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Complete List of Authors:	Zhu, Lemei; Hunan Key Laboratory of the Research and Development of Novel Pharmaceutical Preparations; Changsha Medical University Zhang, Yuan; Hunan Key Laboratory of the Research and Development of Novel Pharmaceutical Preparations Li, Xi; Hunan Key Laboratory of the Research and Development of Novel Pharmaceutical Preparations; Changsha Medical University Zou, Xuemin; Hunan Key Laboratory of the Research and Development of Novel Pharmaceutical Preparations; Changsha Medical University Bing, Pingping; Hunan Key Laboratory of the Research and Development of Novel Pharmaceutical Preparations Qi, Mingxu; University of South China, Department of Cardiovascular Medicine He, Binsheng; Hunan Key Laboratory of the Research and Development of Novel Pharmaceutical Preparations
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Vitamin D Supplementation for Managing COVID-19 in Patients with Vitamin D Deficiency : Meta-analysis and Trial Sequential Analysis of Randomized Controlled Trials

Lemei Zhu[1,2], Yuan Zhang[1], Xi Li[1,2], Xuemin Zou[1,2], Pingping Bing[1*], Mingxu Qi[3*], Binsheng He[1*]

1. Hunan Key Laboratory of the Research and Development of Novel Pharmaceutical Preparations, Changsha, 410219, China.

2. School of Public Health, Changsha Medical University, Changsha, 410219, China.

3. Department of Cardiovascular Medicine, Affiliated Nanhua Hospital, University of South China, Hengyang,421001, China

Corresponding Author:

Pingping Bing, Hunan Key Laboratory of the Research and Development of Novel Pharmaceutical Preparations, Changsha, 410219, China. (Email: bpping@163.com)

Mingxu Qi, Department of Cardiovascular Medicine, Affiliated Nanhua Hospital, University of South China, Hengyang,421001, China. (Email: qimingxuqi@163.com)

Binsheng He, unan Key Laboratory of the Research and Development of Novel Pharmaceutical Preparations, Changsha, 410219, China. (Email: hbscsmu@163.com)

Abstract

Background Vitamin D deficiency was prevalent among population. Former studies showed that vitamin D supplementation might be useful for treating COVID-19 infection. Therefore, we performed a meta-analysis to explore vitamin D supplementation efficacy in treating COVID-19 patients with vitamin D deficiency.

Method PubMed, Cochrane Library, Embase and Web of Science was lastly searched on June 1, 2024 to identifying randomized controlled trials exploring vitamin D supplementation for patients with COVID-19 and vitamin D deficiency. The primary outcomes included mortality during follow-up, 28-day mortality, need for mechanical ventilation and ICU.

Result A total of nine studies, comprising 814 participants, were included in the analysis. The pooled results indicated that vitamin D supplementation was associated with a lower risk of mortality (RR 0.76; 95% CI 0.59 to 0.96). However, this apparent benefit was not robust when examined through subgroup analysis, the leave-one-out method, and trial sequential analysis. Consequently, the role of vitamin D supplementation in treating COVID-19 patients with vitamin D deficiency remains inconclusive. Regarding other outcomes, there was no statistically significant difference between vitamin D supplementation and no supplementation in terms of 28-day mortality, the need for mechanical ventilation and ICU admission, or the length of stay in the ICU and hospital.

Conclusion Vitamin D supplementation couldn't effectively improve clinical outcomes of COVID-19 patients with vitamin D deficiency. As a result, our results didn't strongly support its use as a specific therapeutic measure against COVID-19.

Keywords: Vitamin D supplementation; Vitamin D deficiency; COVID-19; Meta-analysis; Trial sequential analysis

Strengths and limitations of this study

1. This study is the first meta-analysis specifically targeting patients with vitamin D deficiency and COVID-19.
2. This meta-analysis conducted subgroup analyses based on the severity of COVID-19, supplementation frequency, definition of vitamin D deficiency, development level of the country, risk of bias, and sample size.
3. This study used trial sequential analysis to examine the robustness of the meta-analysis results.

Introduction

COVID-19, caused by the SARS-CoV-2 virus, is a highly transmissible and potentially severe respiratory illness that has resulted in a global pandemic, affecting millions of people worldwide with varying morbidity and mortality rates[1].

Vitamin D, a steroid hormone derived from cholesterol, plays a significant role in regulating the expression of various genes, including those in immune cells[2]. Vitamin D deficiency is widespread across the globe; for example, 40% of the European population is reported to lack sufficient vitamin D[3]. Maintaining appropriate levels of vitamin D is essential for optimal respiratory immune function[2 4-6]. Despite this, the precise impact of vitamin D supplementation on preventing and treating COVID-19 remains a topic of debate. According to a systematic review, vitamin D supplementation can significantly reduce the severity of COVID-19 infection, suggesting its use as a supplementary treatment for COVID-19[7]. In contrast, a 2021 meta-analysis that included eight randomized controlled trials (RCTs) found that vitamin D supplementation did not enhance clinical outcomes in patients infected with SARS-CoV-2[8].

Currently, no meta-analysis specifically focuses on COVID-19 patients with vitamin D deficiency. To investigate the role of vitamin D supplementation in these patients, we conducted a meta-analysis of randomized controlled trials to determine whether vitamin D supplementation improves clinical outcomes in COVID-19 patients with vitamin D deficiency.

Methods

This meta-analysis of RCTs was performed following the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) checklist[9]. The study protocol was registered on PROSPERO (CRD42024573791).

Search strategy and selection criteria

A comprehensive literature search was conducted on June 1, 2024 across several databases including PubMed, Cochrane Library, Embase, and Web of science with Mesh and broad search terms. We also manually searched the reference lists of relevant review articles. After completing the initial research, we conducted the same search again to include the latest published studies. The detailed search strategy was in the appendix.

The retrieved literature was imported into EndNote X9. After removing duplicate references, it was assessed for eligibility by two reviewers. According to the PICO principle, inclusion criteria were: COVID-19 patients with vitamin D deficiency, intervention group using vitamin D supplementation, and the control group not using vitamin D supplementation, with reported relevant clinical outcomes. Exclusion criteria were: non-randomized controlled trials, and studies for which full text could not be retrieved. The definition of vitamin D deficiency was according to previous studies[10-13].

Data extraction

A comprehensive data extraction form was developed based on the guidelines outlined in the Cochrane Handbook for Systematic Reviews of Interventions. The

form was piloted on a subset of the included studies before extracting the following data: author details, participant characteristics, intervention details (type, duration, frequency, and other details), primary and secondary outcomes, follow-up times.

The consistency between data extractors was measured using the Kappa value.

Quality assessment

Potential sources of bias in RCTs were assessed using Risk of Bias 2 (Rob2), a revised tool for assessing the risk of bias in randomized trials. Rob2 encompasses five key domains: 1. Randomization process;2. Deviations from intended interventions;3. Missing outcome data;4. Measurement of the outcome;5. Selection of the reported result. Within each domain, bias was evaluated and categorized as either low risk, some concerns, or high risk, depending on the circumstances and relevant evidence. Ultimately, the overall bias of each study was classified as either low risk, some concerns, or high risk, based on the comprehensive assessment of bias across the five domains. When there was a discrepancy in the assessment results for a certain domain, the outcome was resolved through discussion.

The quality of evidence was assessed in line with the GRADE tools.

Outcomes

The primary outcomes were mortality during follow up and 28-day mortality. The secondary outcomes included need for mechanical ventilation and ICU admission, length of stay in hospital and ICU.

Statistically analysis

Dichotomous variables were presented as event number and total number. The

Mantel-Haenszel model was used for analyzing dichotomous variables. Continuous variables were presented as mean and standard deviation. The Inverse-variance method was used for analyzing continuous variables. The DerSimonian-Laird was used to assess the statistical heterogeneity across studies. $I^2 \geq 50\%$ was deemed as the existence of statistical heterogeneity. Considering the potential clinical heterogeneity, random-effects model was used for analysis. Subgroup analysis according to different characteristic (severity of COVID-19, vitamin D supplement, definition of vitamin D deficiency and so on) was conducted on mortality during follow-up. Sensitivity analysis was conducted through leave-one-out method. Trial sequential analysis was also performed to explore the robust of result. In trial sequential analysis, the statistical power was set to 80%. The funnel plot and Egger's test were used to assess the publication bias. In this study, trial sequential analysis was performed by Trial sequential Analysis software (Copenhagen Trial Unit, Centre for Clinical Intervention Research, Rigshospitalet). The meta-analysis was performed by Stata17. The level of statistical significance was set as $P<.05$. All statistical tests performed were 2 sided.

Results

Literature search

A total of 178 studies were initially found across all databases, with 64 identified as duplicates. After screening titles and abstracts, 78 studies were excluded. The remaining 36 studies were then assessed for full text. Ultimately, 9 studies[10-18] met the inclusion criteria and were included in the analysis (Figure1).

Baseline study characteristics

A total of 9 studies[10-18], encompassing 814 participants, were included. The vitamin D dosage ranged from 3,000 IU to 60,000 IU. Two studies used a single high dose of vitamin D supplementation, while seven studies employed a continuous dosing regimen. Six studies defined vitamin D deficiency as <20 ng/ml, two studies as <30 ng/ml, and one study as <10 ng/ml. Additionally, two studies focused on severe COVID-19, and one study examined moderate to severe COVID-19 cases.

Quality assessment

Three studies had some concerns of bias, primarily due to their open-label design and lack of blinding. Six studies were assessed to have a low risk of bias. The detailed distribution of bias is shown in Figure 2.

The Kappa value, used to estimate the equivalence of data extraction in this study, was 0.86.

Mortality

Eight studies reported the mortality during follow-up. The pooled result showed that vitamin D supplementation had statistically significantly lower risk of mortality than no vitamin D supplementation (RR 0.76; 95%CI 0.59 to 0.96) (Figure3A).

To assess the vitamin D's role in reducing hospitalization mortality, we analyzed 28-day mortality. The pooled result showed that there was no statistically significantly difference between vitamin D supplementation and no vitamin D supplementation (RR 0.79; 0.49 to 1.26) (Figure3B).

Need for ICU admission and mechanical ventilation

Three studies reported on the need for mechanical ventilation, and the pooled results showed no statistically significant difference between vitamin D supplementation and no vitamin D supplementation (RR 0.90; 95% CI 0.69 to 1.17) (Figure4A).

Four studies reported on the need for ICU admission, and the pooled results showed no statistically significant difference between vitamin D supplementation and no vitamin D supplementation (RR 0.84; 95% CI 0.45 to 1.56) (Figure4B).

Length of stay in ICU and hospital

Six studies reported on the length of stay in the ICU, and the pooled results showed no statistically significant difference between vitamin D supplementation and no vitamin D supplementation (MD -0.41; 95% CI -1.09 to 0.28) (Figure5A).

Four studies reported on the length of stay in the hospital, and the pooled results showed no statistically significant difference between vitamin D supplementation and no vitamin D supplementation (MD -0.07; 95% CI -0.61 to 0.46) (Figure5B).

Subgroup analysis

Considering the limited number of included studies, we performed a subgroup analysis only on mortality during follow-up. The subgroups were defined based on the severity of COVID-19, supplementation frequency, definition of vitamin D deficiency, development level of the country, risk of bias, and sample size. No statistically significant differences were observed in any subgroup, except for the developing country group (RR 0.70; 95% CI 0.50 to 0.98) (Figure6).

Sensitivity analysis

Sensitivity analysis was performed on morality during follow-up by leave-one-out

method and trail sequential analysis.

Sensitivity analysis was performed on mortality during follow-up using the leave-one-out method and trial sequential analysis (eFigure1).

Using the leave-one-out method, we found that excluding the studies by Burgarin et al., Bychinin et al.[10], Maghbooli et al.[11], and Singh et al.[13] resulted in no statistically significant difference between vitamin D supplementation and no vitamin D supplementation.

We also performed a trial sequential analysis on mortality during follow-up. With 80% power, the pooled result showed no statistically significant difference (RR 0.74; α-spending adjusted CI 0.46 to 1.19). The required sample size (RSA) was determined to be 1874 (eFigure2).

Publication bias

The funnel plot of the above outcomes was symmetric. To more objectively assess publication bias, we also performed Egger's test, which showed no significant evidence of publication bias ($P > 0.05$).

Grade assessment

The quality of evidence for the above outcomes ranged from very low to moderate (Table2).

Discussion

Our study was the first to explore the efficacy of vitamin D in treating COVID-19 patients with vitamin D deficiency. We found that vitamin D supplementation could

reduce mortality during follow-up. However, this result should be interpreted with caution for the following reasons. Firstly, the leave-one-out method showed that nearly half of the studies could change the conclusion, indicating that the result was not robust. Secondly, in the subgroup analysis, most groups showed no statistically significance difference between vitamin D supplementation and no vitamin D supplementation. Thirdly, trial sequential analysis revealed no statistically significant difference between vitamin D supplementation and no vitamin D supplementation when adjusted confidence intervals were considered. The analysis also indicated that a larger sample size is needed to determine the true effect of vitamin D.

Regarding other outcomes in our study, vitamin D did not appear to reduce the need for mechanical ventilation and ICU admission or shorten the length of stay in the ICU and hospital. Overall, the efficacy of vitamin D in treating COVID-19 patients with vitamin D deficiency remains inconclusive. More studies are needed to explore this further.

In 2023, Meng et al.'s meta-analysis[19] explored the efficacy of vitamin D in treating COVID-19. Their results showed that while vitamin D supplementation couldn't reduce mortality, it might be beneficial in reducing the severity of illness caused by SARS-CoV-2, particularly in vitamin D-deficient patients. Additionally, their study indicated that vitamin D supplementation could reduce the need for ICU admission. However, they did not analyze the data based on follow-up time, and new research has since been published. Our study results show that vitamin D supplementation does not reduce the need for ICU admission. Recently, a review also showed that vitamin

D deficiency is linked to an increased risk of acquiring SARS-CoV-2 infection and poor COVID-19 prognosis, however, available evidence with regard to improved clinical outcomes with vitamin D supplementation is inconsistent[20]. Furthermore, whether vitamin D can reduce mortality still requires further exploration.

The relationship between vitamin D and COVID-19 has been a subject of extensive research, with mixed findings regarding its efficacy in preventing or treating the disease. Observational studies that initially suggested a link between low vitamin D levels and worse COVID-19 outcomes may have been confounded by other factors such as age, comorbidities, and socioeconomic status[21-25]. These factors themselves are risk factors for both vitamin D deficiency and severe COVID-19, complicating the interpretation of results[26-31]. A number of clinical trials have produced mixed results, with some showing no significant difference in outcomes between those receiving vitamin D supplementation and those who did not[32-36]. This inconsistency suggests that vitamin D may not have a substantial impact on COVID-19 outcomes.

However, our study has certain limitations. First, the number of studies included is relatively small, with only nine randomized controlled trials and small sample sizes. Second, although there was no significant statistical heterogeneity, clinical heterogeneity among the studies cannot be ignored. The severity of patients' diseases and the frequency and dosage of vitamin D supplementation varied among the studies. To address this, we conducted a subgroup analysis and found that vitamin D supplementation did not reduce mortality in different subgroups. Third, although our conclusions suggest that vitamin D supplementation may reduce mortality, sensitivity

analysis revealed that the conclusions are not reliable. Therefore, more high-quality research is needed in the future to further explore the role of vitamin D supplementation in vitamin D deficient COVID-19 patients.

Conclusion

Our results showed that vitamin D supplementation does not significantly reduce mortality, the need for mechanical ventilation and ICU admission, or the length of stay in ICU and hospital. While vitamin D is essential for overall health and maintaining adequate levels is beneficial, current evidence does not strongly support its use as a specific therapeutic measure against COVID-19.

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AUTHOR CONTRIBUTIONS

LMZ, PPB, MXQ and BSH: proposed the design, searched the literature, collected, analysed and interpret the data, and wrote the report; LMZ, XMZ, YZ, and XL searched and collected the literature; LMZ, YZ, XMZ, XL and BSH analysed and interpreted the data.

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Declaration of competing interest

The authors declare that they have no known competing finical interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table1. Characteristic of included randomized controlled trials

Table2. Quality of evidence

Figure1. Flowchart of literature search

Figure2. Risk of bias of included studies by Risk of Bias Tool 2

Figure3. Vitamin D supplementation versus no vitamin D supplementation on mortality (A) during follow-up and 28-day mortality (B).

Figure4. Vitamin D supplementation versus no vitamin D supplementation on need for mechanical ventilation (A) and ICU admission (B).

Figure5. Vitamin D supplementation versus no vitamin D supplementation on length of stay in ICU (A) and hospital (B).

Figure6.Subgroup analysis of mortality during follow-up.

Table1. Characteristic of included randomized controlled trials

Study	Country	Severity of COVID-19	Intervention group	Control group	Definition of Vitamin D deficiency	Follow-up
Bugarin2023	Croatia	Severe COVID-19	10,000 IU of cholecalciferol daily during ICU stay	Standard care	<20ng/ml	3 months
Bychinin2022	Russia	Severe COVID-19	60,000 IU of cholecalciferol once per seven days followed by daily maintenance doses of 5000 IU. The high dose repeated on day 8, 16, 24, 32. The supplementation was administered until the patients was discharge from ICU.	Placebo	<20ng/ml	During hospitalization
Cervero2022	Spain	NA	10,000 IU of cholecalciferol daily for 14 days	Standard care	<30ng/ml	28 days
Dilokpattanamongkol2024	Thailand	NA	2 mcg of alfacalcidol daily during the hospitalization	Standard care	<20ng/ml	During hospitalization

Maghbooli2021	Iran	NA	3000-6000 IU per day of vitamin D3 for 30 days	Placebo	<30ng/L	2 months
Murai2021	Brazil	Moderate to severe COVID-19	Single dose of 200,000 IU of vitamin D3	Placebo	<20ng/L	4 months
Niet2022	Belgium	NA	25,000 IU of vitamin D3 per day over 4 consecutive days, followed by 25,000 IU per week up to 6 weeks	Placebo	<20ng/L	9 weeks
Rastogi2022	India	NA	Daily 60000 IU of cholecalciferol for 7 days , and a weekly supplementation of 60000IU provided to those with 25(OH)D > 50 ng/ml or else continued on daily vitamin D 60,000 IU supplementation for another 7 days up until day 14	Placebo	<20ng/L	3 weeks

Singh2024	India	Severe	A single dose of 60,000 IU of cholecalciferol	Placebo	<10 ng/ml	During hospitalization

Table2 Quality of evidence

Outcomes	No. of participants (No. of trials)	Risk ratio (95%CI)	Mean difference (95%CI)	Risk of bias[a]	Inconsistency[b]	Imprecision[c]	Small study effects[d]	Certainty of evidence
Mortality during follow-up	737 (8)	0.76 (0.59,0.96)		Down graded	Not down graded	Down graded	Not down graded	Low
28-day mortality	442 (4)	0.79 (0.49,1.26)		Down graded	Not down graded	Down graded	Not down graded	Low
Need for mechanical ventilation	327 (3)	0.90 (0.69,1.17)		Down graded	Not down graded	Down graded	Not down graded	Low
Need for ICU admission	349 (4)	0.84 (0.45,1.56)		Not down graded	Not down graded	Down graded	Not down graded	Moderate
Length of stay in ICU	582 (6)		-0.41 (-1.09,0.28)	Down graded	Down graded	Down graded	Not down graded	Very low
Length of stay in hospital	378 (4)		-0.07 (-0.61,0.46)	Not down graded	Down graded	Down graded	Not down graded	Low

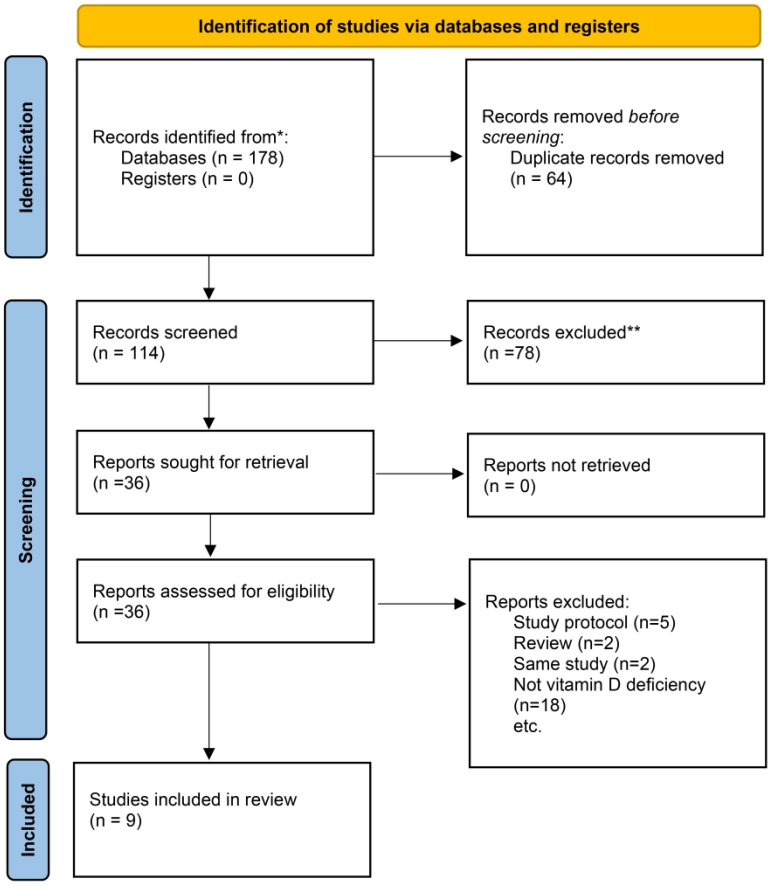
ICU, intensive care unit

[a] Downgraded by one level because >25% of participants in this comparison were from studies at high risk of bias.

