

# The role of vitamin D in prevention of COVID-19 and its severity: an umbrella review

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**Abstract.** *Background and aim:* Vitamin D is known to modulate immune response and its deficiency was associated with respiratory distress in patients hospitalized for pneumonia. Nevertheless, numerous reviews on vitamin D in COVID-19 patients have shown conflicting results, as previously reported also for other respiratory diseases (e.g., influenza). *Methods:* This umbrella review aims to assess whether low serum 25-OHD is associated with susceptibility to COVID 19, their severity, and mortality. A total of 1559 studies were excluded after the title, abstract and full-text articles screening and 9 papers were included in this review: 2 systematic reviews and 7 metanalysis. *Results:* The findings of this review that summarized studies from 5 WHO regions (European Region, Region of the Americas, South-East Asia Region, Eastern Mediterranean Region, Western Pacific Region) to exclusion only African region, show that low serum 25-OHD levels are associated with higher infection risks for COVID-19. *Conclusions:* Although the umbrella findings indicate a potential role of vitamin D deficiency in COVID-19 severity in hospitalized patients and showing an association between Vitamin D supplementation and COVID-19 severity, however, more robust data from randomized controlled trials are further needed to confirm a possible association with the mortality rates. ([www.actabiomedica.it](http://www.actabiomedica.it))

**Key words:** SARS-COV-2, COVID-19, Review, Prevention, Cause of Death, Quality of Life, Vitamin D

## Introduction

The associations between vitamin D levels and diseases have been assessed in a large and rapidly expanding literature (1-3). Numerous studies have examined the effect of vitamin D supplementation on a range of pathologies and health conditions (3-5). Vitamin D seems linked to skeletal diseases including osteoporosis and involving calcium, phosphorus and bone metabolism or several risk conditions related to fractures, muscle strength, falls; more recently, also cancer, cardiovascular diseases, and metabolic disorders were considered (5-14). Indeed, the vitamin D receptor and the

enzyme that activates vitamin D (1 $\alpha$ -hydroxylase) have been identified in many organs, leading to claims of its extra-skeletal effects, even if often unconvincing (15). An exception to the weakness of these reports seems to be showed by the observation of a more consistent effect of vitamin D on the immune system, and its possible role in increasing the defence against infectious diseases as well as the decrease in autoimmunity conditions (15, 16). Several randomized clinical trials have shown a reduced incidence in acute respiratory infections when a supplementation with vitamin D is present (17-19).

In the 2020s, after the spreading of SARS-CoV-2 pandemic, a growing scientific attention turned to

evaluate the association between COVID-19 and vitamin D deficiency (20-23). In this perspective, achieving optimal levels may represent a challenging objective for prevention and treatment of COVID-19 (24). Unravelling these associations might be of great importance for public health, as vitamin D deficiency has been found to be highly prevalent in populations residing at high latitudes or leading an indoors oriented lifestyle (25). The available literature contains contrasting data and is often unclear, leading to still open debates about the optimal concentrations of vitamin D and related guidelines for supplementation (24, 26).

To provide an overview of the breadth and validity of the claimed associations of vitamin D with COVID-19 disease, we have done an umbrella review of the available evidences across existing systematic reviews and meta-analyses focused to address the role and optimal concentrations of vitamin D.

## Material and Methods

A detailed protocol for the review has been registered with the International Prospective Register of Systematic Reviews (PROSPERO CRD42021255767). The preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement and the guidelines developed by Aromataris and colleagues were followed to perform this umbrella review (27).

### *Study design*

Systematic reviews and meta-analyses were considered in this study. Studies not following a systematic review approach, narrative reviews and primary studies were excluded. Studies including in this review aimed to investigate the association between Vitamin D and COVID-19 under different social and clinical conditions. Only reviews published in English language were included in this review.

### *Exclusion Criteria*

Reviews not meeting the predefined criteria mentioned above and reviews about other diseases but not including an intervention or with interventions other than Vitamin D supplementation were excluded.

### *Search Methods for Identification of Studies*

Relevant systematic reviews and meta-analyses according to inclusion criteria were identified through systematic searches of the following electronic databases: PubMed, Scopus and the Web of Science. The full search string is ["systematic review" OR "systematic literature review" OR "evidence-based review" OR "meta-analysis" OR "meta-analytic" OR "meta-regression" OR "pooled effect" OR "pooled estimate" OR "scoping review" OR "rapid review" OR "evidence based practice" OR "systematized review" OR "literature review" OR "review of the literature" "Vitamin" AND "D" OR "Cholecalciferol" OR "D3" OR "D2" AND ("COVID" OR "SARS")]. Studies published from January 2020 until March 2021 were included. Reference lists of identified studies were checked.

