission, between patient who developed COVID-19 related pneumonia and those who did not [38]. Maghbooli et al. showed no significant association between vitamin D status, measured on admission, and acute kidney injury or multi-organ damage [35]. Vassiliou et al. reported a median of 17 days in ICU in patients with serum 25(OH)D < 15.2 ng/ml and 35 in those with levels >15.2 ng/ml, however this difference was not significant [48].

We did not identify any data regarding time on respirators, time to seronegative conversion, time to symptomatic recovery and risk of seropositive conversion in family members.

3.1.3. Serum 25(OH)D levels in COVID-19 infected patients compared to those not infected

Seven studies assessed serum 25(OH)D level in patients with COVID-19 infection as compared to those without COVID-19 [37,58,61–65]. Two were cross-sectional studies [37,62], three were case-control studies [58,61,63], and two were cohort studies [64,65]. The sample size ranged from 105 to 4510. The mean age varied between 42 and 81 years, and the proportion of women was between 46 and 67%.

Two cohort studies were conducted in the same population [64,65], the UK biobank, and one study did not provide the standard deviation for the mean 25(OH)D levels [62]. We therefore included only 5 studies in the primary analysis [37,58,61,63,64]. Combing data from these 5 studies (N = 5266) revealed that serum 25(OH)D level in COVID-19 patients was 5.9 ng/ml lower than that in individuals without COVID-19 (MD -5.9, 95% CI [−9.5, −2.3]; I^2 = 94%) (Fig. 3). If we were to exclude the study of Baktash et al. from the analysis, because of the lower quality of cross sectional-studies, the mean difference becomes 4.9 ng/ml, and is still significant (data not shown) [37]. Timing on blood draw with relation to COVID testing was 10 years prior in the cohort study [64], within 7 weeks of PCR in one case control study [63], and during acute illness in the cross-sectional study [37]. Four studies were of poor quality, while only one was a cohort of good quality [64] (Appendix C.1).

3.2. Clinical trials

3.2.1. Completed trials

A pilot study, recently published preliminary data on a subset of patients (76 out of the 1008 target participants) based on the COVIDIOL trial protocol conducted in Spain (NCT04366908, EudraCT2020-001717-20) [67]. The study was a randomized double blinded controlled trial, in which hospitalized COVID-19 patients received standard care (hydroxychloroquine and azithromycin) alone (N = 26) or with calcifediol (N = 50). The treatment group received 0.532 mg of calcifediol on admission, 0.266 mg on days 3 and 7, and then weekly. The mean age of participants was 53 ± 10 years, 31% and 46% of the participants were women in the treatment and control groups respectively. Baseline characteristics were similar between treatment groups. Only one out of the 50 patients receiving calcifediol required ICU admission while 50% of those not receiving vitamin D required ICU admission. The OR ratio of ICU admission in patients with calcifediol treatment v/s those with no treatment was 0.03 (95% CI: 0.003–0.25) [67]. The overall risk of bias in this study was uncertain mainly stemming from the unclear description of allocation concealment and blinding of participants (Appendix C.2).

A small (non-registered) trial from India, randomized COVID-19 patients with vitamin D deficiency (25(OH)D < 20 ng/ml) to receive either 60,000 IU/day of cholecalciferol (N = 16) or placebo (N = 24) for 7 days [68]. At 7 days if the 25(OH)D serum level did not reach 50 ng/ml, participants in the intervention arm continued the same supplementation for 7 additional days. If serum 25(OH)D exceeded 50 ng/ml they were supplemented with 60,000 IU/week. The median age of participants was 50 in the intervention arm and 47 in the placebo arm. Baseline characteristics were similar between treatment groups except for serum calcium levels which were higher in the intervention group. At the 14 days follow up, 62.5% of the participants in the intervention arm became SARS-CoV-2 negative compared to only 20.8% in the control arm. In addition, there was a significant increase in serum 25(OH)D levels (+42.4 v/s +5.1 ng/ml) and a significant decrease in fibrinogen levels (−0.9 v/s −0.04 ng/ml) in the intervention group compared to placebo. However, there was no significant difference in CRP, procalcitonin, ferritin and D-dimer levels [68]. The overall risk of bias in this study was unclear mainly due to the unclear description of sequence generation, allocation concealment, blinding, and selective outcome reporting (Appendix C.2).