[b] Downgraded by one level because heterogeneity (I^2) >50%.

[c] Downgraded by one level because the limits of the 95% confidence interval were 20% different to the point estimates.

[D] Downgraded by one level owing to small study bias.

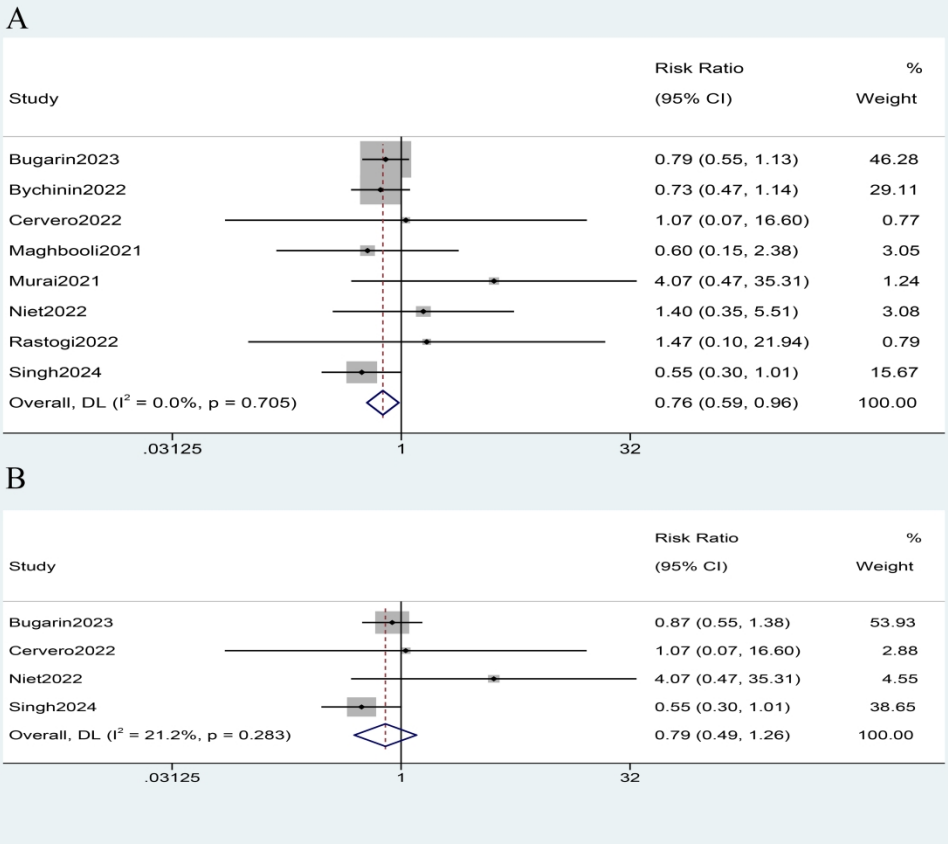


Flowchart of literature search

1389x2060mm (72 x 72 DPI)

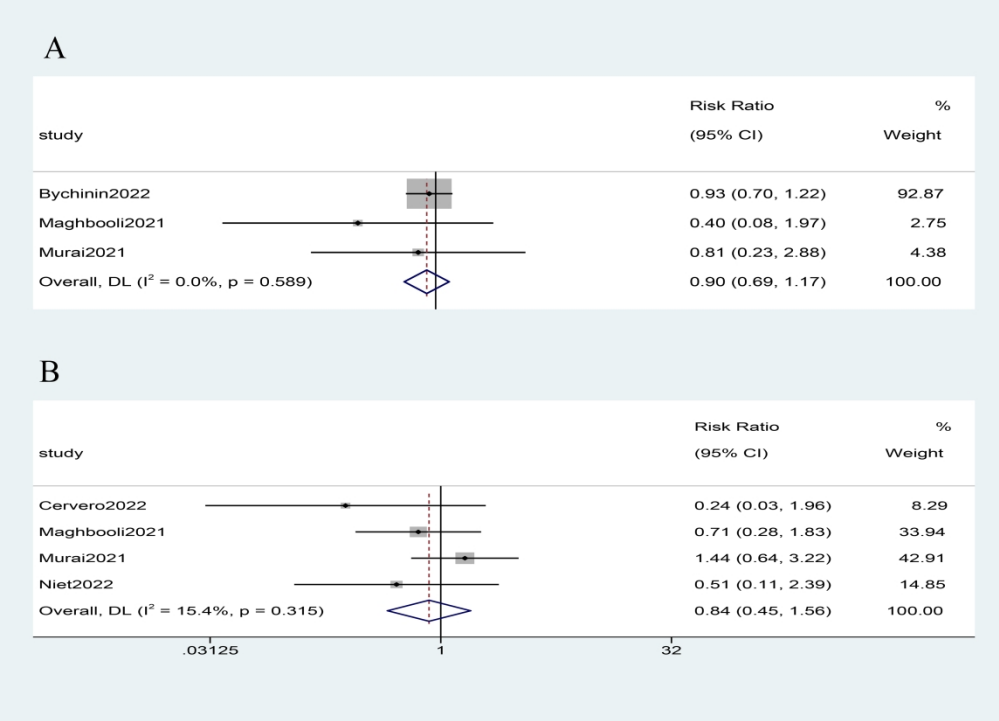
Risk of bias of included studies by Risk of Bias Tool 2

2116x1695mm (72 x 72 DPI)



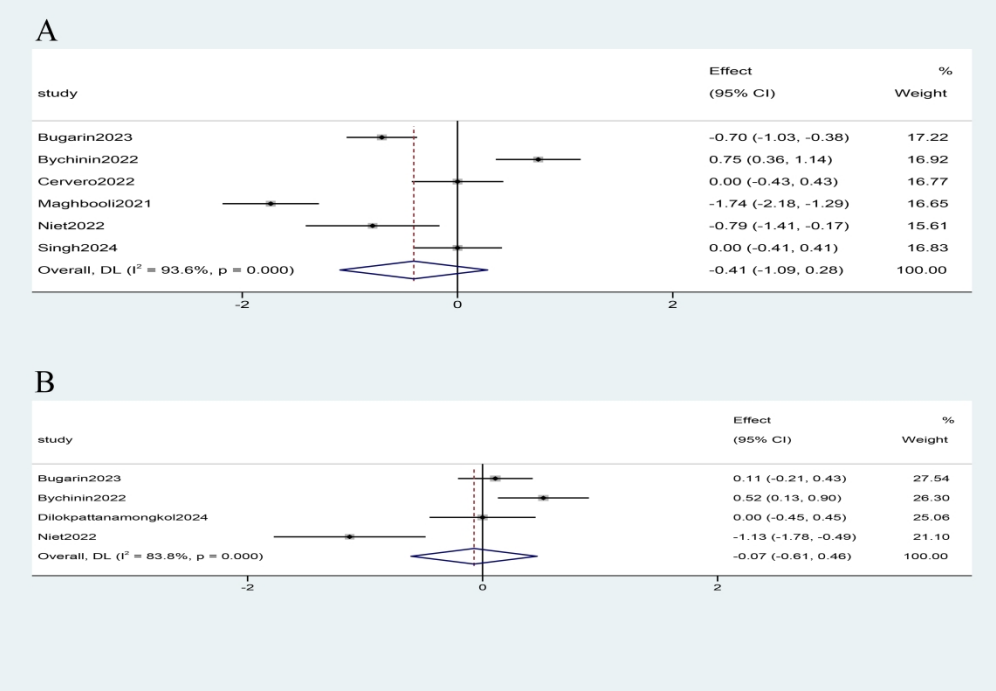
Vitamin D supplementation versus no vitamin D supplementation on mortality (A) during follow-up and 28-day mortality (B).

2116x1806mm (72 x 72 DPI)



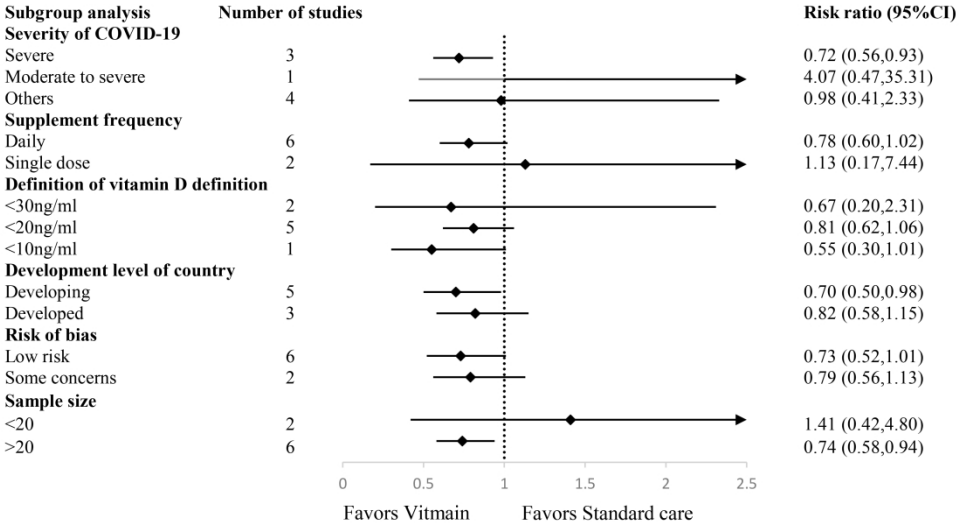
Vitamin D supplementation versus no vitamin D supplementation on mortality (A) during follow-up and 28-day mortality (B).

1763x1274mm (72 x 72 DPI)



Vitamin D supplementation versus no vitamin D supplementation on length of stay in ICU (A) and hospital (B)

2469x1709mm (72 x 72 DPI)



Subgroup analysis of mortality during follow-up.

1244x648mm (72 x 72 DPI)

Supplement

Search strategy

PubMed

1. “COVID-19”[Mesh] OR "COVID-19"[tiab] OR “COVID 19” [tiab] OR “2019-nCoV Infection” [tiab] OR “2019 nCoV Infection” [tiab] OR “2019-nCoV Infections” [tiab] OR “Infection, 2019-nCoV” [tiab] OR “SARS-CoV-2 Infection” [tiab] OR “Infection, SARS-CoV-2” [tiab] OR “SARS CoV 2 Infection” [tiab] OR “SARS-CoV-2 Infections” [tiab] OR “2019 Novel Coronavirus Disease” [tiab] OR “2019 Novel Coronavirus Infection” [tiab] OR “COVID-19 Virus Infection” [tiab] OR “COVID 19 Virus Infection” [tiab] OR “COVID-19 Virus Infections” [tiab] OR “Infection, COVID-19 Virus” [tiab] OR “Virus Infection, COVID-19” [tiab] OR “COVID19” [tiab] OR “Coronavirus Disease 2019” [tiab] OR “Disease 2019, Coronavirus” [tiab] OR “Coronavirus Disease-19” [tiab] OR “Coronavirus Disease 19” [tiab] OR “Severe Acute Respiratory Syndrome Coronavirus 2 Infection” [tiab] OR “COVID-19 Virus Disease” [tiab] OR “COVID 19 Virus Disease” [tiab] OR “COVID-19 Virus Diseases” [tiab] OR “Disease, COVID-19 Virus” [tiab] OR “Virus Disease, COVID-19” [tiab] OR “SARS Coronavirus 2 Infection” [tiab] OR “2019-nCoV Disease” [tiab] OR “2019 nCoV Disease” [tiab] OR “2019-nCoV Diseases” [tiab] OR “Disease, 2019-nCoV” [tiab] OR “COVID-19 Pandemic” [tiab] OR “COVID 19 Pandemic” [tiab] OR “Pandemic, COVID-19” [tiab] OR “COVID-19 Pandemics” [tiab]
2. "Vitamin D"[Mesh] OR "vitamin D"[tiab] OR "vitamin D3"[tiab] OR "vit D"[tiab] OR "vit D3"[tiab] OR "calciferol"[tiab] OR "cholecalciferol"[tiab] OR "calcidiol"[tiab] OR "calcitriol"[tiab] OR "25 hydroxyvitamin d"[tiab] OR "25 hydroxyvitamin D3"[tiab] OR "25 hydroxycalciferol"[tiab] OR "1,25 dihydroxyvitamin D"[tiab] OR "1,25 dihydroxyvitamin D3"[tiab] OR "calcifediol"[tiab]
3. ((compar*[tiab]) OR ((singl*[tiab] or doubl*[tiab] or tripl*[tiab]) and (mask*[tiab] or blind*[tiab]))) OR (random*[tiab] or placebo[tiab] or controlled[tiab] or trial*[tiab])
4. #1 And #2 And #3

Cochrane Library

1. MeSH descriptor: [COVID-19] explode all trees
2. (COVID-19 OR COVID 19 OR 2019 nCoV Infection OR SARS-CoV-2 Infection OR Infection, SARS-CoV-2 OR SARS CoV 2 Infection OR SARS-CoV-2 Infections OR 2019 Novel Coronavirus Disease OR 2019 Novel Coronavirus Infection OR COVID-19 Virus Infection OR COVID 19 Virus Infection OR COVID-19 Virus Infections OR Infection, COVID-19 Virus OR Virus Infection, COVID-19 OR COVID19 OR Coronavirus Disease 2019 OR Disease 2019, Coronavirus OR Coronavirus Disease-19 OR Coronavirus Disease 19 OR Severe Acute Respiratory Syndrome Coronavirus 2 Infection OR COVID-19 Virus Disease OR COVID 19 Virus Disease OR COVID-19 Virus Diseases OR Disease, COVID-19 Virus OR Virus Disease, COVID-19 OR SARS Coronavirus 2 Infection OR 2019 nCoV Disease OR COVID-19 Pandemic

OR COVID 19 Pandemic OR Pandemic, COVID-19 OR COVID-19 Pandemics):ti,ab
3. #1 OR #2
4. MeSH descriptor: [Vitamin D] explode all trees
5. (vitamin D OR vitamin D3 OR vit D OR vit D3 OR calciferol OR cholecalciferol OR calcidiol OR calcitriol OR 25 hydroxyvitamin d OR 25 hydroxyvitamin D3 OR 25 hydroxycalciferol OR 1,25 dihydroxyvitamin D OR 1,25 dihydroxyvitamin D3 OR calcifediol):ti,ab
6. #4 OR #5
7. ((compar*) OR ((singl* or doubl* or tripl*) and (mask* or blind*))) OR (random* or placebo or controlled or trial*):ti,ab
8. #3 AND #6 AND #7

Embase

1. 'coronavirus disease 2019'/exp
2. ((Covid-19) OR (Covid 19) OR (2019-nCoV Infection) OR (SARS-CoV-2 Infections)):ti,ab
3. #1 OR #2
4. 'vitamin d'/exp
5. ((vitamin D) OR (vitamin D3) OR (25 hydroxycalciferol) OR (1,25 dihydroxyvitamin D3)):ti,ab
6. #4 OR #5
7. #3 AND #6
8. compar* OR ((singl* OR doubl* OR tripl*) AND (mask* OR blind*)) OR random*:ti,ab OR placebo:ti,ab OR controlled:ti,ab OR trial*:ti,ab
9. #7 AND #8

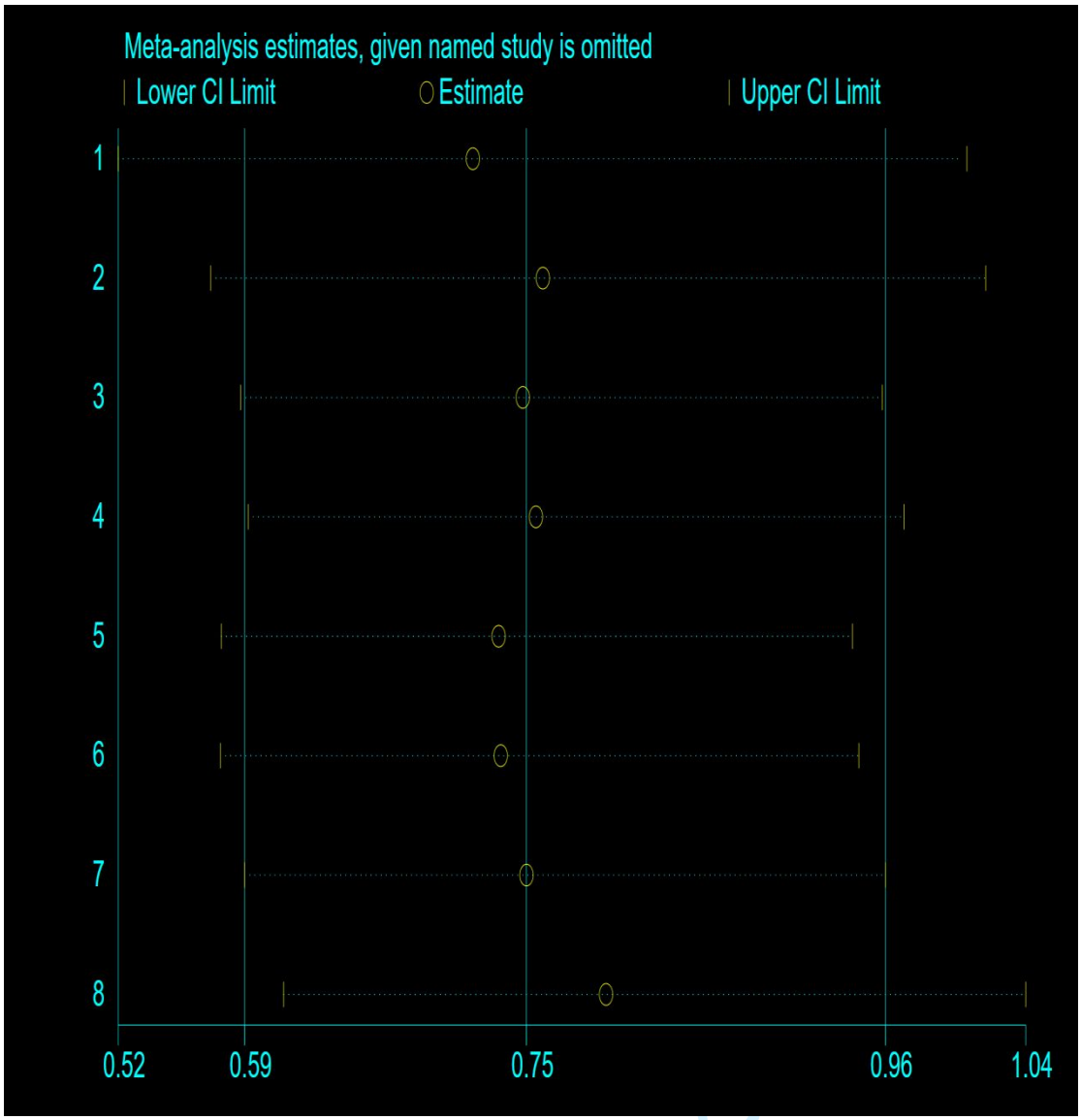
Web of Science

1. TS=(COVID-19 OR COVID 19 OR 2019-nCoV Infection OR 2019 nCoV Infection OR 2019-nCoV Infections OR Infection, 2019-nCoV OR SARS-CoV-2 Infection OR Infection, SARS-CoV-2 OR SARS CoV 2 Infection OR SARS-CoV-2 Infections OR 2019 Novel Coronavirus Disease OR 2019 Novel Coronavirus Infection OR COVID-19 Virus Infection OR COVID 19 Virus Infection OR COVID-19 Virus Infections OR Infection, COVID-19 Virus OR