### *Data Collection and Analysis*

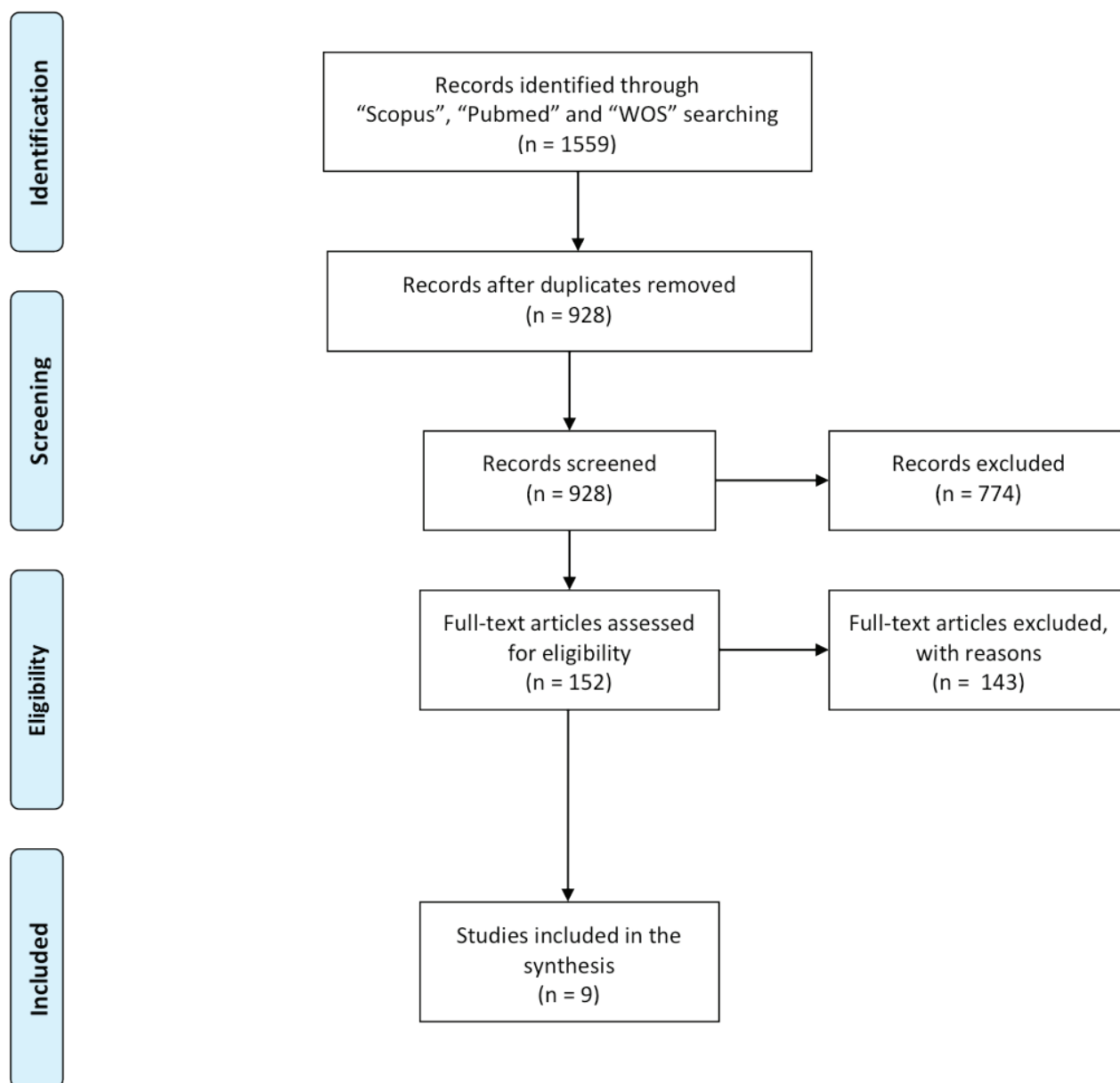
Titles and abstracts obtained from the search were transferred to a reference site (Covidence – Better systematic review management. <https://www.covidence.org/>) for relevance assessment. Potentially eligible studies were screened by title and abstract to evaluate if they met the inclusion criteria by three authors (L.M.M., F.V., G.G.) independently. Potentially relevant studies were independently screened through full-text reading by three authors independently (L.M.M., F.V., G.G.) and a decision was made regarding their inclusion. Disagreements were settled by consensus among the authors. Figure 1 shows the review process.

### *Data Extraction and Management*

Data were independently extracted by two reviewers (L.M.M., F.V.) and the following information was considered for each article: i) first author and month/year of publication, ii) title, iii) study designs (systematic review or meta-analysis) and number and type of studies included, iv) participants' age, v) brief description of the study and vi) main finding of study.