A newly published trial from Brazil randomized 240 COVID-19 patients to receive either a single oral dose of vitamin D3 at a dose of 200,000 IU (N = 120) or placebo (N = 120) [69]. Baseline characteristics were comparable between intervention groups. The mean age of participants was 56 in both arms and 41.2% and 46.6% of participants were women in the intervention and placebo arm respectively. The length of hospital stay was not statistically different between both arms. In addition, supplementation of 200,000 IU of vitamin D3 did not improve COVID-19 related health outcomes such as mortality, ICU admission and need for mechanical ventilation compared to placebo. Although the risk of bias was low in most of the items assessed, the overall risk of bias in this study was unclear due to the lack of description of the allocation concealment method (Appendix C.2).

3.2.2. On-going trials

We identified 32 trials protocols, including two of the above trials, from the clinicaltrials.gov (n = 23) and WHO registries, including the European (n = 4) and Iranian registries (n = 5) (search current until January 20th 2021) (Appendix E). Three were expected to be completed in the summer of 2020, an additional four by December 2020. One trial was completed in November 2020, and results have been recently published as discussed above [69]. The remaining studies identified in clinicaltrials.gov are expected to be completed in 2021. The expected completion date was not specified in trials identified in the European and Iranian registers (Appendix E). The on-going trials are being conducted across continents, in the USA, Canada, UK, Argentina, Brazil, Spain, France, Belgium, Switzerland, India, Jordan, and Iran. The Vitamin D formulation is D3 in 22 studies, calcifediol in two studies, and not mentioned in the other 9 protocols. Vitamin D is administered as daily (N = 17), weekly (N = 4), or in large boluses once or in discrete repeated timings (N = 12). The doses range is between a daily equivalent of 357 and 60,000 IU/day. Study duration ranges between one week and 12 months. 26 trials aim to study the effect of vitamin D treatment in COVID-19 patients while 6 studies aim to identify a role in prevention of COVID-19 infection with supplementing healthy patients (n = 4) or health care workers (n = 2) with vitamin D. Primary outcomes include COVID-19 infection, infection duration, hospitalization, hospital stay, ICU admission, ICU stay, mortality, symptoms severity, symptoms recovery, seronegative conversion, respiratory failure, and laboratory measurements including CRP, Il-6, IL-1, TNFa, 25(OH)D, and serum COVID-19 antibodies levels (Appendix E).

We identified 8 mega-trials with a sample size ranging between 1008 and 5440, that span between 2 weeks and 24 weeks of vitamin D supplementation, and doses between 1000 IU/day and 9600 IU/day. Of these, four are conducted in outpatient COVID-19 patients and primarily assess the effect of vitamin D on health-related outcomes such as hospitalization, ICU admission and mortality. The other four assess the effect of vitamin D in preventing infection in healthy volunteers (n = 2) or health care workers (n = 2).

4. Discussion

This systematic review and meta-analysis reveals very uncertain evidence for an association between serum 25(OH)D levels <20 ng/ml and risk of mortality, ICU admission, mechanical ventilation, non-invasive ventilation and testing positive for SARS-CoV-2. However, serum 25(OH)D levels were 6 ng/ml lower in COVID-19 patients as compared to those without COVID-19 infection, this difference was significant. Increasing the cutoff of low 25(OH)D levels to 30 ng/ml in our sensitivity analysis, revealed a significantly increased risk of mortality and testing positive for SARS-CoV-2 in patients with such levels. However, there were no associations with risk of disease severity and ARDS, and length of hospital stay. We only identified three completed clinical trials of uncertain quality. A pilot trial was the first to demonstrate that supplementation with calcifediol, a non-traditional form of vitamin D, may improve clinical outcomes specifically ICU admissions in COVID-19 hospitalized patients. The second small study suggests that