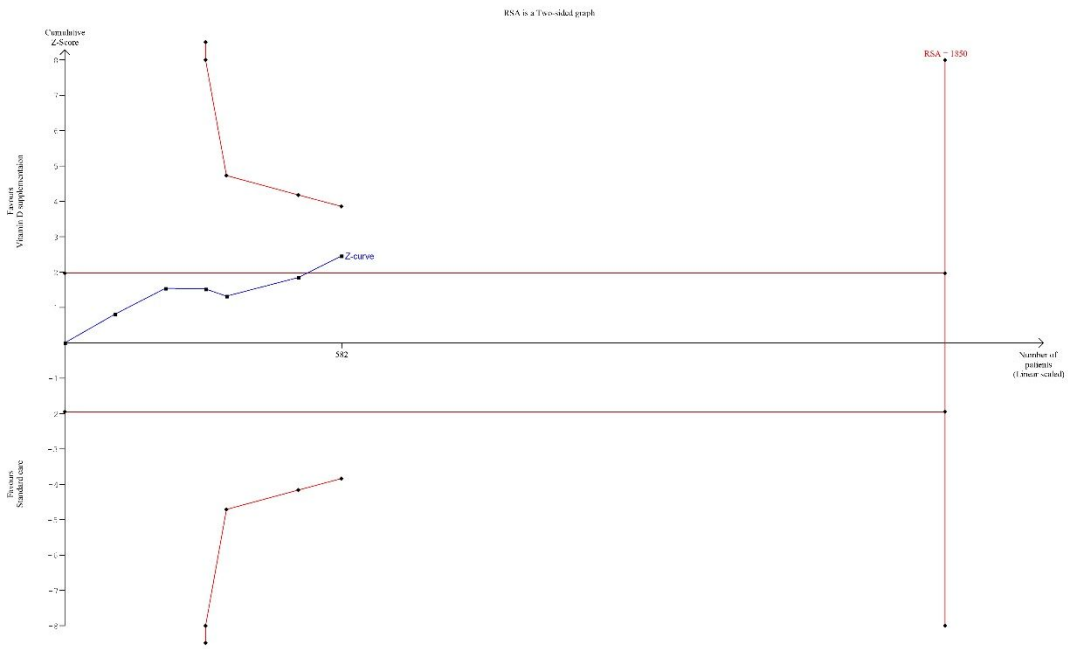
Virus Infection, COVID-19 OR COVID19 OR Coronavirus Disease 2019 OR Disease 2019, Coronavirus OR Coronavirus Disease-19 OR Coronavirus Disease 19 OR Severe Acute Respiratory Syndrome Coronavirus 2 Infection OR COVID-19 Virus Disease OR COVID 19 Virus Disease OR COVID-19 Virus Diseases OR Disease, COVID-19 Virus OR Virus Disease, COVID-19 OR SARS Coronavirus 2 Infection OR 2019-nCoV Disease OR 2019 nCoV Disease OR 2019-nCoV Diseases OR Disease, 2019-nCoV OR COVID-19 Pandemic OR COVID 19 Pandemic OR Pandemic, COVID-19 OR COVID-19 Pandemics)

2. TS= (vitamin D OR vitamin D3 OR vit D OR vit D3 OR calciferol OR cholecalciferol OR calcidiol OR calcitriol OR 25 hydroxyvitamin d OR 25 hydroxyvitamin D3 OR 25 hydroxycalciferol OR 1,25 dihydroxyvitamin D OR 1,25 dihydroxyvitamin D3 OR calcifediol)
3. #1 AND #2
4. TS=(((compar*) OR ((singl* or doubl* or tripl*) and (mask* or blind*))) OR (random* or placebo or controlled or trial*))
5. #3 AND #4

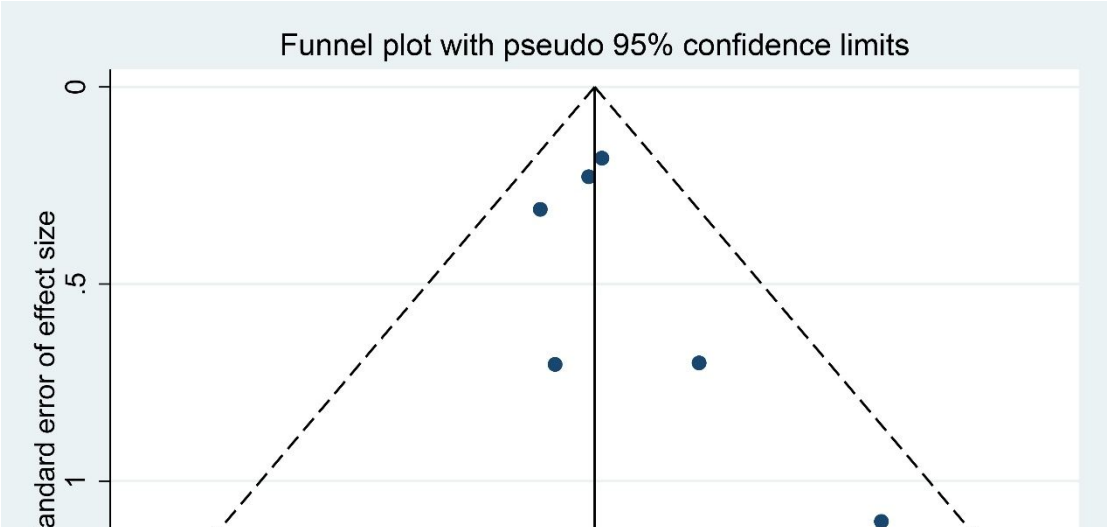
eFigure1. Leave-one-out on mortality during follow-up



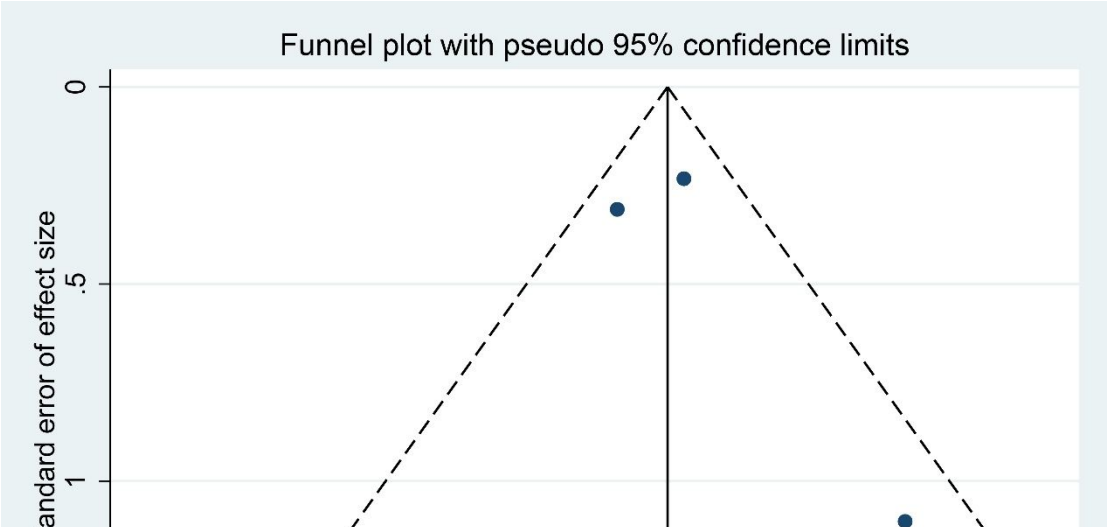
eFigure2. Trial sequential analysis on mortality during follow-up



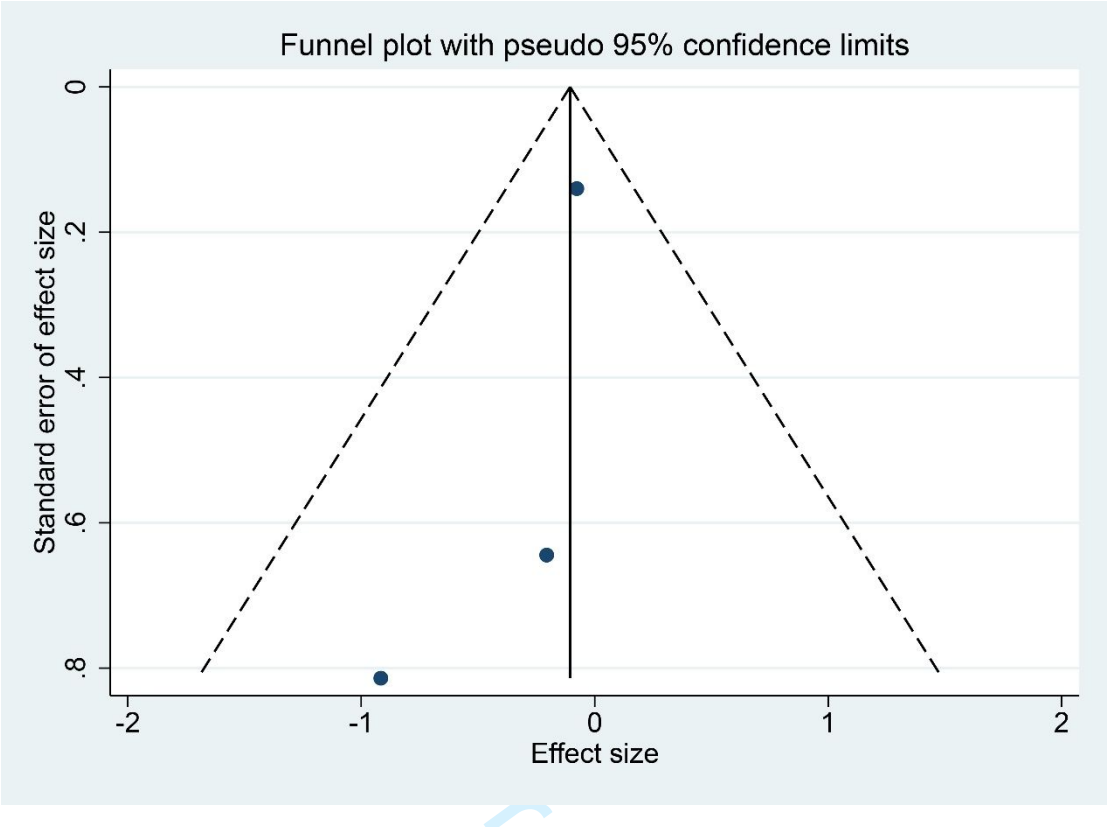
eFigure3. Funnel plot of mortality during follow-up



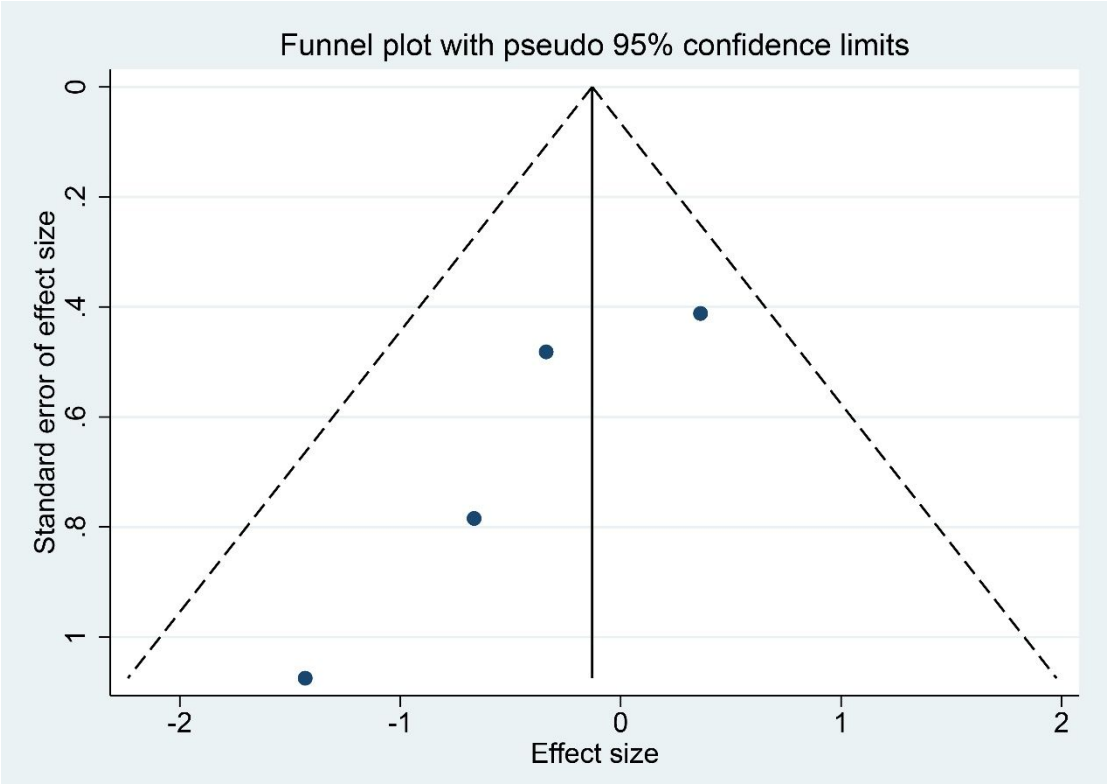
eFigure4. Funnel plot of 28day-mortality



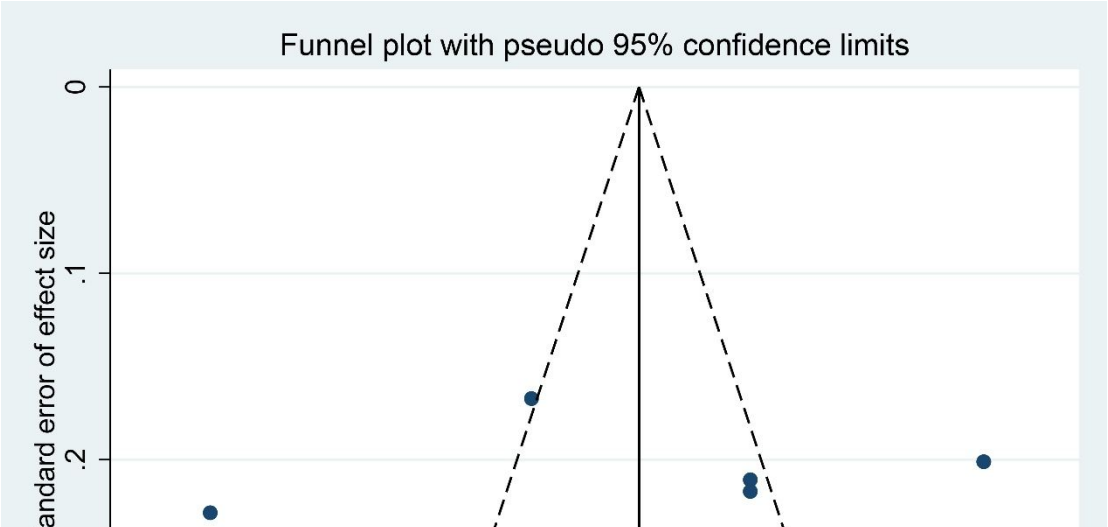
eFigure5. Funnel plot of need for mechanical ventilation



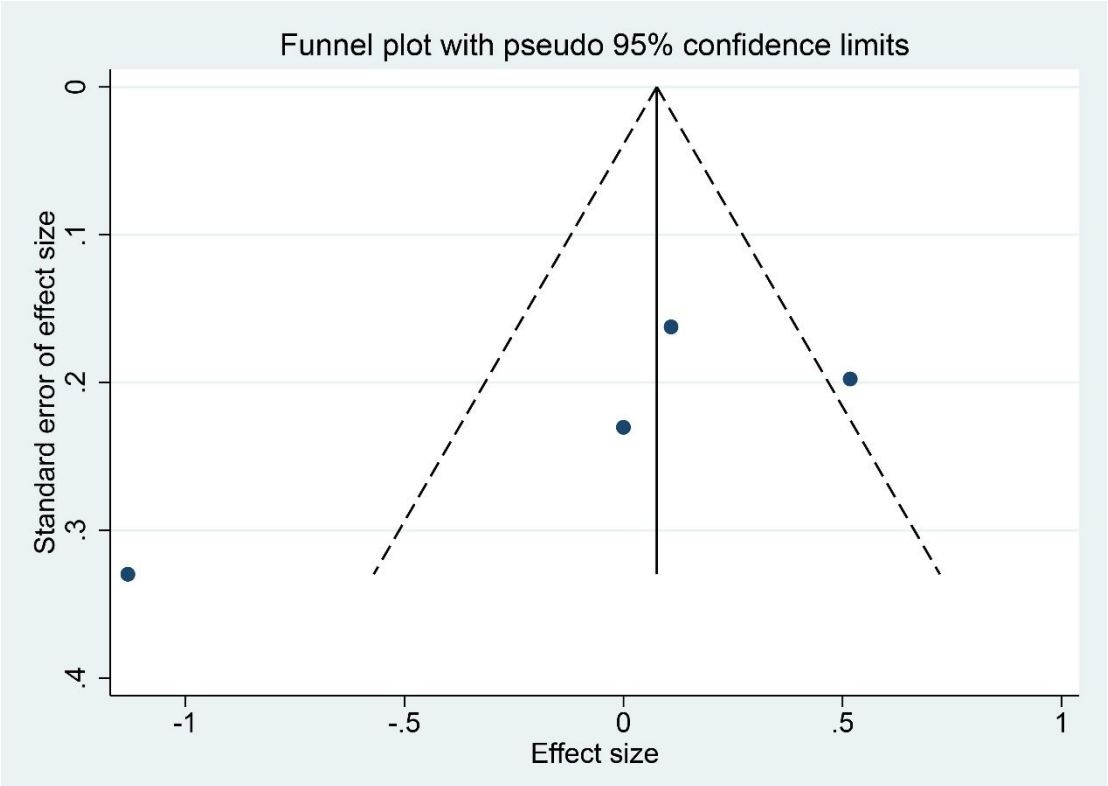
eFigure6. Funnel plot of need for ICU admission



eFigure7. Funnel plot of length of stay in ICU



eFigure8. Funnel plot of length of stay in hospital



BMJ Open

Vitamin D Supplementation for Managing COVID-19 in Patients with Vitamin D Deficiency：A systematic review and Meta-analysis of Randomized Controlled Trials

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Primary Subject Heading:	Respiratory medicine
Secondary Subject Heading:	Global health
Keywords:	COVID-19, Meta-Analysis, NUTRITION & DIETETICS, Health

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Vitamin D Supplementation for Managing COVID-19 in Patients with Vitamin D Deficiency：A systematic review and Meta-analysis of Randomized Controlled Trials

Lemei Zhu[1,2], Yuan Zhang[1], Xi Li[1,2], Xuemin Zou[1,2], Pingping Bing[1*], Mingxu Qi[3*], Binsheng He[1* 5]

1. Hunan Key Laboratory of the Research and Development of Novel Pharmaceutical Preparations, Changsha Medical University, Changsha, 410219, China.

2. School of Public Health, Changsha Medical University, Changsha, 410219, China.

3. Department of Cardiovascular Medicine, Affiliated Nanhua Hospital, University of South China, Hengyang,421001, China

Corresponding Author:

Pingping Bing, Hunan Key Laboratory of the Research and Development of Novel Pharmaceutical Preparations, Changsha, 410219, China. (Email: bpping@163.com)

Mingxu Qi, Department of Cardiovascular Medicine, Affiliated Nanhua Hospital, University of South China, Hengyang,421001, China. (Email: qimingxuqi@163.com)

Binsheng He, unan Key Laboratory of the Research and Development of Novel Pharmaceutical Preparations, Changsha, 410219, China. (Email: hbscsmu@163.com)

Abstract

Objectives Vitamin D deficiency was prevalent among population. Former studies showed that vitamin D supplementation might be useful for treating COVID-19 infection. Therefore, we performed a meta-analysis to explore vitamin D supplementation efficacy in treating COVID-19 patients with vitamin D deficiency.