### *Quality Assessment Tools*

Since flaws in the design, conduct, analysis, and reporting of studies can cause to be under or



**Figure 1.** PRISMA flow diagram of the systematic review process.

overestimated, two independent reviewers (L.M.M., F.V.) extracted data on the quality of evidence as well as on the risk of bias. The Covidence – Better systematic review management was used to evaluate the methodological quality and risk of bias of studies included in the systematic review (28). The overall final rate of each systematic review was judged as high, moderate, low, or critically low. Disagreements between the two reviewers (L.M.M, F.V.) were resolved in a consensus meeting.

## Results

The electronic search initially resulted in 1559 citations (Figure 1). A total of 1407 studies were excluded after the title and abstract screening and 152 full-text articles were selected and read. A total of 9 papers were included in this umbrella review (29-37). In table 1, the selected papers for this review were shown and all studies were published in 2021 except for one (33), which was published in 2020.

**Table 1.** The list of review studies included in the umbrella review.

Author (Ref)	Year of Publication	Month of Publication
Akbar et al. (27)	2021	Mar
Bassatne et al. (28)	2021	Jun
Dramè et al. (29)	2021	Apr
Liu et al. (30)	2021	Jan
Pereira et al. (31)	2020	Nov
Petrelli et al. (32)	2021	Mar
Shah et al. (33)	2021	Jan
Teshome et al. (34)	2021	Mar
Yisak et al. (35)	2021	May

Table 2 reported the aims of these studies: principally, the purpose was to assess the Vitamin D level and the association with susceptibility, severity, and mortality related to COVID-19 (29-37). The association of Vitamin D and risk of COVID-19 or adverse outcome in people aged 60 years or over was analysed in only three studies (30, 31, 33). Finally, in this umbrella was included 2 systematic reviews (31, 37)

and 7 meta-analyses (29, 30, 31-36). At least three databases were used in all reviews, except Dramè, Shah and Yisak that used only two databases (31, 35, 37). There is a heterogeneity in date range of database searching: 4 of 9 papers had set up the data range until December 2020 (29, 30, 35, 36), 4 reviews until September – November 2020 (31-33, 37), and only a paper until 31 January 2021 (34).

**Table 2.** Summary of selected article characteristics for the umbrella review regarding aims of included studies, type of review and list and date range of database searching used.

Author (REF)	Objective/s of study	Type of review	Types of databases	Date range of database searching
Akbar et al. (29)	This systematic review and meta-analysis aimed to assess whether low serum 25-hydroxyvitamin D (25-OHD) level is associated with susceptibility to COVID-19, severity, and mortality related to COVID-19.	A systematic review and meta-analysis	PubMed, Scopus, and Embase database	until 9 December 2020
Bassatne et al. (30)	Vitamin D deficiency is associated with an increased risk of COVID-19 related health outcomes and that vitamin D supplementation would decrease these risks.	A systematic review and meta-analysis	Medline (OVID), Embase.com, CINAHL (EBSCO), and Cochrane	until December 18th 2020
Dramè et al. (31)	This systematic review aimed to determine whether there is any available evidence on the association between vitamin D deficiency (compared to non-vitamin D deficiency) or vitamin D supplementation (compared to non-vitamin D supplementation), and risk of COVID-19 or adverse outcome, in people aged 60 years or over.	A systematic review	PubMed and Scopus	All publications up to and, including 5 November, 2020, with no specific start date specified

Table 2 (continued)

Author (REF)	Objective/s of study	Type of review	Types of databases	Date range of database searching
Liu et al. (32)	To assess the relationship between low vitamin D status and COVID-19 risk, authors have performed a meta-analysis of published studies to provide a clinical reference.	A systematic review and meta-analysis	PubMed, Embase, and Cochrane Library databases	from database inception to September 25, 2020,
Pereira et al. (33)	Systematic review and meta-analysis, we analyze the association between vitamin D deficiency and COVID-19 severity, via an analysis of the prevalence of vitamin D deficiency and insufficiency in people with the disease	Meta-analysis	Embase, PubMed, Scopus, Web of Science, ScienceDirect and pre-print Medrevix were searched.	studies published up to October 9, 2020
Petrelli et al. (34)	The study's primary outcome was COVID-19 infection risk in vitamin D-deficient vs non deficient patients. Secondary endpoints were severity (intensive care unit and/or mechanical ventilation), death, and therapeutic effect of vitamin D supplementation in COVID-19-affected patients	A systematic review and meta-analysis	PubMed, the Cochrane Library, and EMBASE	studies published until January 31, 2021
Shah et al. (35)	Meta-analysis aims to understand the effect of oral supplementation of vitamin D on intensive care unit (ICU) requirement and mortality in hospitalized COVID-19 patients.	Meta-analysis	PubMed, preprint servers, and google scholar were searched	From December 2019 to December 2020.
Teshome et al. (36)	The present review aimed to summarize the available evidence regarding the association between Vitamin D levels and the risk of COVID-19 infection.	A systematic review and meta-analysis	PUBMED/MEDLINE, Cochrane/Wiley library, Scopus, and SciELO)	from May 15, 2020, to December 20, 2020
Yisak et al. (37)	The purpose of this study was to undertake a systematic review to summarize and determine whether there is a relation between vitamin D status and COVID-19 infection and prognosis	A systematic review	PubMed and Google Scholar	search from August 2020 to September 2020