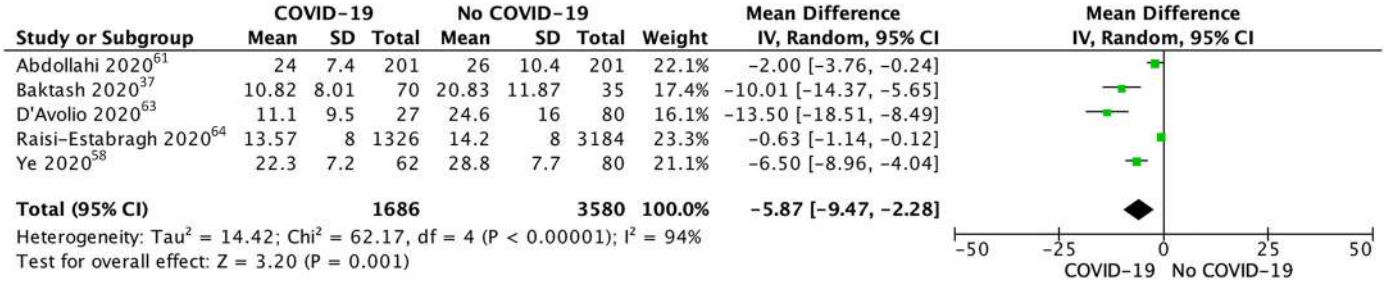

Fig. 3. Forest plot for the mean difference in 25(OH)D levels (ng/ml) between COVID-19 infected and non-infected patients.

supplementation with cholecalciferol may lead to a faster recovery from COVID-19. The third study did not show any effect of vitamin D supplementation on COVID-19 related health outcomes.

The putative protective effects of vitamin D on COVID related health outcomes are stipulated to be mediated by several mechanisms. These include modulating the cytokine storm resulting from activation of the RAS system subsequent to activation of the ACE receptor by the coronavirus, modulating neutrophil activity, maintaining the pulmonary epithelial barrier, and stimulating epithelial repair [70,71]. This mechanism might lead to the speculated protective role of vitamin D supplementation. This hypothesis is supported by the results of the pilot clinical trial which revealed that supplementation of calcifediol reduced risk of ICU admission from COVID-19 [67]. In our meta-analysis, there was a trend of increased risk of ICU admission with low 25(OH)D levels. The above-described pathophysiology may also explain a potential decrease in other COVID related health outcomes including mortality. However, our findings on mortality in the primary analysis based on seven studies were not significant.

In our meta-analysis of 8209 patients, patients with serum 25(OH)D levels <30 ng/ml were 1.5 times more likely to test positive for COVID-19 compared to patients with desirable 25(OH)D levels. In addition, the difference in 25(OH)D levels based on COVID-19 positivity might suggest a role for calcitriol, the active metabolite of vitamin D in inhibiting post-entry viral replication in nasal epithelial cells, leading to decreased SARS-CoV-2 viral titers and thus a lower risk of testing positive for COVID-19 in vitamin D replete individuals [72]. These findings are in line with those from previous studies reporting on the increased risk of viral respiratory infections with vitamin D deficiency, and the efficacy of vitamin D supplementation in reducing that risk [13,21,73,74]. In fact, vitamin D's role in immunity is well established [17,75].