Design Systematic review and meta-analysis

Data sources PubMed, Cochrane Library, Embase and Web of Science.

Eligibility Criteria Randomized controlled trials exploring vitamin D supplementation for patients with COVID-19 and vitamin D deficiency.

Data extraction and synthesis Two independent reviewers employed standardized methods to search, screen, and code the included studies. The primary outcomes included mortality during follow-up, 28-day mortality, need for mechanical ventilation and ICU. The secondary outcome included length of stay in hospital and ICU. The risk of bias was assessed using the Risk of Bias 2 tool. Depending on the level of heterogeneity, either a random-effects model or a fixed-effects model was applied. The findings were summarized using GRADE evidence profiles and synthesized qualitatively.

Results A total of nine studies, comprising 870 participants, were included in the analysis. The pooled results indicated that vitamin D supplementation was associated with a lower risk of mortality (Risk ratio 0.76; 95% CI 0.60 to 0.97). However, this apparent benefit was not robust when examined through the leave-one-out method, and trial sequential analysis. Regarding other outcomes, there was no statistically

significant difference between vitamin D supplementation and no supplementation in terms of 28-day mortality, the need for mechanical ventilation and ICU admission. Vitamin D supplementation was associated with a 0.41-day shorter length of stay in the ICU (Mean difference -0.41; 95%CI -1.09 to 0.28) and a 0.07-day shorter length of stay in the hospital (Mean difference -0.07; 95%CI -0.61 to 0.46) compared to no supplementation; however, neither difference was statistically significant.

Conclusion Based on evidence of low to moderate quality, vitamin D supplementation reduced the mortality rate during follow-up in COVID-19 patients with vitamin D deficiency. However, it did not improve 28-day mortality, nor did it reduce the need for mechanical ventilation and ICU admission, or the length of stay in the ICU and hospital.

Keywords: Vitamin D supplementation; Vitamin D deficiency; COVID-19; Meta-analysis; Trial sequential analysis

Strengths and limitations of this study

- This meta-analysis of RCTs was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist.
- Comprehensive literature search across multiple databases to identify relevant studies.
- Rigorous inclusion criteria to ensure the quality and relevance of studies.
- Use of trial sequential analysis and sensitivity analysis to assess the statistical robustness of the results.
- The number of studies included is limited, with only nine randomized controlled trials and relatively small sample sizes.

Introduction

COVID-19, caused by the SARS-CoV-2 virus, is a highly transmissible and potentially severe respiratory illness that has resulted in a global pandemic, affecting millions of people worldwide with varying morbidity and mortality rates[1 2].

Vitamin D, a steroid hormone derived from cholesterol, plays a significant role in regulating the expression of various genes, including those in immune cells[3]. In hospitalized COVID-19 patients, vitamin D also showed anti-inflammatory effects[4]. Vitamin D deficiency is widespread across the globe; for example, 40% of the European population is reported to lack sufficient vitamin D, and vitamin D deficiency is also common in high-altitude regions such as Nepal, the Andes, and Tibet[5 6]. Maintaining appropriate levels of vitamin D is essential for optimal respiratory immune function[3 7-11]. Despite this, the precise impact of vitamin D supplementation on preventing and treating COVID-19 remains a topic of debate. According to a systematic review, vitamin D supplementation can significantly reduce the severity of COVID-19 infection, as measured by outcomes such as hospitalization rates, the need for mechanical ventilation, and mortality, suggesting its use as a supplementary treatment for COVID-19[12]. In contrast, a 2021 meta-analysis that included eight randomized controlled trials (RCTs) found that vitamin D supplementation did not enhance clinical outcomes in patients infected with SARS-CoV-2[13]. Recently, a meta-analysis conducted by Meng et al. explored the role of vitamin D in the prevention and treatment of SARS-CoV-2 infection. Their results suggested that vitamin D supplementation may have some beneficial impact on the

severity of illness caused by SARS-CoV-2, particularly in vitamin D deficient patients. Although they specifically analyzed patients with vitamin D deficiency, the studies they included were limited, and the analysis focused solely on mortality as the outcome. Moreover, they did not perform comprehensive subgroup analyses, such as based on the severity of vitamin D deficiency.

Amrein et al. raised another important point, namely that vitamin D is clearly not a cure-all and is likely effective only when there is a deficiency[6]. To comprehensively investigate the role of vitamin D supplementation in these patients, we conducted a meta-analysis of randomized controlled trials to determine whether vitamin D supplementation improves clinical outcomes (mortality during follow-up, 28-day mortality, need for mechanical ventilation and ICU and length of stay in hospital and ICU) in COVID-19 patients with vitamin D deficiency.

Methods

This meta-analysis of RCTs was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) checklist[14]. The study protocol was registered on PROSPERO (CRD42024573791).

Search strategy and selection criteria

A comprehensive literature search was conducted on June 1, 2024 across several databases including PubMed, Cochrane Library, Embase, and Web of science with Mesh and broad search terms. We also manually searched the reference lists of relevant review articles. After completing the initial research, we conducted the same

search again to include the latest published studies. The detailed search strategy was in the appendix. The retrieved literature was imported into EndNote X9. After removing duplicate references, it was assessed for eligibility by two reviewers. Based on the PICO principle, the inclusion criteria we applied are as follows:

P: COVID-19 patients with vitamin D deficiency;

I: standard care plus vitamin D supplementation;

C: standard care;

O: mortality rate, need for mechanical ventilation or ICU admission, length of stay in ICU and hospital.

Exclusion criteria were: non-randomized controlled trials, and studies for which full text could not be retrieved. The definition of vitamin D deficiency was according to previous studies[6 15-17]. Any disputes will be resolved through discussion.

Data extraction

A comprehensive data extraction form was developed based on the guidelines outlined in the Cochrane Handbook for Systematic Reviews of Interventions. The form was piloted on a subset of the included studies before extracting the following data: author details, participant characteristics, intervention details (type, duration, frequency, and other details), primary and secondary outcomes, follow-up times.

The consistency between data extractors was measured using the Kappa value. Any disputes will be resolved through discussion.

Quality assessment

Potential sources of bias in RCTs were assessed using Risk of Bias 2 (Rob2), a revised tool for assessing the risk of bias in randomized trials[18]. Rob2 encompasses five key domains: 1. Randomization process;2. Deviations from intended interventions;3. Missing outcome data;4. Measurement of the outcome;5. Selection of the reported result. Within each domain, bias was evaluated and categorized as either low risk, some concerns, or high risk, depending on the circumstances and relevant evidence. Ultimately, the overall bias of each study was classified as either low risk, some concerns, or high risk, based on the comprehensive assessment of bias across the five domains. When there was a discrepancy in the assessment results for a certain domain, the outcome was resolved through discussion.

Outcomes

The primary outcomes were mortality during follow up and 28-day mortality. The secondary outcomes included need for mechanical ventilation and ICU admission, length of stay in hospital and ICU. Mortality during follow-up refers to the deaths that occurred during the follow-up period in each study. Since the follow-up durations vary across studies, the time frame for mortality during follow-up is not consistent. 28-day mortality specifically refers to the mortality rate from the start of the study up to day 28. Need for mechanical ventilation and ICU admission refers to patients who initially did not require mechanical ventilation or ICU admission but received mechanical ventilation or were admitted to the ICU during the study. Length of stay in hospital and ICU refers to the duration of hospitalization and ICU stay for patients who received different treatments.

Statistically analysis

Dichotomous variables were presented as event numbers and total numbers, with combined outcomes expressed as Risk Ratio (RR) with 95% Confidence Intervals (CIs). Continuous variables were presented as mean and standard deviation, with combined outcomes expressed as Mean Difference (MD) with 95% Confidence Intervals (CIs). The choice of analysis model was based on the level of heterogeneity. If $I^2 \geq 50\%$, heterogeneity was considered significant, and the DerSimonian-Laird method combined with a random-effects model was used for analysis. If $I^2 < 50\%$, no significant heterogeneity was assumed, and the Inverse-variance method combined with a fixed-effects model was used for analysis[19]. Subgroup analysis according to different characteristics (severity of COVID-19, vitamin D supplementation, definition of vitamin D deficiency, and so on) was conducted on mortality during follow-up. Sensitivity analysis was performed using the leave-one-out method. A funnel plot was generated to subjectively assess publication bias, and Egger's test was also conducted to objectively test for publication bias; if $p > 0.05$, no significant publication bias was assumed. In this study, trial sequential analysis was performed using Trial Sequential Analysis software (Copenhagen Trial Unit, Centre for Clinical Intervention Research, Rigshospitalet) (http://ctu.dk/tsa/). The meta-analysis was performed using Stata 17 (STATA Corporation, Texas, USA) (https://www.stata.com/stata17/). The quality of evidence was assessed by GRADE guidelines[20].

Patient and public involvement

None.

Results

Literature search

A total of 178 studies were initially found across all databases, with 64 identified as duplicates. After screening titles and abstracts, 78 studies were excluded. The remaining 36 studies were then assessed for full text. Ultimately, 10 studies[15-17 21-27] met the inclusion criteria and were included in the analysis (Figure1).

Baseline study characteristics

A total of 10 studies[15-17 21-26], encompassing 870 participants, were included. The vitamin D dosage ranged from 3,000 IU to 200,000 IU. Three studies used a single high dose of vitamin D supplementation, while seven studies employed a continuous dosing regimen. Seven studies defined vitamin D deficiency as <20 ng/ml, two studies as <30 ng/ml, and one study as <10 ng/ml. Additionally, two studies focused on severe COVID-19, and two study examined moderate to severe COVID-19 cases (Table1).

Quality assessment

We evaluated the outcomes reported in the studies. We found that among the twenty-eight relevant outcomes, fourteen were classified as low risk and fourteen as having some concerns. For example, the study by Soliman et al. did not provide detailed information on the randomization method, which raised concerns about the randomization process. In the studies by Singh et al. and others, vitamin D deficiency

was defined as <10 ng/ml, while Cervero et al. and Maghbooli et al. defined deficiency as <30 ng/ml, which differed from the commonly accepted definition of deficiency. Therefore, these studies also carried an overall risk of bias. The detailed distribution of bias was shown in eTable1.

The Kappa value, used to estimate the equivalence of data extraction in this study, was 0.86.

Mortality

Night studies reported the mortality during follow-up. The pooled result showed that the risk of death in the vitamin D group was 24% lower than in the non-supplementation group (RR 0.76; 95%CI 0.60 to 0.97) (Figure2).

To assess the vitamin D's role in reducing hospitalization mortality, we analyzed 28-day mortality. The pooled result showed that the risk of mortality was 21% lower in the vitamin D group, but this difference was not statistically significant (RR 0.78; 95%CI 0.55 to 1.38) (Figure2).

Need for ICU admission and mechanical ventilation

Three studies reported on the need for mechanical ventilation, and the pooled results showed the need for mechanical ventilation was 10% lower in the vitamin D group, but this difference was not statistically significant (RR 0.90; 95%CI 0.69 to 1.17) (Figure2).

Four studies reported on the need for ICU admission, and the pooled results showed the need for requiring ICU care was 12% lower in the vitamin D group, but this difference was not statistically significant (RR 0.88; 95%CI 0.51 to 1.52) (Figure2).

Length of stay in ICU and hospital

Six studies reported on the length of stay in the ICU, and the pooled results showed the average length of ICU stay was 0.41 days shorter in the vitamin D group, but this difference was not statistically significant (MD -0.41 days; 95%CI -1.09 to 0.28).

Four studies reported on the length of stay in the hospital, and the pooled results showed the average hospital stay was 0.07 days shorter in the vitamin D group, but this difference was also not statistically significant (MD -0.07 days; 95%CI -0.61 to 0.46) (Figure3).

Subgroup analysis

Considering the limited number of included studies, we performed a subgroup analysis only on mortality during follow-up. Considering that participants' responses to vitamin D may vary due to differences in the severity of COVID-19, supplementation frequency, degree of vitamin D deficiency, development level of the country, risk of bias, and sample size across studies, we performed subgroup analyses based on these characteristics (Figure4). There were no statistically significant group differences within any of the subgroups, so these results do not support an effect of the aforementioned characteristics on vitamin D.

Sensitivity analysis

Sensitivity analysis was performed on morality during follow-up by leave-one-out method and trail sequential analysis.

Sensitivity analysis was performed on mortality during follow-up using the leave-one-out method and trial sequential analysis (eFigure1).

Using the leave-one-out method, we found that excluding the studies by Burgarin et al., Bychinin et al.[21], Maghbooli et al.[15], and Singh et al.[17] resulted in no statistically significant difference between vitamin D supplementation and no vitamin D supplementation. This suggests that the result was not robust.

We also performed a trial sequential analysis on mortality during follow-up. With 80% power, the pooled result showed no statistically significant difference (RR 0.74; α-spending adjusted CI 0.46 to 1.19). The required sample size (RSA) was determined to be 1874 (eFigure2).

Publication bias

We plotted funnel plots for the aforementioned outcomes (eFigure3-8). However, due to the limited number of included studies, there is a considerable risk of bias when evaluating the symmetry of the funnel plots. To more objectively assess publication bias, we also performed Egger's test. The p-values for Egger's test for the above outcomes were all greater than 0.05, indicating no significant evidence of publication bias.

Grade assessment

The quality of evidence for the above outcomes ranged from very low to moderate (eTable2). Specifically, the quality of evidence was moderate for mortality during follow-up, 28-day mortality, need for mechanical ventilation, and need for ICU admission. In contrast, the quality of evidence was low for length of stay in ICU and length of stay in hospital.

Discussion

Our study comprehensively explored the efficacy of vitamin D in treating COVID-19 patients with vitamin D deficiency. We found that vitamin D supplementation could reduce mortality during follow-up. However, this result should be interpreted with caution for the following reasons. Firstly, the leave-one-out method showed that nearly half of the studies could change the conclusion, indicating that the result was not robust. Secondly, in the subgroup analysis, most groups showed no statistically significance difference between vitamin D supplementation and no vitamin D supplementation. This may be due to the limited number of studies included in the subgroup analysis, which may not accurately reflect the true effect. Thirdly, trial sequential analysis revealed no statistically significant difference between vitamin D supplementation and no vitamin D supplementation when adjusted confidence intervals were considered. The analysis also indicated that a larger sample size is needed to determine the true effect of vitamin D.

Regarding other outcomes in our study, vitamin D did not appear to reduce the need for mechanical ventilation and ICU admission or shorten the length of stay in the ICU and hospital. Overall, the efficacy of vitamin D in treating COVID-19 patients with vitamin D deficiency remains inconclusive. Due to the potential exclusion of vulnerable groups and the variability in the definitions of vitamin D deficiency, the interpretation of the results should be made with caution. More studies are needed to explore this further.

In 2023, Meng et al.'s meta-analysis[28] explored the efficacy of vitamin D in treating

COVID-19. Their results showed that while vitamin D supplementation couldn't reduce mortality, it might be beneficial in reducing the severity of illness caused by SARS-CoV-2, particularly in vitamin D-deficient patients. Additionally, their study indicated that vitamin D supplementation could reduce the need for ICU admission. However, they did not analyze the data based on follow-up time, and new research has since been published. Our study results show that vitamin D supplementation does not reduce the need for ICU admission. Recently, a review also showed that vitamin D deficiency is linked to an increased risk of acquiring SARS-CoV-2 infection and poor COVID-19 prognosis, however, available evidence with regard to improved clinical outcomes with vitamin D supplementation is inconsistent[29]. Furthermore, whether vitamin D can reduce mortality still requires further exploration.

The relationship between vitamin D and COVID-19 has been a subject of extensive research, with mixed findings regarding its efficacy in preventing or treating the disease. Observational studies that initially suggested a link between low vitamin D levels and worse COVID-19 outcomes may have been confounded by other factors such as age, comorbidities, and socioeconomic status[30-34]. These factors themselves are risk factors for both vitamin D deficiency and severe COVID-19, complicating the interpretation of results[35-40]. A number of clinical trials have produced mixed results, with some showing no significant difference in outcomes between those receiving vitamin D supplementation and those who did not[41-45]. This inconsistency suggests that vitamin D may not have a substantial impact on COVID-19 outcomes. Another possible explanation is that the design and interpretation of some studies may be

problematic. It is well known that RCTs for vitamin D should be designed based on the criteria for nutrients, rather than using the pharmaceutical standards applied to drugs. As mentioned in the "Guidelines for optimizing design and analysis of clinical studies of nutrient effects", and as noted by Pilz S et al., designing an appropriate study protocol is key to accurately assessing the impact of vitamin D on health outcomes[46 47]. Therefore, optimizing the study design is not only crucial for ensuring the reliability of the results, but also determines whether the evaluation of vitamin D intervention reflects its true effects.

The role of vitamin D in regulating the immune system has been extensively studied, especially in the context of viral infections[48 49]. The onset and severity of COVID-19 are closely linked to the host's immune response, and vitamin D is believed to enhance the immune system's defense through multiple mechanisms[48]. Specifically, vitamin D helps boost the innate immune response by enhancing the function of macrophages, monocytes, and dendritic cells, all of which play crucial roles in antiviral immunity[49]. Additionally, vitamin D regulates T cell differentiation, promoting cell-mediated immune responses against infections, while also suppressing excessive immune reactions, such as cytokine storms, thereby reducing the severity of the COVID-19 disease course[50].

The role of vitamin D is particularly critical in the early stages of disease onset[51]. Studies have shown that early intervention can significantly improve immune function and slow disease progression[21 52]. For instance, supplementing vitamin D before or at the early onset of symptoms helps to promptly regulate the immune response and

enhance the body's ability to combat the virus[53]. In contrast, if intervention occurs later, after symptoms have manifested or during the later stages of the disease, the effects of vitamin D may be greatly diminished[54 55]. By this point, the immune system may already be in a dysregulated state, particularly under the influence of high viral loads or cytokine storms, making it difficult for vitamin D alone to quickly restore immune function.

Moreover, using high doses or active forms of vitamin D, such as 25(OH)D (calcidiol), may further enhance its therapeutic effects[56]. 25(OH)D is the active form of vitamin D, and it works more rapidly than regular vitamin D3[57]. High-dose vitamin D interventions have shown promising clinical effects during the early stages of the pandemic[57]. In particular, for high-risk patients, timely high-dose vitamin D supplementation can significantly reduce the risk of disease worsening, especially in populations with low vitamin D levels[58].

Regarding high-risk groups, those at higher risk of COVID-19-related death include elderly patients, individuals with comorbidities, and patients with serum 25(OH)D concentrations below 20 ng/mL[59]. The immune systems of older adults and those with chronic diseases are generally weaker, and their vitamin D levels are often lower, making them more susceptible to severe complications or death after infection[60]. Additionally, studies have shown that if hospitalized patients have low vitamin D levels, their immune function is impaired, leading to more severe clinical outcomes[59]. Therefore, for these high-risk groups, timely and appropriate vitamin D intervention could be a critical measure to reduce the mortality rate and severity of the COVID-19

disease course[61].

However, it is important to note that vitamin D supplementation may also have potential adverse effects, such as hypercalcemia and hypoparathyroidism, particularly when taken in excessive doses[62 63]. These adverse effects should be considered when evaluating the overall benefits and risks of vitamin D supplementation, especially in vulnerable populations.

In summary, vitamin D supplementation has the potential to reduce the incidence, severity, and mortality of COVID-19. However, its effectiveness depends on multiple factors, particularly the timing and dosage of intervention. Moreover, factors such as the economic status, sex, and age of patients may serve as effect modifiers that could influence the outcomes, which were not thoroughly analyzed in our study. Future research is needed to further clarify the optimal timing and dosage for vitamin D intervention, and whether personalized treatment plans based on patients' underlying conditions and vitamin D levels are necessary. Furthermore, during the pandemic, it is important to encourage high-risk populations (such as older adults and individuals with chronic diseases) to maintain adequate vitamin D levels to enhance immunity and improve the body's ability to combat COVID-19.