In Table 3 the details of these studies were described. The whole of the studies included in each of the considered reviews, showed a wide coverage of geographical areas: in particular 5 works present investigations from at least 5 regions out of 6 of the World Health Organisation (WHO), including European Region, Region of the Americas, South-East Asia Region, Eastern Mediterranean Region, Western Pacific Region (29, 30, 33, 34, 36), while

two papers selected investigations from 2 regions out of 6 (European Region and Region of the Americas or Western Pacific Region) (31, 35) and only one work collected studies from 3 regions out of 6 (European Region, Western Pacific Region, South-East Asia Region) (32). No study analysed works from the African Region. Risk of bias assessment was performed by two independent authors in all papers (29-37).

**Table 3.** Summary of selected article characteristics for the umbrella review regarding the number and type of included study in each review, WHO Regions and instrument used to appraise the primary studies and the rating of quality.

Author (REF)	Number of included study	WHO Regions Included in these studies	Type of studies	Instrument used to appraise the primary studies and the rating of quality
Akbar et al. (29)	14	5/6 (European Region, Region of the Americas, South-East Asia Region, Eastern Mediterranean, Region Western Pacific Region)	2 Cross-Sectional, 1 Observational 2 Prospective Observational, 1 Restrospective Observational (case-control), 8 Retrospective observational	This study follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting guideline. Risk of bias assessment was performed by two independent authors using the Newcastle-Ottawa Scale (NOS).
Bassatne et al. (30)	34	5/6 (European Region, Region of the Americas, South-East Asia Region, Eastern Mediterranean, Region Western Pacific Region)	31 observational studies 3 RCT	The New Castle-Ottawa quality scale for observational studies and the Cochrane Risk of bias tool, version 1 for RCT.
Dramè et al. (31)	11	2/6 (European Region, Western Pacific Region)	4 Prospective cohort 1 Retrospective case control 6 Retrospective cohort	Study selection was performed following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. The quality of included studies was assessed independently by two researchers (LG MD) using the Newcastle-Ottawa scale (NOS) for cohort studies and a modified version of the Newcastle-Ottawa scale for cross-sectional studies.
Liu et al. (32)	10	3/6 (European Region, Western Pacific Region, South-East Asia Region)	Case control studies	The methodological quality of the included study was assessed by the modified Newcastle-Ottawa scale (NOS)
Pereira et al. (33)	27	5/6 (European Region Region of the Americas, South-East Asia Region, Eastern Mediterranean, Region Western Pacific Region)	8 cohort studies 1 Retrospective multicenter 6 retrospective 2 retrospective cross-sectional 4 cross-sectional 2 retrospective cohort 1 population basic 1 case series 1 clinical retrospective 1prospective cohort	Methodological quality was assessed according to the Research Triangle Institute Item Bank (RTI-Item Bank) scale, which assesses the risk of bias.

Table 3 (continued)

Author (REF)	Number of included study	WHO Regions Included in these studies	Type of studies	Instrument used to appraise the primary studies and the rating of quality
Petrilli et al. (34)	43	5/6 (European Region Region of the Americas, South-East Asia Region, Eastern Mediterranean, Region Western Pacific Region)	2 Case-control 1 Cross-sectional 1 Observational 1 Population-based study 5 Prospective 2 Prospective cohort 2 Prospective observational 1 Randomized 1 Registry data 21 Retrospective 2 Retrospective case-control 1 Retrospective cross-sectional 3 Retrospective observational	The study adhered to the Meta-analysis of Observational Studies in Epidemiology (MOOSE) reporting guidelines. Risk of bias assessment was performed by two independent authors using the Newcastle-Ottawa Scale (NOS).
Shah et al. (35)	3	2/6 (European Region, Region of the Americas)	2 randomized controlled trials 1 retrospective case-control study	Systematic review and meta-analysis were conducted in accordance with the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA)  Cochrane RevMan tool was used for quantitative assessment of the data. For the observational study "Risk of Bias Assessment tool for Nonrandomized Studies (RoBANS)" for the controlled observational studies, as recommended by the Cochrane Handbook for Systematic Reviews of Interventions was used.
Teshome et al. (36)	14	5/6 (European Region Region of the Americas, South-East Asia Region, Eastern Mediterranean, Region Western Pacific Region)	3 Cross-sectional 5 Case-control 5 Cohort studies 1 Interim audit	JBI tools
Yisak et al. (37)	9	2/6 (European Region Region of the Americas)	4 cross-sectional 1 randomized controlled trial 1 retrospective cohort 1 prospective cohort 1 retrospective, observational study 1 case- controlled survey	To determine the quality of studies included in this review, the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) system was used.