This systematic review and meta-analysis has several limitations with regards to its conclusions, that stems from the studies themselves, namely the low quality and certainty of the evidence available to-date. Limitations include the scarce evidence provided from published observational studies, and from the heterogeneity in the definition of vitamin D deficiency, and the timing of blood withdrawal in relation to the diagnosis of COVID-19. We identified results from only three randomized controlled trials, and we could only meta-analyze results from few observational studies, which downgrades the level of evidence. Furthermore, only 14 studies adjusted for important confounders of low vitamin D, such as season, age and BMI, comorbidities, known predictors which may have introduced information bias [35,38,39,41,45,49,52,53,56–58,60,64,65]. Importantly, we scrutinized the timing of serum 25(OH)D measurement with regards to the timing of assessment of COVID related outcomes, and types of assays used. This is crucial considering the impact of acute illness on serum 25(OH)D levels [76–80], and thus the potential for reverse causality in such observational studies. One study assessed the correlation between COVID related health outcomes and vitamin D levels measured within one year prior to the study [60]. Several studies assessed the correlation between vitamin D status and mortality, ICU admission, hospitalization and disease severity, but did not mention the timing of 25(OH)D levels measurement [40,44,46,47,49–51,55,56,58,61,62]. In addition, 25(OH)D levels were measured in acute settings in 12 studies [35–39,41–43,45,48,53,59] and more than 2/3 of the studies were conducted in the inpatient COVID-19 patients [35–38,40–53,55,57–59,61]. Serum 25(OH)D levels in acutely ill patients are usually lower than those of healthy individuals due to inflammation [75–78]. In fact, 25(OH)D levels may decrease by 40% within the first 24 h of acute illness [77,78]. Injection of E. coli lipopolysaccharides induced inflammation in normal volunteers and resulted in an acute decrease in mean serum 25(OH)D levels by 2.6 ng/ml, as measured by mass spectroscopy, 2–3 h later [81,82]. Furthermore, decreased synthesis of vitamin D binding proteins and increased 25(OH)D renal excretion, may play a significant role in regulating vitamin D levels in critically ill patients [79,80]. Therefore, the low levels measured during the illness reported in the included studies might be due to the acute illness. Interestingly, low serum calcium and phosphate levels have been reported in COVID-19 patients suffering from severe disease [83–85], and low 25(OH)D levels, although not reported [84,85], could be one potential contributing factor for such low levels. Only three studies used liquid chromatography mass spectroscopy, which may provide more accurate results, as compared to NIST standards [38,53,63]. The accuracy of the serum 25(OH)D cutoffs used in various studies is therefore unclear, and evidence for quality assurance protocol for assay standardization was mentioned in only three studies [43,48,52].

To our knowledge, only one systematic-review and meta-analysis was published on this topic [86]. However, the authors only focused on COVID-19 severity, mortality and hospitalization, did not assess certainty of the evidence, combined studies with heterogeneous definitions of disease severity, and their conclusions are limited by considerations of reverse causality. We are unaware of any other systematic review and meta-analysis that aims to assess the impact of low serum 25(OH)D levels on additional important outcomes such as ICU admission, mechanical and non-invasive ventilation, ARDS. In addition, the strengths of our study lie in its extensive and rigorous search, outreach to investigators for missing data, granular analysis, use of accepted instruments to assess quality of studies and strength of the evidence reported, as well as scrutiny of the type of vitamin D assay used and timing of measurement of serum 25(OH)D levels in relation to COVID-19 related outcomes. We also excluded non peer-reviewed articles to enhance the quality of the evidence. The quality of the research is crucial and due to the rapid dissemination of articles during the COVID-19 pandemic, it has been subjected to many flaws and weaknesses [87]. This systematic review and meta-analysis therefore presents a thorough and reliable review and analysis of the available evidence, and a comprehensive overview of on-going clinical trials, based on a systematic search of the major trial registries.

In conclusion, our systematic review reveals that none of the outcomes evaluated revealed a clear and strong direction for a cause effect relationship of vitamin D status on COVID-19 health related outcomes. The evidence available to-date is insufficient to make any recommendations for high doses of vitamin D to either prevent or treat COVID-19 complications. Doses below the Upper Tolerable Level set by the IOM of 4000 IU/day, are most prudent for now [22,26,75,88]. Clear evidence-based recommendations on vitamin D supplementation, timing and dosing regimen can only be determined based on results from several on-going vitamin D randomized controlled trials on COVID-19 related health outcomes.

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Credit authorship contribution statement

AB, MB, OEZ, MC and GEHF designed the search strategy; AB and MB screened titles, abstracts and full texts, and abstracted data from included studies; AB, MB and MR screened and abstracted data from clinical trials protocols; AB and MB performed statistical analysis; MC and GEHF provided input on statistical analysis and quality assessment; AB and MB wrote the paper; MC and GEHF provided major input on the paper; All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

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