In this study, we found significant differences in the definition of "vitamin D deficiency" across studies, which may introduce selection bias. Some studies defined deficiency as a serum vitamin D level below 30 ng/ml, while others used 20 ng/ml, which could lead to overdiagnosis or underdiagnosis of vitamin D deficiency. Specifically, for elderly patients, a higher threshold (e.g., 25 ng/ml) might result in

their exclusion from studies, thus affecting the study conclusions. We recommend that future research adopt standardized definitions of vitamin D deficiency and adjust the criteria based on patient characteristics (such as age, sex, and comorbidities) to reduce potential selection bias and misdiagnosis.

Moreover, the variability in vitamin D categorization may impact the assessment of treatment efficacy. Due to the inconsistent standards for defining vitamin D deficiency across studies, some studies may have underestimated the effect of vitamin D on treatment outcomes. To improve the accuracy of results, we suggest that future studies consider individualized vitamin D deficiency criteria based on different population characteristics and further explore the impact of these criteria on treatment efficacy, ensuring that all patients with true vitamin D deficiency are included in the analysis.

However, our study also has other limitations. Firstly, the number of studies included is relatively small, with only nine randomized controlled trials and small sample sizes. Secondly, although there was no significant statistical heterogeneity, clinical heterogeneity among the studies cannot be ignored. The severity of patients' diseases and the frequency and dosage of vitamin D supplementation varied among the studies. To address this, we conducted a subgroup analysis and found that vitamin D supplementation did not reduce mortality in different subgroups. Thirdly, there is a potential risk of publication bias in our study. Although Egger's test did not show significant publication bias, the number of studies included in our analysis is relatively small, so caution is still needed when interpreting the risk of publication

bias. Lastly, although our conclusions suggest that vitamin D supplementation may reduce mortality, sensitivity analysis revealed that the conclusions are not reliable. Therefore, more high-quality research is needed in the future to further explore the role of vitamin D supplementation in vitamin D deficient COVID-19 patients.

Conclusion

This study suggested that vitamin D supplementation might have reduced mortality during follow-up, but no significant difference was observed in mortality at 28 days. Additionally, vitamin D supplementation did not significantly improve the need for mechanical ventilation, ICU admission rate, or reduce hospital and ICU length of stay. While these results indicated that vitamin D might have had some impact on mortality in COVID-19 patients with vitamin D deficiency, the findings should be interpreted cautiously due to variations in the studies and potential selection biases. Future research should focus on high-quality clinical trials, particularly those considering individual differences, study design, and follow-up duration, to draw more reliable and consistent conclusions.

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Author Contributions

Binsheng He is the guarantor of this study. LMZ, PPB, MXQ and BSH: proposed the design, searched the literature, collected, analysed and interpret the data, and wrote the report; LMZ, XMZ, YZ, and XL searched and collected the literature; LMZ, YZ, XMZ, XL and BSH analysed and interpreted the data.

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Declaration of competing interest

The authors declare that they have no known competing finical interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval

Ethical approval was not required for this study, since all data came from published articles.

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685 Table1. Characteristic of included randomized controlled trials

686 Figure1. Flowchart of literature search

687 Figure2. Vitamin D supplementation versus no vitamin D supplementation on
688 mortality during follow-up, 28-day mortality, need for mechanical ventilation and
689 need for ICU admission.

690 Figure3. Vitamin D supplementation versus no vitamin D supplementation on length
691 of stay in ICU and hospital.

692 Figure4.Subgroup analysis of mortality during follow-up.

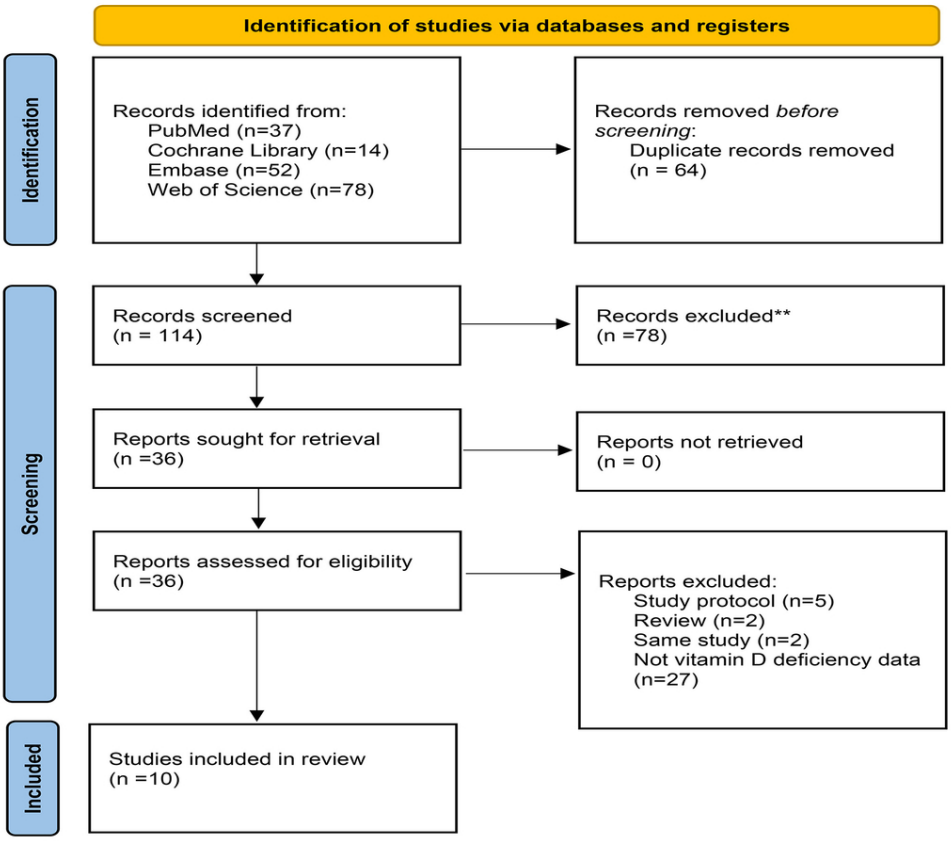
693

694 Table1.

Study	Country	Severity of COVID-19	Intervention group	Control group	Definition of Vitamin D deficiency	Follow-up
Bugarin2023	Croatia	Severe COVID-19	10,000 IU of cholecalciferol daily during ICU stay	Standard care	<20ng/ml	3 months
Bychinin2022	Russia	Severe COVID-19	60,000 IU of cholecalciferol once/ 7days followed by daily maintenance doses of 5000 IU. The high dose repeated on day 8, 16, 24, 32.	Placebo	<20ng/ml	During hospitalization
Cervero2022	Spain	NA	10,000 IU of cholecalciferol daily for 14 days	Standard care	<30ng/ml	28 days
Dilokpattanamongkol2024	Thailand	NA	2 mcg of alfacalcidol daily during the hospitalization	Standard care	<20ng/ml	During hospitalization
Maghbooli2021	Iran	NA	3000-6000 IU per day of vitamin D3 for 30 days	Placebo	<30ng/ml	2 months
Murai2021	Brazil	Moderate to severe COVID-19	Single dose of 200,000 IU of vitamin D3	Placebo	<20ng/ml	4 months
Niet2022	Belgiu	NA	25,000 IU	Placeb	<20ng/	9 weeks

	m		of vitamin D3 per day over 4 consecutive days, followed by 25,000 IU per week up to 6 weeks	o	ml	
Rastogi2022	India	NA	Daily 60000 IU of cholecalciferol for 7 days，and a weekly supplementation of 60000IU provided to those with 25(OH)D > 50 ng/ml or else continued on daily vitamin D 60,000 IU supplementation for another 7 days up until day 14	Placebo	<20ng/ml	3 weeks
Singh2024	India	Severe	A single dose of 60,000 IU of cholecalciferol	Placebo	<10 ng/ml	During hospitalization
Soliman2022	Egypt	Moderate to severe COVID-19	200.000 units intramuscularly once as a single dose	placebo	<20ng/ml	6 weeks

695



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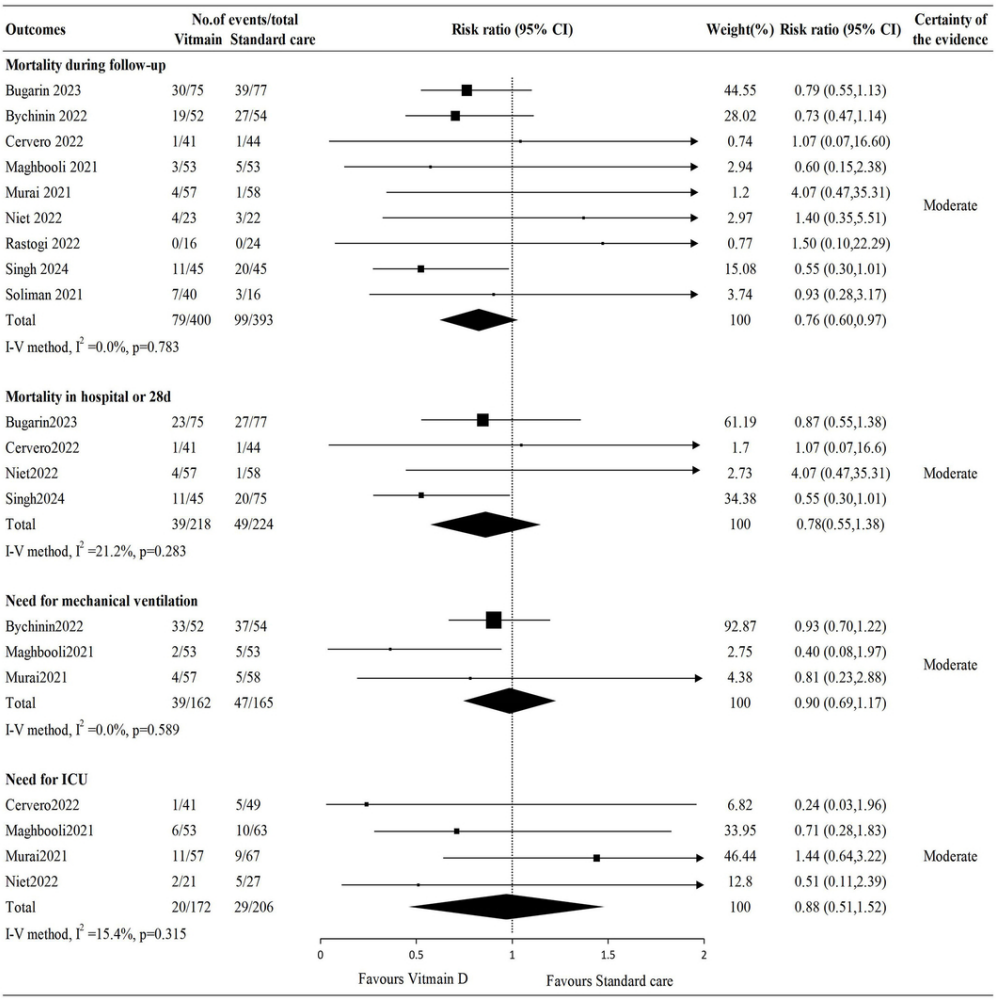

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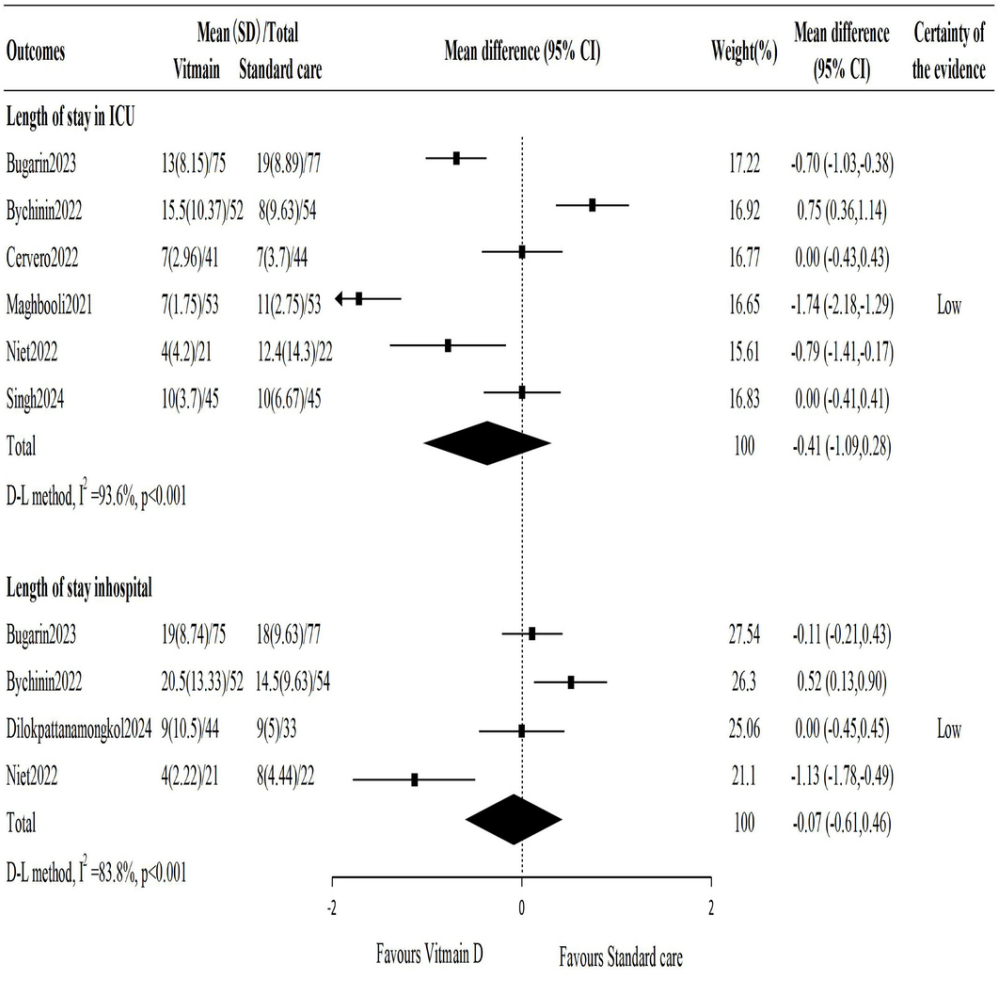

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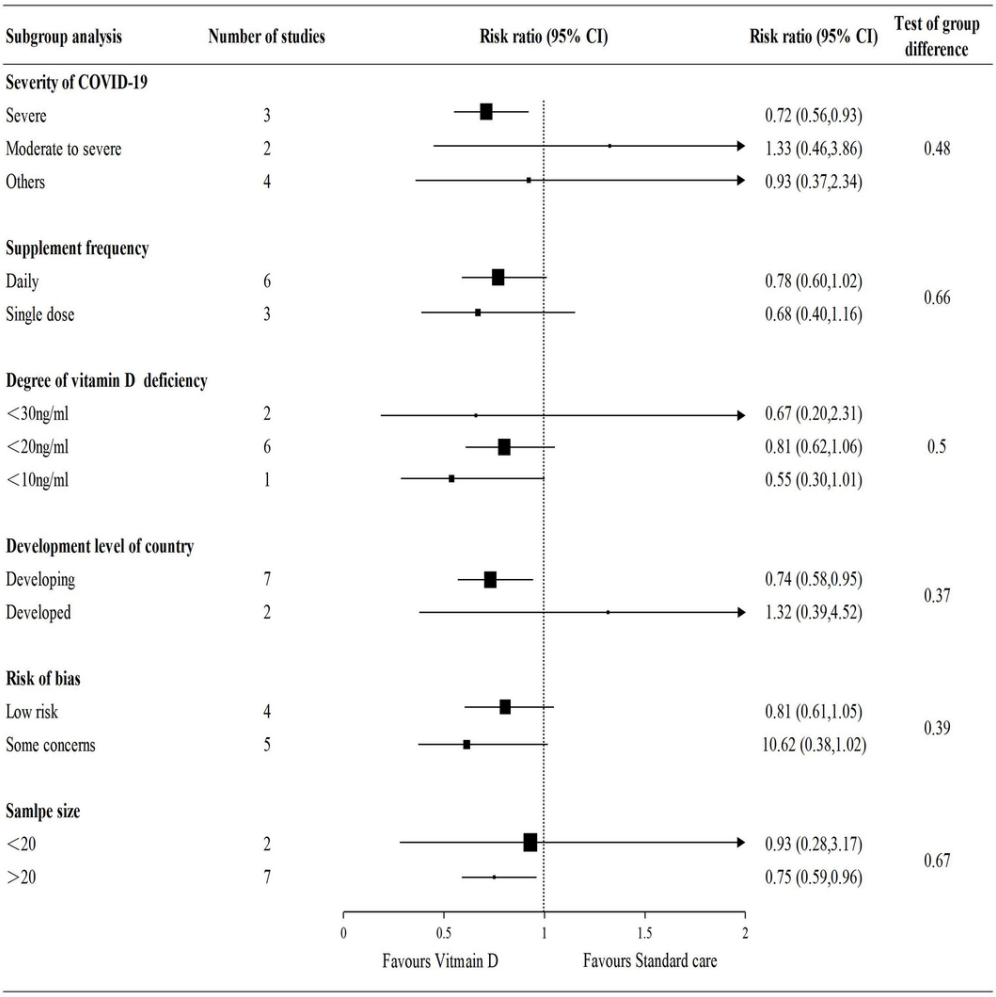


90x90mm (300 x 300 DPI)

Supplement

Search strategy

PubMed

1. “COVID-19”[Mesh] OR "COVID-19"[tiab] OR “COVID 19” [tiab] OR “2019-nCoV Infection” [tiab] OR “2019 nCoV Infection” [tiab] OR “2019-nCoV Infections” [tiab] OR “Infection, 2019-nCoV” [tiab] OR “SARS-CoV-2 Infection” [tiab] OR “Infection, SARS-CoV-2” [tiab] OR “SARS CoV 2 Infection” [tiab] OR “SARS-CoV-2 Infections” [tiab] OR “2019 Novel Coronavirus Disease” [tiab] OR “2019 Novel Coronavirus Infection” [tiab] OR “COVID-19 Virus Infection” [tiab] OR “COVID 19 Virus Infection” [tiab] OR “COVID-19 Virus Infections” [tiab] OR “Infection, COVID-19 Virus” [tiab] OR “Virus Infection, COVID-19” [tiab] OR “COVID19” [tiab] OR “Coronavirus Disease 2019” [tiab] OR “Disease 2019, Coronavirus” [tiab] OR “Coronavirus Disease-19” [tiab] OR “Coronavirus Disease 19” [tiab] OR “Severe Acute Respiratory Syndrome Coronavirus 2 Infection” [tiab] OR “COVID-19 Virus Disease” [tiab] OR “COVID 19 Virus Disease” [tiab] OR “COVID-19 Virus Diseases” [tiab] OR “Disease, COVID-19 Virus” [tiab] OR “Virus Disease, COVID-19” [tiab] OR “SARS Coronavirus 2 Infection” [tiab] OR “2019-nCoV Disease” [tiab] OR “2019 nCoV Disease” [tiab] OR “2019-nCoV Diseases” [tiab] OR “Disease, 2019-nCoV” [tiab] OR “COVID-19 Pandemic” [tiab] OR “COVID 19 Pandemic” [tiab] OR “Pandemic, COVID-19” [tiab] OR “COVID-19 Pandemics” [tiab]
2. "Vitamin D"[Mesh] OR "vitamin D"[tiab] OR "vitamin D3"[tiab] OR "vit D"[tiab] OR "vit D3"[tiab] OR "calciferol"[tiab] OR "cholecalciferol"[tiab] OR "calcidiol"[tiab] OR "calcitriol"[tiab] OR "25 hydroxyvitamin d"[tiab] OR "25 hydroxyvitamin D3"[tiab] OR "25 hydroxycalciferol"[tiab] OR "1,25 dihydroxyvitamin D"[tiab] OR "1,25 dihydroxyvitamin D3"[tiab] OR "calcifediol"[tiab]
3. Deficiency[tiab]
4. “Mortality”[tiab] OR “Mechanical ventilation”[tiab] OR “Intensive care unit”[tiab] OR “Length of stay”[tiab]
5. ((compar*[tiab]) OR ((singl*[tiab] or doubl*[tiab] or tripl*[tiab]) and (mask*[tiab] or blind*[tiab]))) OR (random*[tiab] or placebo[tiab] or controlled[tiab] or trial*[tiab])
6. #1 AND #2 AND #3 AND #4 AND $5