In table 4, the main findings in all studies were described. Akbar et al. summarized 14 papers, including cross-sectional, prospective, and retrospective studies, and reported that the higher rate of severe COVID-19 was observed in patients with serum 25-OHD level below a cut-off point ranging from 20 to 30 ng/mL (Odd Ratio (OR)= 1.90 (1.24 to 2.93)). Moreover, low serum 25-OHD was associated with higher mortality (OR = 3.08 (1.35 to 7.00)) (29). Basatne et al. (30) selected 34 papers (31 peer-reviewed observational studies and 3 Randomized Control Trials - RCT). The results of observational studies showed a positive trend between serum 25(OH)D level <20 ng/ml and an increased risk of mortality (Relative Risk (RR) = 2.09 (0.92 to 4.77)), Intensive Care Unit (ICU) admission (RR= 4.89 (0.54 to 44.26)), invasive ventilation (RR= 1.34 (0.64 to 2.79)), non-invasive ventilation (RR= 1.08 (0.30 to 3.80)) or SARS-CoV-2 positivity (RR= 1.35 (0.93 to 1.96)). However, these associations are not statistically significant. In this paper, also three RCTs were investigated. The first trial is a pilot study where the treatment group received 0.532 mg of calcifediol on admission, 0.266 mg on days 3 and 7, and then weekly. The OR ratio of ICU admission in patients with calcifediol treatment v/s those with no treatment was 0.03 (0.003–0.25). The second RCT is 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 or placebo for 7 days. At the 14 days followed up, 62.5% of the participants in the intervention arm became SARS-CoV-2 negative compared to only 20.8% in the control arm. The third trial did not find any effect of vitamin D supplementation on COVID-19 related health outcomes. 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.

Dramè et al. summarized 11 papers, including 7 retrospective and 4 prospective studies (31). 4/11 studies compared vitamin D-supplemented patients to non-supplemented patients, while 7/11 compared patients with vs without vitamin D deficiency. In all four studies, patients with vitamin D supplementation had better rates of primary clinical outcomes

(e.g., death, the severity of the disease, oxygen therapy requirement). However, the ideal supplementation regimen (dose, frequency of administration, duration) remains unclear. Moreover, in the seven studies that compared patients with vs without vitamin D deficiency, serum vitamin D level was significantly higher in COVID-19-negative patients compared to COVID-19-positive patients.

Liu et al. selected a total of 10 articles (32). Overall, the pooled OR in the fixed-effect model showed that vitamin D deficiency (< 25 ng/ml) or insufficiency was associated with an increased risk of COVID-19 (OR = 1.43 (1.00–2.05)). In addition, COVID-19-positive individuals had lower vitamin D levels than COVID-19-negative individuals (Standardized Mean Difference (SMD) = -0.37 (-0.52 to -0.21)).

Pereira et al. included in this review 27 papers (33). The main outcome of this papers was the prevalence of vitamin D deficiency in severe cases of COVID-19. Vitamin D deficiency was little associated with a higher chance of infection by COVID-19 (OR = 1.35 (0.80–1.88)) but linked to severe cases of COVID-19 (OR = 1.64 (1.30–2.09)), where 64% of cases had the vitamin D deficiency compared with mild cases. Moreover, a vitamin D concentration insufficiency increased hospitalization (OR = 1.81 (1.41–2.21)) and mortality from COVID-19 (OR = 1.82 (1.06–2.58)).

Petrelli et al. selected 43 papers for qualitative synthesis and 17 for quantitative synthesis (34). An OR > 1 was associated with the worst outcome in deficient compared with nondeficient patients. Among subjects with deficient vitamin D values, risk of COVID-19 infection was higher compared to those with replete values (OR = 1.26 (1.19–1.34)). Vitamin D deficiency (<20 ng/ml) was also associated with worse severity and higher mortality than in nondeficient patients (OR = 2.6 (1.84–3.67) and OR = 1.22 (1.04–1.43), respectively).

Shah et al. selected for final synthesis 3 papers, including 2 RCTs and 1 retrospective case-control study (35). The results of meta-analysis involved pooled data of 532 hospitalized patients (189 on vitamin D supplementation and 343 on usual care/placebo) of COVID-19 from three studies (two randomized controlled trials, one retrospective case-control study). Statistically ( $p < 0.0001$ ) lower ICU requirement was



**Table 4.** Summary of finding articles selected for the umbrella review.

Author (REF)	Results/Finding	Vitamin D cut-off (ng/mL)
Akbar et al. (29)	This meta-analysis indicates that low serum 25-OHD levels were associated with higher infection, severe COVID-19, and mortality rate.	<20-30 ng/ml
Bassatine et al. (30)	This systematic review and meta-analysis reveal 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.	<20 ng/ml
Dramè et al. (31)	This systematic review supports an association between vitamin D deficiency and the risk of COVID-19 in aged people. In addition, vitamin D deficiency appears to expose these subjects to a greater risk of adverse outcomes. Because of its simplicity of administration, and the rarity of side effects, including vitamin D in preventive strategies for certain viral diseases, it appears to be an attractive option.	<20 ng/ml
Liu et al. (32)	This systematic review and meta-analysis indicated that low vitamin D status might be associated with an increased risk of COVID-19 infection.	< 25 ng/ml
Pereira et al. (33)	This systematic review observes a positive association between vitamin D deficiency and the severity of the disease.	ND
Petrelli et al. (34)	Reduced vitamin D values resulted in a higher infection risk, mortality and severity COVID-19 infection. Supplementation may be considered as preventive and therapeutic measure.	<20 ng/ml
Shah et al. (35)	Final meta-analysis involved pooled data of 532 hospitalized patients (189 on vitamin D supplementation and 343 on usual care/placebo) of COVID-19 from three studies. Statistically ( $p < 0.0001$ ) lower ICU requirement was observed in patients with vitamin D supplementation as compared to patients without supplementations (odds ratio: 0.36; 95% CI: 0.210-0.626).	ND
Teshome et al. (36)	This review showed that improving vitamin D status in the general population has a potential benefit in reducing the risk of acquiring COVID-19 infection.	<20 ng/mL
Yisak et al. (37)	This review shows that blood vitamin D status can determine the risk of being infected with COVID-19, seriousness of COVID-19, and mortality from COVID-19.	<20 ng/ml

observed in patients with vitamin D supplementation as compared to patients without supplementations (OR = 0.36 (0.210-0.626)). In case of mortality, vitamin D supplements had comparable findings with placebo treatment/usual care (OR = 0.93 (0.413-2.113)). Subgroup analysis could not be performed due to limited number of studies and hence dose and duration dependent effect of vitamin D could not be evaluated.

Teshome et al. have selected 14 papers that met the inclusion criteria (36). The qualitative synthesis indicated that vitamin D deficient individuals were at higher risk of COVID-19 infection as compared to vitamin D sufficient patients. The pooled analysis showed that individuals with Vitamin-D deficiency were 80% more likely to acquire COVID-19 infection as compared to those who had sufficient Vitamin D levels (OR = 1.80 (1.72 - 1.88)).

Yisak et al. included 9 studies in the review (37). Risk Factors of COVID-19 Infection, Severity, and Mortality data from COVID-19 patients were associated with vitamin D low level (<20 ng/ml) (OR = 1.77 (1.07– 2.93)). In addition, disease severity was associated with vitamin D deficiency (OR = 1.95 (1.07– 3.56)), and odds of admission to ICU were higher in vitamin D-deficient individuals (OR = 2.55 (1.28–5.08)).

Finally, 7 papers out of 9 defined Vitamin D cut-off, expressed in ng/mL, below which the vitamin D level can be considered insufficient (29-32, 34, 36, 37). In 5 reviews the cut off is <20 ng/ml (28, 29, 32, 34, 35), in one <20-30 ng/ml (29), while in another is < 25 ng/ml (32).

The methodological quality of included reviews was generally high. Six studies were judged to be of high quality (29-32, 36), 2 of moderate quality (35, 37) and 1 studies of low quality (34). The systematic review and meta-analysis conducted by Petrelli and colleagues (34) failed to meet nearly 5, 6 and 7 items of AMSTAR, perhaps due to a lack of clarifications in the description of the article. Findings of the quality assessment were presented in Supplemental Table S1.