Cochrane Library

1. MeSH descriptor: [COVID-19] explode all trees
2. (COVID-19 OR COVID 19 OR 2019 nCoV Infection OR SARS-CoV-2 Infection OR Infection, SARS-CoV-2 OR SARS CoV 2 Infection OR SARS-CoV-2 Infections OR 2019 Novel Coronavirus Disease OR 2019 Novel Coronavirus Infection OR COVID-19 Virus Infection OR COVID 19 Virus Infection OR COVID-19 Virus Infections OR Infection, COVID-19 Virus OR Virus Infection, COVID-19 OR COVID19 OR Coronavirus Disease 2019 OR Disease 2019, Coronavirus OR Coronavirus Disease-19 OR Coronavirus Disease 19 OR Severe Acute Respiratory Syndrome Coronavirus 2 Infection OR

COVID-19 Virus Disease OR COVID 19 Virus Disease OR COVID-19 Virus Diseases OR Disease, COVID-19 Virus OR Virus Disease, COVID-19 OR SARS Coronavirus 2 Infection OR 2019 nCoV Disease OR COVID-19 Pandemic OR COVID 19 Pandemic OR Pandemic, COVID-19 OR COVID-19 Pandemics):ti,ab

3. #1 OR #2
4. MeSH descriptor: [Vitamin D] explode all trees
5. (vitamin D OR vitamin D3 OR vit D OR vit D3 OR calciferol OR cholecalciferol OR calcidiol OR calcitriol OR 25 hydroxyvitamin d OR 25 hydroxyvitamin D3 OR 25 hydroxycalciferol OR 1,25 dihydroxyvitamin D OR 1,25 dihydroxyvitamin D3 OR calcifediol):ti,ab
6. #4 OR #5
7. (Deficiency):ti,ab
8. (mortality or mechanical ventilation or intensive care unit):ti,ab
9. ((compar*) OR ((singl* or doubl* or tripl*) and (mask* or blind*))) OR (random* or placebo or controlled or trial*):ti,ab
10. #3 AND #6 AND #7 AND #8 AND #9

Embase

1. 'coronavirus disease 2019'/exp
2. ((Covid-19) OR (Covid 19) OR (2019-nCoV Infection) OR (SARS-CoV-2 Infections)):ti,ab
3. #1 OR #2
4. 'vitamin d'/exp
5. ((vitamin D) OR (vitamin D3) OR (25 hydroxycalciferol) OR (1,25 dihydroxyvitamin D3)):ti,ab
6. #4 OR #5
7. (Deficiency):ti,ab
8. (mortality or mechanical ventilation or intensive care unit):ti,ab
9. compar* OR ((singl* OR doubl* OR tripl*) AND (mask* OR blind*)) OR random*:ti,ab OR placebo:ti,ab OR controlled:ti,ab OR trial*:ti,ab
10. #3 AND #6 AND #7 AND #8 AND #9

Web of Science

1. TS=(COVID-19 OR COVID 19 OR 2019-nCoV Infection OR 2019 nCoV Infection OR 2019-nCoV Infections OR Infection, 2019-nCoV OR SARS-CoV-2 Infection OR Infection, SARS-CoV-2 OR SARS CoV 2 Infection OR SARS-CoV-2 Infections OR 2019 Novel Coronavirus Disease OR 2019 Novel Coronavirus Infection OR COVID-19 Virus Infection OR COVID 19 Virus Infection OR COVID-19 Virus Infections OR Infection, COVID-19 Virus OR Virus Infection, COVID-19 OR COVID19 OR Coronavirus Disease 2019 OR Disease 2019, Coronavirus OR Coronavirus Disease-19 OR Coronavirus Disease 19 OR Severe Acute Respiratory Syndrome Coronavirus 2 Infection OR COVID-19 Virus Disease OR COVID 19 Virus Disease OR COVID-19 Virus Diseases OR Disease, COVID-19 Virus OR Virus Disease, COVID-19 OR SARS Coronavirus 2 Infection OR 2019-nCoV Disease OR 2019 nCoV Disease OR 2019-nCoV Diseases OR Disease, 2019-nCoV OR COVID-19 Pandemic OR COVID 19 Pandemic OR Pandemic, COVID-19 OR COVID-19 Pandemics)
2. TS= (vitamin D OR vitamin D3 OR vit D OR vit D3 OR calciferol OR cholecalciferol OR calcidiol OR calcitriol OR 25 hydroxyvitamin d OR 25 hydroxyvitamin D3 OR 25 hydroxycalciferol OR 1,25 dihydroxyvitamin D OR 1,25 dihydroxyvitamin D3 OR calcifediol)
3. TS= (Deficiency)
4. TS= (mortality or mechanical ventilation or intensive care unit)
5. TS=(((compar*) OR ((singl* or doubl* or tripl*) and (mask* or blind*))) OR (random* or placebo or controlled or trial*))
6. #1 AND #2 AND #3 AND #4 AND #5

eTable1. Risk of bias of included studies

Outcome	D1	D2	D3	D4	D5	Overall bias
Study: Bugarin2023						
Mortality during follow up	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
28-day mortality	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Length of stay in ICU	Low risk	Some concerns	Low risk	Low risk	Low risk	Some concerns
Length of stay in hospital	Low risk	Some concerns	Low risk	Low risk	Low risk	Some concerns
Study: Bychinin2022						
Mortality during follow up	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Need for mechanical ventilation	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Length of stay in ICU	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Study: Cervero2022						
Mortality during follow up	Low risk	Low risk	Low risk	Low risk	Some concerns	Some concerns
28-day mortality	Low risk	Low risk	Low risk	Low risk	Some concerns	Some concerns
Length of stay in ICU	Low risk	Some concerns	Low risk	Low risk	Some concerns	Some concerns
Study: Dilokpattanamongkol2024						
Length of stay in hospital	Low risk	Some concerns	Low risk	Low risk	Low risk	Some concerns
Study: Maghbooli2021						
Mortality during follow up	Low risk	Low risk	Low risk	Low risk	Some concerns	Some concerns
Need for mechanical ventilation	Low risk	Low risk	Low risk	Low risk	Some concerns	Some concerns
Need for ICU admission	Low risk	Low risk	Low risk	Low risk	Some concerns	Some concerns

Length of stay in ICU	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Study: Murai2021						
Mortality during follow up	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Need for mechanical ventilation	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Need for ICU admission	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Study: Niet2022						
Mortality during follow up	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
28-day mortality	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Need for ICU admission	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Length of stay in ICU	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Length of stay in hospital	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Study: Rastogi2022						
Mortality during follow up	Low risk	Low risk	Low risk	Some concerns	Low risk	Some concerns
Study: Singh2024						
Mortality during follow up	Low risk	Low risk	Low risk	Low risk	Some concerns	Some concerns
28-day mortality	Low risk	Low risk	Low risk	Low risk	Some concerns	Some concerns
Length of stay in ICU	Low risk	Low risk	Low risk	Low risk	Some concerns	Some concerns
Study: Soliman2021						
Mortality during follow up	Some concerns	Low risk	Low risk	Low risk	Some concerns	Some concerns

D1: Randomisation process

D2: Deviations from the intended interventions

D3: Missing outcome data

D4: Measurement of the outcome
D5: Selection of the reported result
eTable2.

Outcomes	No. of participants (No. of trials)	Risk ratio (95%CI)	Mean difference (95%CI)	Risk of bias[a]	Inconsistency[b]	Imprecision[c]	Small study effects[d]	Certainty of evidence
Mortality during follow-up	737 (8)	0.76 (0.59,0.96)		Not Down graded	Not down graded	Down graded	Not down graded	Moderate
28-day mortality	442 (4)	0.79 (0.49,1.26)		Not Down graded	Not down graded	Down graded	Not down graded	Moderate
Need for mechanical ventilation	327 (3)	0.90 (0.69,1.17)		Not Down graded	Not down graded	Down graded	Not down graded	Moderate
Need for ICU admission	349 (4)	0.84 (0.45,1.56)		Not down graded	Not down graded	Down graded	Not down graded	Moderate
Length of stay in ICU	582 (6)		-0.41 (-1.09,0.28)	Not Down graded	Down graded	Down graded	Not down graded	Low
Length of stay in hospital	378 (4)		-0.07 (-0.61,0.46)	Not down graded	Down graded	Down graded	Not down graded	Low

ICU, intensive care unit

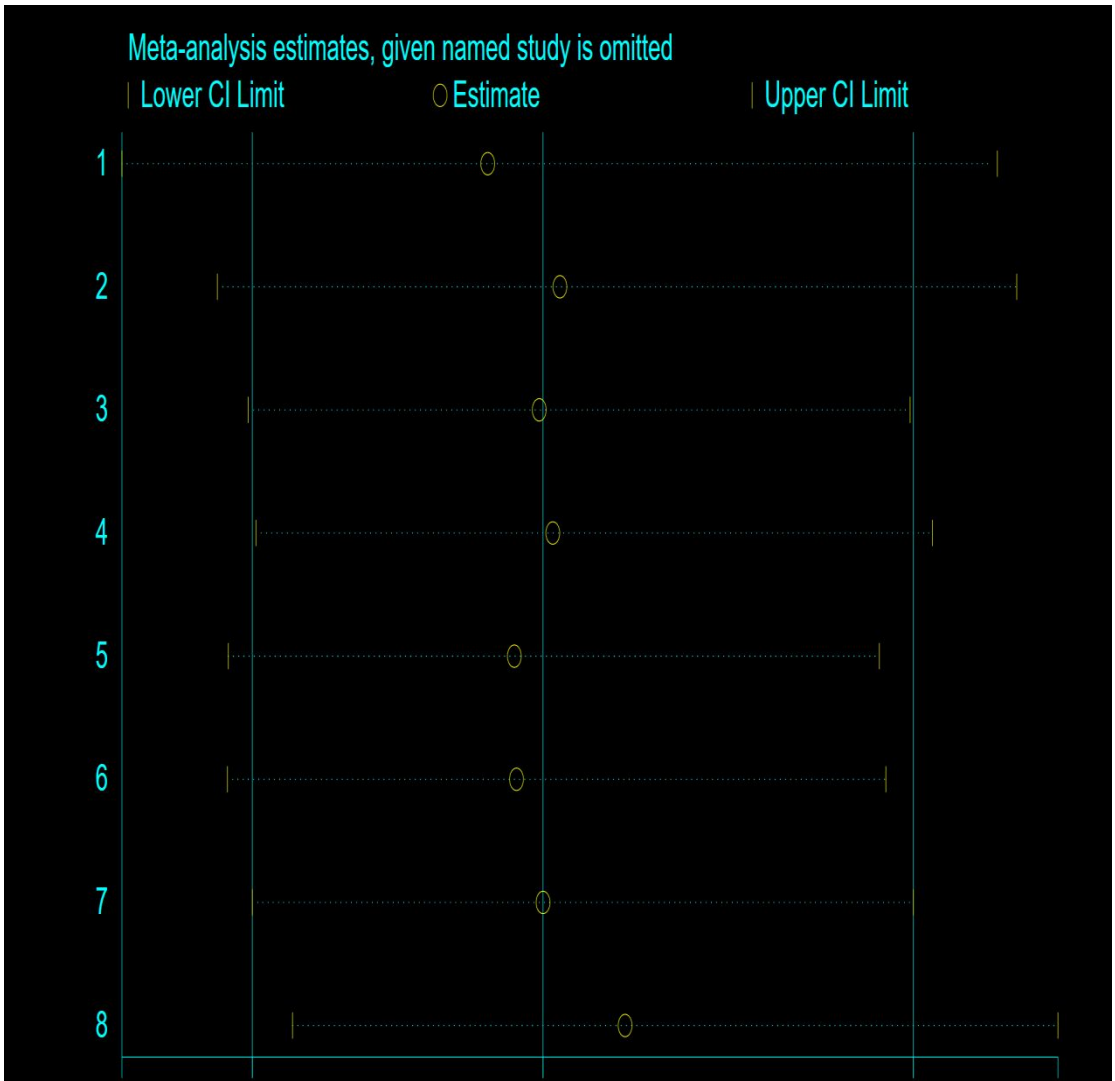
[a] Downgraded by one level because >25% of participants in this comparison were from studies at high risk of bias.

[b] Downgraded by one level because heterogeneity (I^2) >50%.

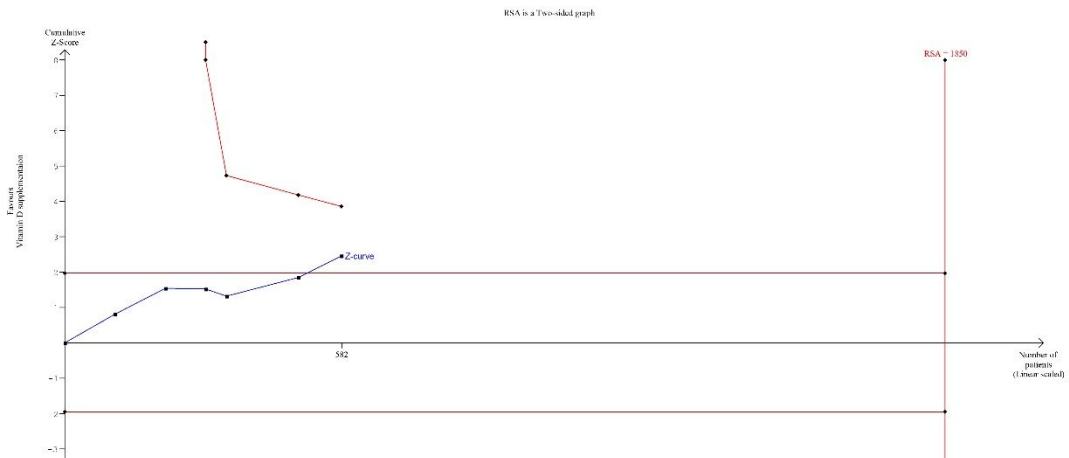
[c] Downgraded by one level because the limits of the 95% confidence interval were 20% different to the point estimates.

[D] Downgraded by one level owing to small study bias.

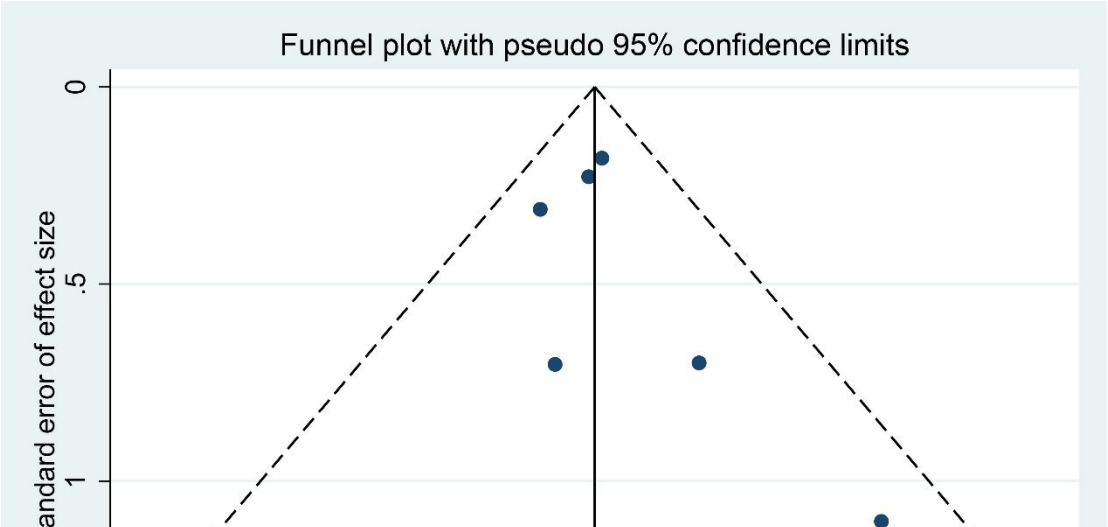
eFigure1. Leave-one-out on mortality during follow-up



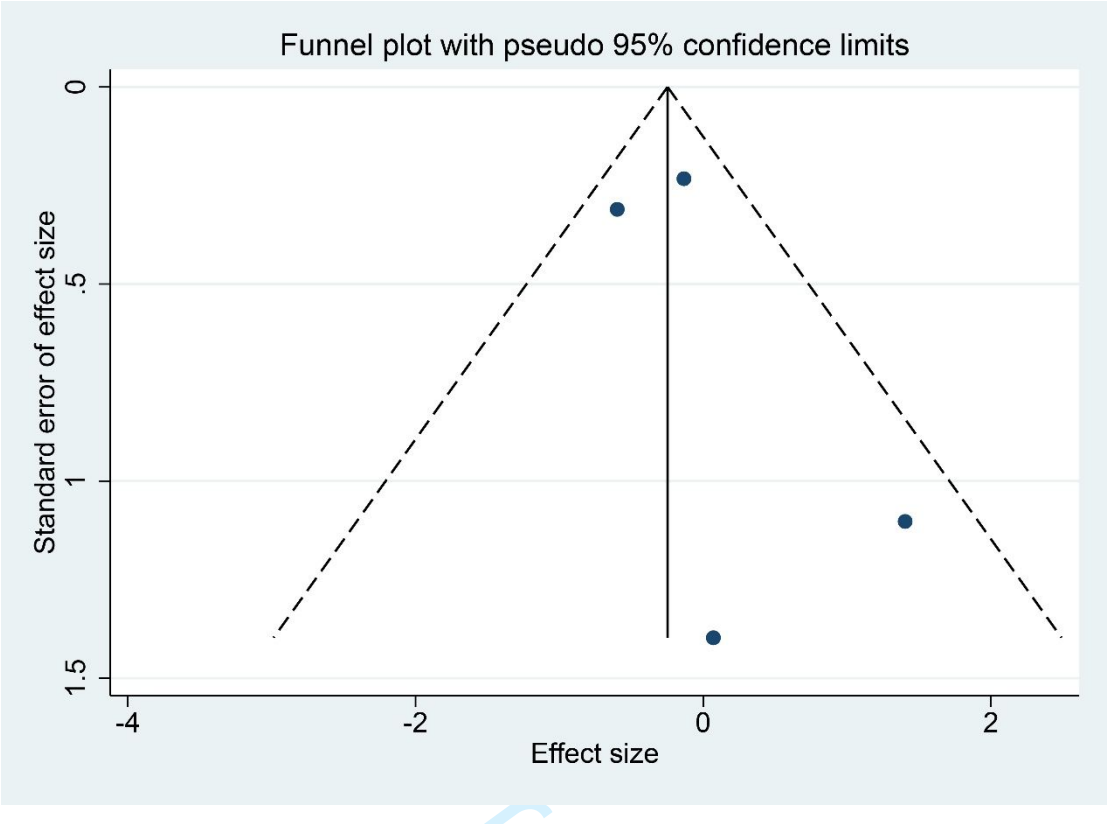
eFigure2. Trial sequential analysis on mortality during follow-up



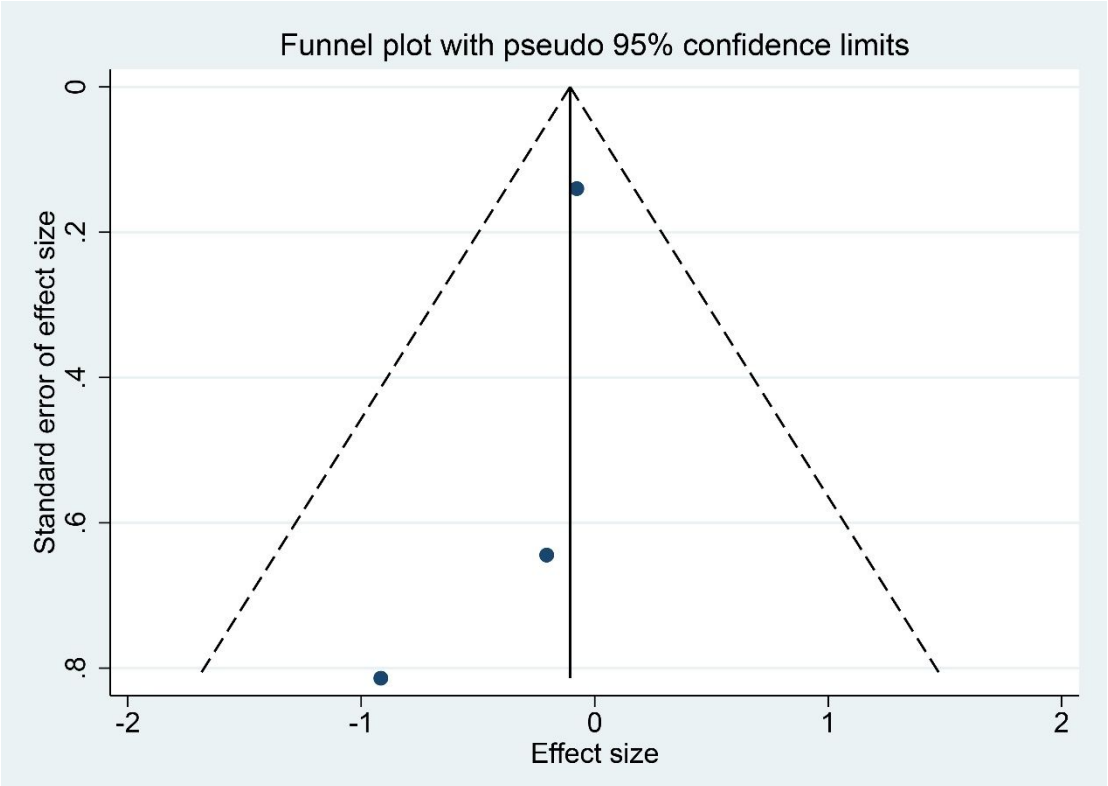
eFigure3. Funnel plot of mortality during follow-up



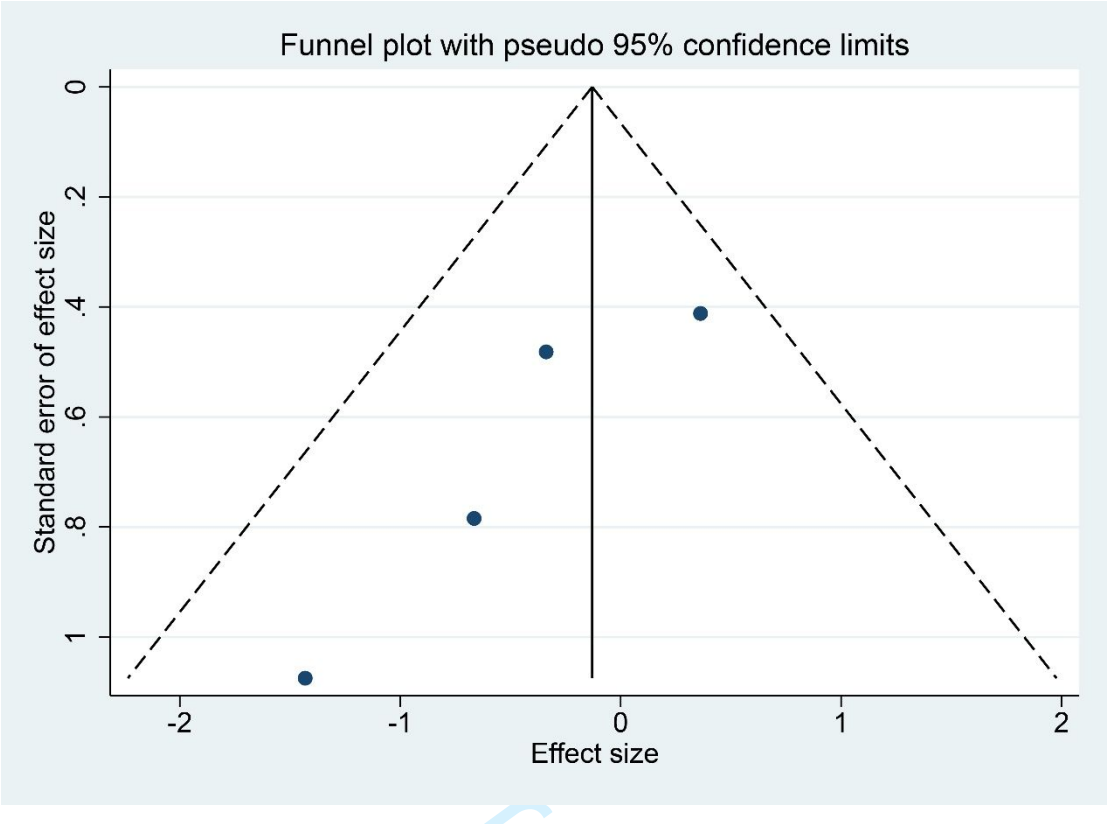
eFigure4. Funnel plot of 28day-mortality



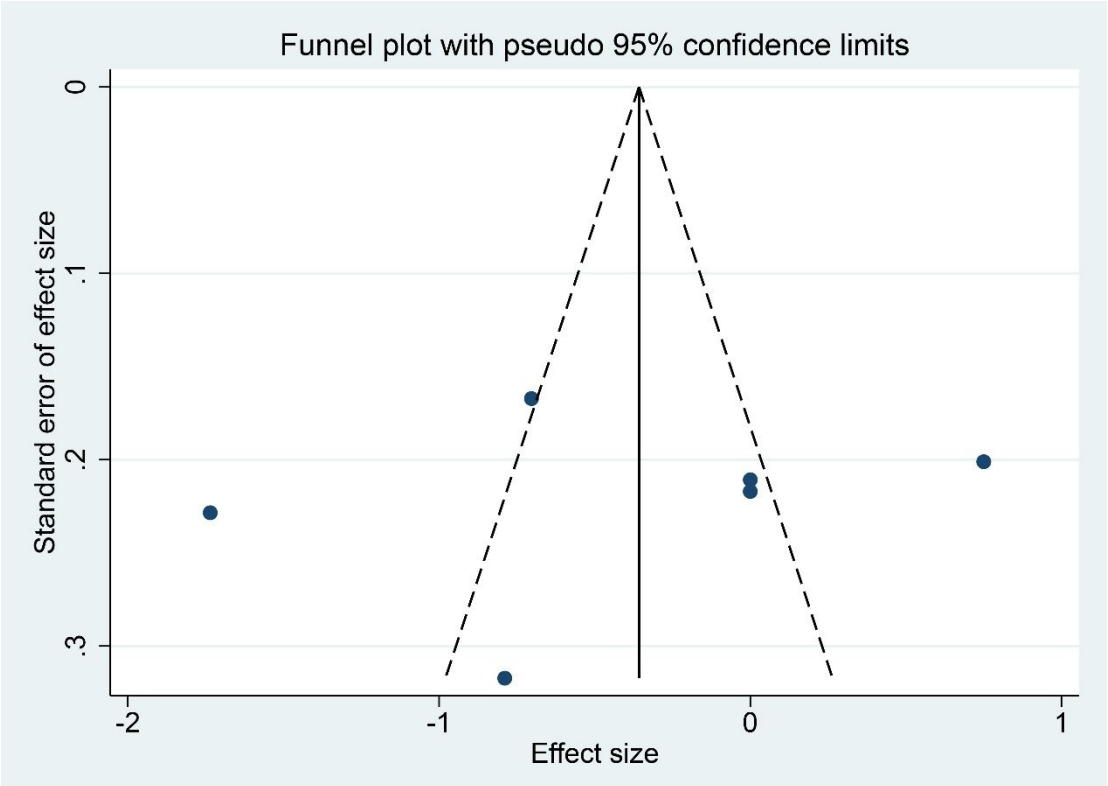
eFigure5. Funnel plot of need for mechanical ventilation



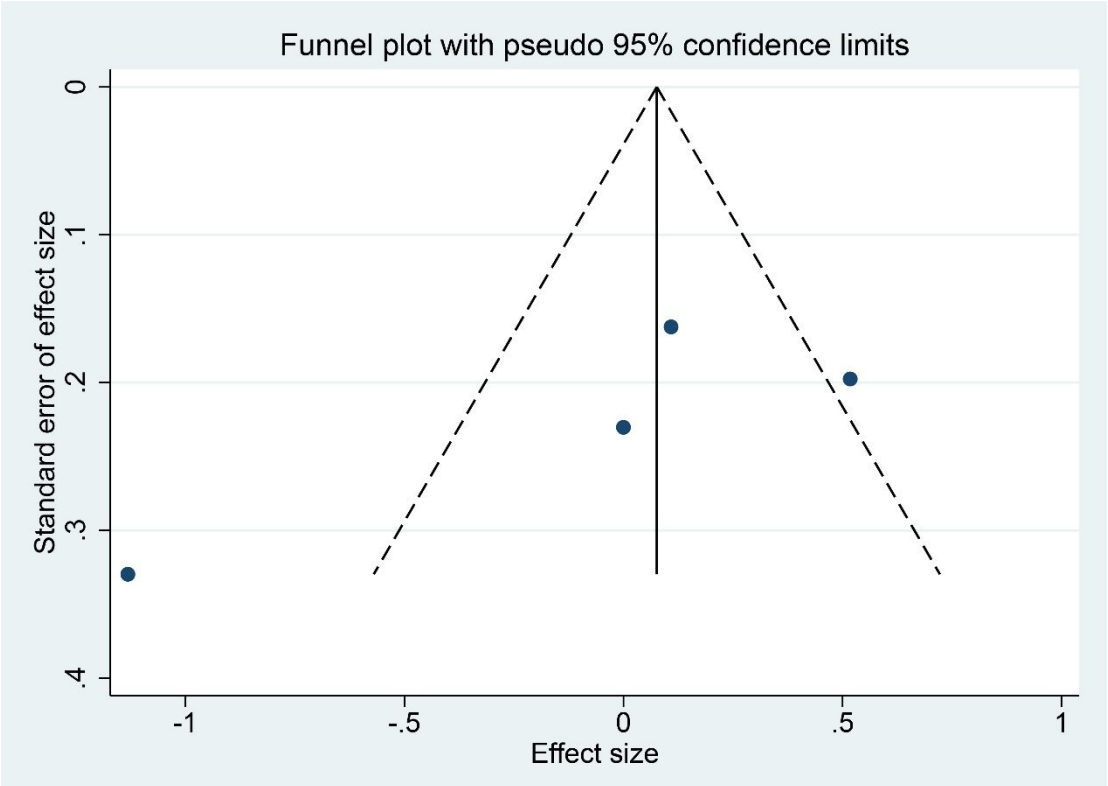
eFigure6. Funnel plot of need for ICU admission



eFigure7. Funnel plot of length of stay in ICU



eFigure8. Funnel plot of length of stay in hospital



BMJ Open

Vitamin D Supplementation for Managing COVID-19 in Patients with Vitamin D Deficiency：A systematic review and Meta-analysis of Randomized Controlled Trials

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Manuscript ID	bmjopen-2024-091903.R2
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Primary Subject Heading:	Respiratory medicine
Secondary Subject Heading:	Global health
Keywords:	COVID-19, Meta-Analysis, NUTRITION & DIETETICS, Health

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Vitamin D Supplementation for Managing COVID-19 in Patients with Vitamin D Deficiency：A systematic review and Meta-analysis of Randomized Controlled Trials

Lemei Zhu[1,2], Yuan Zhang[1], Xi Li[1,2], Xuemin Zou[1,2], Pingping Bing[1*], Mingxu Qi[3*], Binsheng He[1*]

1. Hunan Key Laboratory of the Research and Development of Novel Pharmaceutical Preparations, Changsha Medical University, Changsha, 410219, China.

2. School of Public Health, Changsha Medical University, Changsha, 410219, China.

3. Department of Cardiovascular Medicine, Affiliated Nanhua Hospital, University of South China, Hengyang,421001, China

Corresponding Author:

Pingping Bing, Hunan Key Laboratory of the Research and Development of Novel Pharmaceutical Preparations, Changsha, 410219, China. (Email: bpping@163.com)

Mingxu Qi, Department of Cardiovascular Medicine, Affiliated Nanhua Hospital, University of South China, Hengyang,421001, China. (Email: qimingxuqi@163.com)

Binsheng He, Hunan Key Laboratory of the Research and Development of Novel Pharmaceutical Preparations, Changsha, 410219, China. (Email: hbscsmu@163.com)

Abstract

Objectives Vitamin D deficiency is prevalent among population. Previous studies have shown that vitamin D supplementation might be useful for treating COVID-19 infection. Therefore, we performed a meta-analysis to explore vitamin D supplementation efficacy in treating COVID-19 patients with vitamin D deficiency.

Design Systematic review and meta-analysis

Data sources PubMed, Cochrane Library, Embase and Web of Science.

Eligibility Criteria Randomized controlled trials exploring vitamin D supplementation for patients with COVID-19 and vitamin D deficiency.

Data extraction and synthesis Two independent reviewers employed standardized methods to search, screen, and code the included studies. The primary outcomes included mortality during follow-up, 28-day mortality, need for mechanical ventilation and intensive care unit (ICU). The secondary outcome included length of stay in hospital and ICU. The risk of bias was assessed using the Risk of Bias 2 tool. Depending on the level of heterogeneity, either a random-effects model or a fixed-effects model was applied. The findings were summarized using GRADE evidence profiles and synthesized qualitatively.

Results A total of nine studies, comprising 870 participants, were included in the analysis. The pooled results indicated that vitamin D supplementation was associated with a lower risk of mortality (Risk ratio 0.76; 95% CI 0.60 to 0.97). However, this apparent benefit was not robust when examined through the leave-one-out method, and trial sequential analysis. Regarding other outcomes, there was no statistically

significant difference between vitamin D supplementation and no supplementation in terms of 28-day mortality, the need for mechanical ventilation and ICU admission. Vitamin D supplementation was associated with a 0.41-day shorter length of stay in the ICU (Mean difference -0.41; 95%CI -1.09 to 0.28) and a 0.07-day shorter length of stay in the hospital (Mean difference -0.07; 95%CI -0.61 to 0.46) compared to no supplementation; however, neither difference was statistically significant.

Conclusion Based on evidence of low to moderate quality, vitamin D supplementation reduced the mortality rate during follow-up in COVID-19 patients with vitamin D deficiency. However, it did not improve 28-day mortality, nor did it reduce the need for mechanical ventilation and ICU admission, or the length of stay in the ICU and hospital.

Keywords: Vitamin D supplementation; Vitamin D deficiency; COVID-19; Meta-analysis; Trial sequential analysis

Strengths and limitations of this study

- This meta-analysis of RCTs was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist.
- A comprehensive literature search was performed across multiple databases to identify relevant studies.
- Rigorous inclusion criteria were applied to ensure the quality and relevance of studies.
- Trial sequential analysis and sensitivity analysis were used to assess the statistical robustness of the results.
- The number of studies included was limited, with only nine RCTs and relatively small sample sizes, which may affect the generalizability of the results.

Introduction

COVID-19, caused by the SARS-CoV-2 virus, is a highly transmissible and potentially severe respiratory illness that has resulted in a global pandemic, affecting millions of people worldwide with varying morbidity and mortality rates[1 2].

Vitamin D, a steroid hormone derived from cholesterol, plays a significant role in regulating the expression of various genes, including those in immune cells[3]. In hospitalized COVID-19 patients, vitamin D also showed anti-inflammatory effects[4]. Vitamin D deficiency is widespread across the globe; for example, 40% of the European population is reported to lack sufficient vitamin D, and vitamin D deficiency is also common in high-altitude regions such as Nepal, the Andes, and Tibet[5 6]. Maintaining appropriate levels of vitamin D is essential for optimal respiratory immune function[3 7-11]. Despite this, the precise impact of vitamin D supplementation on preventing and treating COVID-19 remains a topic of debate. According to a systematic review, vitamin D supplementation can significantly reduce the severity of COVID-19 infection, as measured by outcomes such as hospitalization rates, the need for mechanical ventilation, and mortality, suggesting its use as a supplementary treatment for COVID-19[12]. In contrast, a 2021 meta-analysis that included eight randomized controlled trials (RCTs) found that vitamin D supplementation did not enhance clinical outcomes in patients infected with SARS-CoV-2[13]. Recently, a meta-analysis conducted by Meng et al. explored the role of vitamin D in the prevention and treatment of SARS-CoV-2 infection. Their results suggested that vitamin D supplementation may have some beneficial impact on the

severity of illness caused by SARS-CoV-2, particularly in vitamin D deficient patients. Although they specifically analyzed patients with vitamin D deficiency, the studies they included were limited, and the analysis focused solely on mortality as the outcome. Moreover, they did not perform comprehensive subgroup analyses, such as based on the severity of vitamin D deficiency.

Amrein et al. raised another important point, namely that vitamin D is clearly not a cure-all and is likely effective only when there is a deficiency[6]. To comprehensively investigate the role of vitamin D supplementation in these patients, we conducted a meta-analysis of randomized controlled trials to determine whether vitamin D supplementation improves clinical outcomes (mortality during follow-up, 28-day mortality, need for mechanical ventilation and ICU and length of stay in hospital and ICU) in COVID-19 patients with vitamin D deficiency.

Methods

This meta-analysis of RCTs was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) checklist[14]. The study protocol was registered on PROSPERO (CRD42024573791).

Search strategy and selection criteria

A comprehensive literature search was conducted on June 1, 2024 across several databases including PubMed, Cochrane Library, Embase, and Web of science with Mesh terms and broad search terms. We also manually searched the reference lists of relevant review articles. After completing the initial research, we conducted the same

search again to include the latest published studies. The detailed search strategy was provided in the appendix. The retrieved literature was imported into EndNote X9. After removing duplicate references, it was assessed for eligibility by two reviewers. Based on the PICO principle, the inclusion criteria we applied are as follows:

P: COVID-19 patients with vitamin D deficiency;

I: standard care plus vitamin D supplementation;

C: standard care;

O: mortality rate, need for mechanical ventilation or ICU admission, length of stay in ICU and hospital.

Exclusion criteria were: non-randomized controlled trials, and studies for which full text could not be retrieved. The definition of vitamin D deficiency was according to previous studies[6 15-17]. Any disputes will be resolved through discussion.

Data extraction

A comprehensive data extraction form was developed based on the guidelines outlined in the Cochrane Handbook for Systematic Reviews of Interventions. The form was piloted on a subset of the included studies before extracting the following data: author details, participant characteristics, intervention details (type, duration, frequency, and other details), primary and secondary outcomes, follow-up times.

The consistency between data extractors was measured using the Kappa value. Any disputes will be resolved through discussion.

Quality assessment

Potential sources of bias in RCTs were assessed using Risk of Bias 2 (Rob2), a revised tool for assessing the risk of bias in randomized trials[18]. Rob2 encompasses five key domains: 1. Randomization process;2. Deviations from intended interventions;3. Missing outcome data;4. Measurement of the outcome;5. Selection of the reported result. Within each domain, bias was evaluated and categorized as either low risk, some concerns, or high risk, depending on the circumstances and relevant evidence. Ultimately, the overall bias of each study was classified as either low risk, some concerns, or high risk, based on the comprehensive assessment of bias across the five domains. When there was a discrepancy in the assessment results for a certain domain, the outcome was resolved through discussion.

Outcomes

The primary outcomes were mortality during follow up and 28-day mortality. The secondary outcomes included need for mechanical ventilation and ICU admission, length of stay in hospital and ICU. Mortality during follow-up refers to the deaths that occurred during the follow-up period in each study. Since the follow-up durations vary across studies, the time frame for mortality during follow-up is not consistent. 28-day mortality specifically refers to the mortality rate from the start of the study up to day 28. Need for mechanical ventilation and ICU admission refers to patients who initially did not require mechanical ventilation or ICU admission but received mechanical ventilation or were admitted to the ICU during the study. Length of stay in hospital and ICU refers to the duration of hospitalization and ICU stay for patients who received different treatments.