## Discussion

The different approaches to cope with COVID-19 pandemic raised several discussions on the optimal

strategies to apply for contrasting infection disease spreading in the population and provide appropriate treatments and effective prevention measures. Although interpersonal distancing, masks, contact tracing and vaccination remain the main tools to protect the population from SARS-CoV-2 infection, additional approaches were considered (42-46). Within this context several studies highlighted also the potential benefits of vitamin D supplementation in preventing and treating infection diseases and in particular COVID-19 (20-23). Vitamin D is a lipid-soluble vitamin that is essential for maintaining good health, growth, and strong bones (5, 7-9). The effect of vitamin D on infections is less clear, however, several studies have underlined that the sufficient blood vitamin D levels seems to play a helpful role in immune system functioning, as cellular response, and protection against the severity of infections caused by microorganisms (38-46). Moreover, several investigations have highlighted the role of Vitamin D levels versus severe COVID-19 raising discussions about the benefits of supplementation of this vitamin when treating the illness caused by SARS-CoV-2, as well as in their prevention (47). However, the studies on vitamin D in COVID-19 patients have shown conflicted results and even if several systematic reviews and meta-analysis have been published they are affected by heterogeneity regarding methodologies, criteria, and the different population target (48). Therefore, the purpose of this umbrella review was to summarize and comprehensively review systematic reviews and meta-analyses on whether low levels of serum 25-OHD are associated with susceptibility to COVID 19, severity, and mortality related to COVID-19. This is the first umbrella review applying the principles of evidence-based medicine to find out a possible role of vitamin D in prevention of COVID-19. A total of 9 studies were selected including investigations in different countries. Here, we summarized investigations covering all WHO regions, with the only exception of the African region, to have a wider view of the world population and reduce possible bias related to local features. Indeed, the levels of vitamin D in serum and their absorption and production may depend by several variables, including genetic determinants (49, 50), demographic factors, as age or gender (51-53), lifestyles (54-59), seasonal and geographical variations (60-62). The results of this study

support the observation that vitamin D deficiency can present an association with COVID-19 severity, independently from the WHO macro-regions where the data were collected. The findings of metanalysis that include populations from 5 WHO regions show that low serum 25-OHD levels are associated with higher infection risks, more severe COVID-19 outcomes, and mortality rates. Moreover, the low levels of Vitamin D are also correlated with the need of ICU admission and mechanical ventilation. (30, 34, 36). The whole of these results supports the role of vitamin D in contrasting COVID-19 infection and the need of supplementation and adequate diets to prevent the disease and its consequences. Our final results are in agreement with previous reports that suggest how vitamin D may play a role in SARS-CoV-2 infection (63, 64). From a mechanistic viewpoint, the hypothesis that vitamin D can modulate host responses to SARS-CoV-2 has relevant plausibility, as many potential links can be considered between respiratory viral infections such as COVID-19 and vitamin D status (65, 66).

Some studies in this umbrella have investigated the association between vitamin D3 and COVID-19 infection risk and severity (30, 34, 37). Although the priority of some systematic review approaches was to include a higher number of studies and enhance the stringency of the analysis by excluding preprint articles that had not been peer reviewed yet and systematically avoiding those investigations with major confounders or a higher risk of bias, studies that have investigated the association between severity and Vitamin D supplementation does not seem conclusive. Particularly, we found low certainty of evidence from RCTs on the use of vitamin D supplementation and reduction of severity of COVID-19, suggesting that further studies are needed to extend this conclusion to COVID-19 patients and its reduction in mortality rate. This observation can be explained from the limitations in several study designs that collected patients with different degree of severity of the disease, age or other demographic and medical conditions that lead some troubles and complications in the interpretations.

The present study selected, with rigorous methodological appraisal, 9 systematic reviews with several study design and collection of different primary papers. Among the results, a critical point seems to be the definition of vitamin D deficiency or of insufficient

level and vitamin D cut-off. In the literature, this question is often discussed, but there are still disagreements about the optimal serum level for 25-hydroxyvitamin D (25(OH)D) and the appropriate supplemental dose (67). We have further searched among the analysed reviews the optimal cut off below which we can say that the level is insufficient. In five of the reviews the cut off is <20 ng/ml (30, 31, 34, 36, 37), while in two is slightly higher different <20-30 ng/ml (29), and < 25 ng/ml (32). This finding is in accordance with the considerable evidence that is available to support a final threshold for sufficiency at least over 20 ng/ml or even 50 nmol/L as indicated by some consensus documents and health authorities (68-73).

With respect to the methodological quality assessment, more than 77 % of the included studies were classified as having a high methodological quality. Most studies missed only item number one, which concerns the inclusion of the components of the PICO process. Some systematic reviews, in fact, have not established some of the specified inclusion criteria, especially the target population (29, 32, 34-37). However, this can, also, be considered as a choice of the study design in order to acquire just a general representation of the population. Furthermore, most of the reviewed studies did not explain or only partially explain the risk of bias (items number 5-7, 9, 11 and 12) or did not consider the impact of bias in the paper discussion. These aspects are fundamental for the quality of a review since they strongly depend on the quality of the primary evidence and most papers have comprehended this aspect, further supporting the stringent selection and elevated level of studies included in this umbrella analysis. However, our study has both strengths and limitations that should be acknowledged when interpreting the results. The main strength of this umbrella review is that it provides a systematic synthesis of studies that involved population on a global scale, within the 5 WHO regions. Moreover, a rigorous quality assessment was performed using the latest version of an effective and recognised tool to assess systematic reviews, namely the AMSTAR 2. Main limits of the work involve the considerable heterogeneity in the study designs included in each single review, and the types of strategies for the interventions, including duration, outcomes, methodology, and other measures whose difference may confound an homogeneous

synthesis and comparison of collected data. This umbrella review summarized available evidences for a possible role of Vitamin D as a tool in prevention of COVID-19 and its severity. Recent *in vitro* study further confirms this hypothesis showing how vitamin D3 is effective in slowing SARS-CoV-2 replication in human cells (74). The whole of the observed results strongly welcomes further epidemiological studies and randomized clinical trials, to confirm potentials for prevention, unravel mechanisms and optimize Vitamin D supplementation as an additional and promising tool in coping with SARS-COV-2 pandemic.

## Conclusion

The recent literature contains contrasting data opening debates about the optimal concentrations of vitamin D and related guidelines for application in dietary supplementation. Within this context, this umbrella study overviews evidences related to a potential role of vitamin D deficiency in COVID-19 severity. Collected data support the association between Vitamin D supplementation and the reduction in the COVID-19 gravity outcomes. Additional and more robust data from randomized controlled trials are needed to substantiate also possible effects on a decrease in COVID-19 mortality. The overall results support the need for further epidemiological studies and clinical trials, in order to strengthen knowledge about possible therapeutic uses, biological mechanisms and optimization of Vitamin D posology. In addition, innovative public health programs and health education campaigns, are desirable to optimize the widespread deficiency of vitamin D in the populations. This umbrella review approached the state of the art summarizing evidences and supporting a possible role of Vitamin D in prevention of COVID-19 and its severity.

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## APPENDIX

Supplemental Table S1. Quality assessment by AMSTAR2

Author	Values* of the items of AMSTAR 2**																Final rate***
(Ref)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Akbar et al. (29)	1	2	2	2	2	2	2	2	2	NA	2	2	2	2	2	NA	High
Bassatne et al. (30)	2	2	2	2	2	2	2	2	2	NA	2	2	2	2	2	NA	High
Dramè et al. (31)	2	2	2	1	2	2	2	2	2	NA	NA	NA	NA	NA	NA	NA	High
Liu et al. (32)	1	2	2	2	2	2	2	2	2	NA	2	2	2	2	2	NA	High
Pereira et al. (33)	2	2	2	2	2	2	2	2	2	NA	2	2	2	2	2	NA	High
Petrelli et al. (34)	1	2	2	2	/	/	/	2	1	NA	1	1	2	2	2	NA	Low
Shah et al. (35)	1	2	2	1	2	2	2	2	2	NA	2	2	2	2	2	NA	Moderate
Teshome et al. (36)	1	2	2	2	2	2	2	2	2	NA	2	2	2	2	2	NA	High
Yisak et al. (37)	1	2	2	1	2	2	2	2	2	NA	NA	NA	NA	NA	NA	NA	Moderate

\*Quantitative value of each item: "NA" non applicable, "/" no, "1" partial yes, "2" yes

\*\*Items of AMSTAR 2

- Did the research questions and inclusion criteria for the review include the components of PICO?
- Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any
- Did the review authors explain their selection of the study designs for inclusion in the review?
- Did the review authors use a comprehensive literature search strategy?
- Did the review authors perform study selection in duplicate?
- Did the review authors perform data extraction in duplicate?
- Did the review authors provide a list of excluded studies and justify the exclusions?
- Did the review authors describe the included studies in adequate detail?
- Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?
- Did the review authors report on the sources of funding for the studies included in the review?
- If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?
- If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?
- Did the review authors account for RoB in individual studies when interpreting/ discussing the results of the review?
- Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?
- If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?
- Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?

\*\*\*Final rate evaluation

High - No or one non-critical weakness: the systematic review provides an accurate and comprehensive summary of the results of the available studies that address the question of interest

Moderate - More than one non-critical weakness\*: the systematic review has more than one weakness but no critical flaws. It may provide an accurate summary of the results of the available studies that were included in the review

Low - One critical flaw with or without non-critical weaknesses: the review has a critical flaw and may not provide an accurate and comprehensive summary of the available studies that address the question of interest

Critically low - More than one critical flaw with or without non-critical weaknesses: the review has more than one critical flaw and should not be relied on to provide an accurate and comprehensive summary of the available studies.