Statistical analysis

Dichotomous variables were presented as event numbers and total numbers, with combined outcomes expressed as Risk Ratio (RR) with 95% Confidence Intervals (CIs). Continuous variables were presented as mean and standard deviation, with combined outcomes expressed as Mean Difference (MD) with 95% Confidence Intervals (CIs). The choice of analysis model was based on the level of heterogeneity. If $I^2 \geq 50\%$, heterogeneity was considered significant, and the DerSimonian-Laird method combined with a random-effects model was used for analysis. If $I^2 < 50\%$, no significant heterogeneity was assumed, and the Inverse-variance method combined with a fixed-effects model was used for analysis[19]. Subgroup analysis according to different characteristics (severity of COVID-19, vitamin D supplementation, definition of vitamin D deficiency, and so on) was conducted on mortality during follow-up. Sensitivity analysis was performed using the leave-one-out method. A funnel plot was generated to subjectively assess publication bias, and Egger's test was also conducted to objectively test for publication bias; if $p > 0.05$, no significant publication bias was assumed. In this study, trial sequential analysis was performed using Trial Sequential Analysis software (Copenhagen Trial Unit, Centre for Clinical Intervention Research, Rigshospitalet) (http://ctu.dk/tsa/). The meta-analysis was performed using Stata 17 (STATA Corporation, Texas, USA) (https://www.stata.com/stata17/). The quality of evidence was assessed by GRADE guidelines[20].

Patient and public involvement

None.

Results

Literature search

A total of 659 studies were initially found across all databases, with 71 identified as duplicates. After screening titles and abstracts, 552 studies were excluded. The remaining 36 studies were then assessed for full text. Ultimately, 10 studies[15-17 21-27] met the inclusion criteria and were included in the analysis (Figure1).

Baseline study characteristics

A total of 10 studies[15-17 21-26], encompassing 870 participants, were included. The vitamin D dosage ranged from 3,000 IU to 200,000 IU. Three studies used a single high dose of vitamin D supplementation, while seven studies employed a continuous dosing regimen. Seven studies defined vitamin D deficiency as <20 ng/ml, two studies as <30 ng/ml, and one study as <10 ng/ml. Additionally, two studies focused on severe COVID-19, and two studies examined moderate to severe COVID-19 cases (Table1).

Quality assessment

We evaluated the outcomes reported in the studies. We found that among the twenty-eight relevant outcomes, fourteen were classified as low risk and fourteen as having some concerns. For example, the study by Soliman et al. did not provide detailed information on the randomization method, which raised concerns about the randomization process. In the studies by Singh et al. and others, vitamin D deficiency

was defined as <10 ng/ml, while Cervero et al. and Maghbooli et al. defined deficiency as <30 ng/ml, which differed from the commonly accepted definition of deficiency. Therefore, these studies also carried an overall risk of bias. The detailed distribution of bias is shown in eTable1.

The Kappa value, used to estimate the equivalence of data extraction in this study, was 0.86.

Mortality

Nine studies reported the mortality during follow-up. The pooled result showed that the risk of death in the vitamin D group was 24% lower than in the non-supplementation group (RR 0.76; 95%CI 0.60 to 0.97) (Figure2).

To assess the vitamin D's role in reducing hospitalization mortality, we analyzed 28-day mortality. The pooled result showed that the risk of mortality was 21% lower in the vitamin D group, but this difference was not statistically significant (RR 0.78; 95%CI 0.55 to 1.38) (Figure2).

Need for ICU admission and mechanical ventilation

Three studies reported on the need for mechanical ventilation, and the pooled results showed the need for mechanical ventilation was 10% lower in the vitamin D group, but this difference was not statistically significant (RR 0.90; 95%CI 0.69 to 1.17) (Figure2).

Four studies reported on the need for ICU admission, and the pooled results showed the need for requiring ICU care was 12% lower in the vitamin D group, but this difference was not statistically significant (RR 0.88; 95%CI 0.51 to 1.52) (Figure2).

Length of stay in ICU and hospital

Six studies reported on the length of stay in the ICU, and the pooled results showed the average length of ICU stay was 0.41 days shorter in the vitamin D group, but this difference was not statistically significant (MD -0.41 days; 95%CI -1.09 to 0.28).

Four studies reported on the length of stay in the hospital, and the pooled results showed the average hospital stay was 0.07 days shorter in the vitamin D group, but this difference was also not statistically significant (MD -0.07 days; 95%CI -0.61 to 0.46) (Figure3).

Subgroup analysis

Considering the limited number of included studies, we performed a subgroup analysis only on mortality during follow-up. Considering that participants' responses to vitamin D may vary due to differences in the severity of COVID-19, supplementation frequency, degree of vitamin D deficiency, development level of the country, risk of bias, and sample size across studies, we performed subgroup analyses based on these characteristics (Figure4). There were no statistically significant group differences within any of the subgroups, so these results do not support an effect of the aforementioned characteristics on vitamin D.

Sensitivity analysis

Sensitivity analysis was performed on morality during follow-up by leave-one-out method and trail sequential analysis.

Sensitivity analysis was performed on mortality during follow-up using the leave-one-out method and trial sequential analysis (eFigure1).

Using the leave-one-out method, we found that excluding the studies by Burgarin et al., Bychinin et al.[21], Maghbooli et al.[15], and Singh et al.[17] resulted in no statistically significant difference between vitamin D supplementation and no vitamin D supplementation. This suggests that the result was not robust.

We also performed a trial sequential analysis on mortality during follow-up. With 80% power, the pooled result showed no statistically significant difference (RR 0.74; α-spending adjusted CI 0.46 to 1.19). The required sample size (RSA) was determined to be 1874 (eFigure2).

Publication bias

We plotted funnel plots for the aforementioned outcomes (eFigure3-8). However, due to the limited number of included studies, there is a considerable risk of bias when evaluating the symmetry of the funnel plots. To more objectively assess publication bias, we also performed Egger's test. The p-values for Egger's test for the above outcomes were all greater than 0.05, indicating no significant evidence of publication bias.

Grade assessment

The quality of evidence for the above outcomes ranged from very low to moderate (eTable2). Specifically, the quality of evidence was moderate for mortality during follow-up, 28-day mortality, need for mechanical ventilation, and need for ICU admission. In contrast, the quality of evidence was low for length of stay in ICU and length of stay in hospital.

Discussion

Our study comprehensively explored the efficacy of vitamin D in treating COVID-19 patients with vitamin D deficiency. We found that vitamin D supplementation could reduce mortality during follow-up. However, this result should be interpreted with caution for the following reasons. Firstly, the leave-one-out method showed that nearly half of the studies could change the conclusion, indicating that the result was not robust. Secondly, in the subgroup analysis, most groups showed no statistically significant difference between vitamin D supplementation and no vitamin D supplementation. This may be due to the limited number of studies included in the subgroup analysis, which may not accurately reflect the true effect. Thirdly, trial sequential analysis revealed no statistically significant difference between vitamin D supplementation and no vitamin D supplementation when adjusted confidence intervals were considered. The analysis also indicated that a larger sample size is needed to determine the true effect of vitamin D.

Regarding other outcomes in our study, vitamin D did not appear to reduce the need for mechanical ventilation and ICU admission or shorten the length of stay in the ICU and hospital. Overall, the efficacy of vitamin D in treating COVID-19 patients with vitamin D deficiency remains inconclusive. Due to the potential exclusion of vulnerable groups and the variability in the definitions of vitamin D deficiency, the interpretation of the results should be made with caution. More studies are needed to explore this further.

In 2023, Meng et al.'s meta-analysis[28] explored the efficacy of vitamin D in treating

COVID-19. Their results showed that while vitamin D supplementation couldn't reduce mortality, it might be beneficial in reducing the severity of illness caused by SARS-CoV-2, particularly in vitamin D-deficient patients. Additionally, their study indicated that vitamin D supplementation could reduce the need for ICU admission. However, they did not analyze the data based on follow-up time, and new research has since been published. Our study results show that vitamin D supplementation does not reduce the need for ICU admission. Recently, a review also showed that vitamin D deficiency is linked to an increased risk of acquiring SARS-CoV-2 infection and poor COVID-19 prognosis, however, available evidence with regard to improved clinical outcomes with vitamin D supplementation is inconsistent[29]. Furthermore, whether vitamin D can reduce mortality still requires further exploration.

The relationship between vitamin D and COVID-19 has been a subject of extensive research, with mixed findings regarding its efficacy in preventing or treating the disease. Observational studies that initially suggested a link between low vitamin D levels and worse COVID-19 outcomes may have been confounded by other factors such as age, comorbidities, and socioeconomic status[30-34]. These factors themselves are risk factors for both vitamin D deficiency and severe COVID-19, complicating the interpretation of results[35-40]. A number of clinical trials have produced mixed results, with some showing no significant difference in outcomes between those receiving vitamin D supplementation and those who did not[41-45]. This inconsistency suggests that vitamin D may not have a substantial impact on COVID-19 outcomes. Another possible explanation is that the design and interpretation of some studies may be

problematic. It is well known that RCTs for vitamin D should be designed based on the criteria for nutrients, rather than using the pharmaceutical standards applied to drugs. As mentioned in the "Guidelines for optimizing design and analysis of clinical studies of nutrient effects", and as noted by Pilz S et al., designing an appropriate study protocol is key to accurately assessing the impact of vitamin D on health outcomes[46 47]. Therefore, optimizing the study design is not only crucial for ensuring the reliability of the results, but also determines whether the evaluation of vitamin D intervention reflects its true effects.

The role of vitamin D in regulating the immune system has been extensively studied, especially in the context of viral infections[48 49]. The onset and severity of COVID-19 are closely linked to the host's immune response, and vitamin D is believed to enhance the immune system's defense through multiple mechanisms[48]. Specifically, vitamin D helps boost the innate immune response by enhancing the function of macrophages, monocytes, and dendritic cells, all of which play crucial roles in antiviral immunity[49]. Additionally, vitamin D regulates T cell differentiation, promoting cell-mediated immune responses against infections, while also suppressing excessive immune reactions, such as cytokine storms, thereby reducing the severity of the COVID-19 disease course[50].

The role of vitamin D is particularly critical in the early stages of disease onset[51]. Studies have shown that early intervention can significantly improve immune function and slow disease progression[21 52]. For instance, supplementing vitamin D before or at the early onset of symptoms helps to promptly regulate the immune response and

enhance the body's ability to combat the virus[53]. In contrast, if intervention occurs later, after symptoms have manifested or during the later stages of the disease, the effects of vitamin D may be greatly diminished[54 55]. By this point, the immune system may already be in a dysregulated state, particularly under the influence of high viral loads or cytokine storms, making it difficult for vitamin D alone to quickly restore immune function.

Moreover, using high doses or active forms of vitamin D, such as 25(OH)D (calcidiol), may further enhance its therapeutic effects[56]. 25(OH)D is the active form of vitamin D, and it works more rapidly than regular vitamin D3[57]. High-dose vitamin D interventions have shown promising clinical effects during the early stages of the pandemic[57]. In particular, for high-risk patients, timely high-dose vitamin D supplementation can significantly reduce the risk of disease worsening, especially in populations with low vitamin D levels[58].

Regarding high-risk groups, those at higher risk of COVID-19-related death include elderly patients, individuals with comorbidities, and patients with serum 25(OH)D concentrations below 20 ng/mL[59]. The immune systems of older adults and those with chronic diseases are generally weaker, and their vitamin D levels are often lower, making them more susceptible to severe complications or death after infection[60]. Additionally, studies have shown that if hospitalized patients have low vitamin D levels, their immune function is impaired, leading to more severe clinical outcomes[59]. Therefore, for these high-risk groups, timely and appropriate vitamin D intervention could be a critical measure to reduce the mortality rate and severity of the COVID-19

disease course[61].

However, it is important to note that vitamin D supplementation may also have potential adverse effects, such as hypercalcemia and hypoparathyroidism, particularly when taken in excessive doses[62 63]. These adverse effects should be considered when evaluating the overall benefits and risks of vitamin D supplementation, especially in vulnerable populations.

In summary, vitamin D supplementation has the potential to reduce the incidence, severity, and mortality of COVID-19. However, its effectiveness depends on multiple factors, particularly the timing and dosage of intervention. Moreover, factors such as the economic status, sex, and age of patients may serve as effect modifiers that could influence the outcomes, which were not thoroughly analyzed in our study. Future research is needed to further clarify the optimal timing and dosage for vitamin D intervention, and whether personalized treatment plans based on patients' underlying conditions and vitamin D levels are necessary. Furthermore, during the pandemic, it is important to encourage high-risk populations (such as older adults and individuals with chronic diseases) to maintain adequate vitamin D levels to enhance immunity and improve the body's ability to combat COVID-19.

In this study, we found significant differences in the definition of "vitamin D deficiency" across studies, which may introduce selection bias. Some studies defined deficiency as a serum vitamin D level below 30 ng/ml, while others used 20 ng/ml, which could lead to overdiagnosis or underdiagnosis of vitamin D deficiency. Specifically, for elderly patients, a higher threshold (e.g., 25 ng/ml) might result in

their exclusion from studies, thus affecting the study conclusions. We recommend that future research adopt standardized definitions of vitamin D deficiency and adjust the criteria based on patient characteristics (such as age, sex, and comorbidities) to reduce potential selection bias and misdiagnosis.

Moreover, the variability in vitamin D categorization may impact the assessment of treatment efficacy. Due to the inconsistent standards for defining vitamin D deficiency across studies, some studies may have underestimated the effect of vitamin D on treatment outcomes. To improve the accuracy of results, we suggest that future studies consider individualized vitamin D deficiency criteria based on different population characteristics and further explore the impact of these criteria on treatment efficacy, ensuring that all patients with true vitamin D deficiency are included in the analysis.

However, our study also has other limitations. Firstly, the number of studies included is relatively small, with only nine randomized controlled trials and small sample sizes. Secondly, although there was no significant statistical heterogeneity, clinical heterogeneity among the studies cannot be ignored. The severity of patients' diseases and the frequency and dosage of vitamin D supplementation varied among the studies. To address this, we conducted a subgroup analysis and found that vitamin D supplementation did not reduce mortality in different subgroups. Thirdly, there is a potential risk of publication bias in our study. Although Egger's test did not show significant publication bias, the number of studies included in our analysis is relatively small, so caution is still needed when interpreting the risk of publication

bias. Lastly, although our conclusions suggest that vitamin D supplementation may reduce mortality, sensitivity analysis revealed that the conclusions are not reliable. Therefore, more high-quality research is needed in the future to further explore the role of vitamin D supplementation in vitamin D deficient COVID-19 patients.

Conclusion

This study suggested that vitamin D supplementation might have reduced mortality during follow-up, but no significant difference was observed in mortality at 28 days. Additionally, vitamin D supplementation did not significantly improve the need for mechanical ventilation, ICU admission rate, or reduce hospital and ICU length of stay. While these results indicated that vitamin D might have had some impact on mortality in COVID-19 patients with vitamin D deficiency, the findings should be interpreted cautiously due to variations in the studies and potential selection biases. Future research should focus on high-quality clinical trials, particularly those considering individual differences, study design, and follow-up duration, to draw more reliable and consistent conclusions.

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Author Contributions

BSH was the guarantor of this work. LMZ, PPB, MXQ and BSH: proposed the design, searched the literature, collected, analysed and interpret the data, and wrote the report; LMZ, XMZ, YZ, and XL searched and collected the literature; LMZ, YZ, XMZ, XL and BSH analysed and interpreted the data.

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Declaration of competing interest

The authors declare that they have no known competing finical interests or personal relationships with any funding sources that could have appeared to influence the work reported in this paper.

Ethical approval

Ethical approval was not required for this study, since all data came from published articles.

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Table1. Characteristic of included randomized controlled trials

Figure1. Flowchart of literature search

Figure2. Vitamin D supplementation versus no vitamin D supplementation on mortality during follow-up, 28-day mortality, need for mechanical ventilation and need for ICU admission.

Figure3. Vitamin D supplementation versus no vitamin D supplementation on length of stay in ICU and hospital.

Figure4.Subgroup analysis of mortality during follow-up.

696 Table1.

Study	Country	Severity of COVID-19	Intervention group	Control group	Definition of Vitamin D deficiency	Follow-up
Bugarin2023	Croatia	Severe COVID-19	10,000 IU of cholecalciferol daily during ICU stay	Standard care	<20ng/ml	3 months
Bychinin2022	Russia	Severe COVID-19	60,000 IU of cholecalciferol once/ 7days followed by daily maintenance doses of 5000 IU. The high dose repeated on day 8, 16, 24, 32.	Placebo	<20ng/ml	During hospitalization
Cervero2022	Spain	NA	10,000 IU of cholecalciferol daily for 14 days	Standard care	<30ng/ml	28 days
Dilokpattanamongkol2024	Thailand	NA	2 mcg of alfacalcidol daily during the hospitalization	Standard care	<20ng/ml	During hospitalization
Maghbooli2021	Iran	NA	3000-6000 IU per day of vitamin D3 for 30 days	Placebo	<30ng/ml	2 months
Murai2021	Brazil	Moderate to severe COVID-19	Single dose of 200,000 IU of vitamin D3	Placebo	<20ng/ml	4 months

Niet2022	Belgium	NA	25,000 IU of vitamin D3 per day over 4 consecutive days, followed by 25,000 IU per week up to 6 weeks	Placebo	<20ng/ml	9 weeks
Rastogi2022	India	NA	Daily 60000 IU of cholecalciferol for 7 days , and a weekly supplementation of 60000IU provided to those with 25(OH)D > 50 ng/ml or else continued on daily vitamin D 60,000 IU supplementation for another 7 days up until day 14	Placebo	<20ng/ml	3 weeks
Singh2024	India	Severe	A single dose of 60,000 IU of cholecalciferol	Placebo	<10 ng/ml	During hospitalization
Soliman2022	Egypt	Moderate to severe COVID-19	200.000 units intramuscularly once as a single dose	placebo	<20ng/ml	6 weeks

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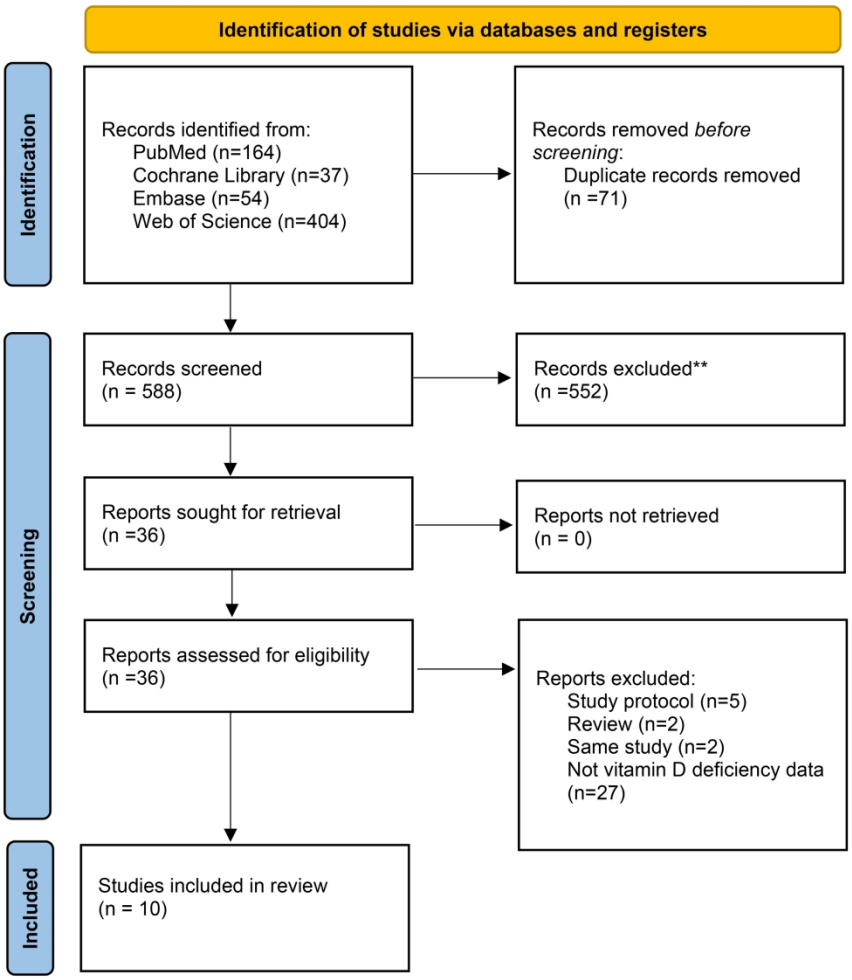

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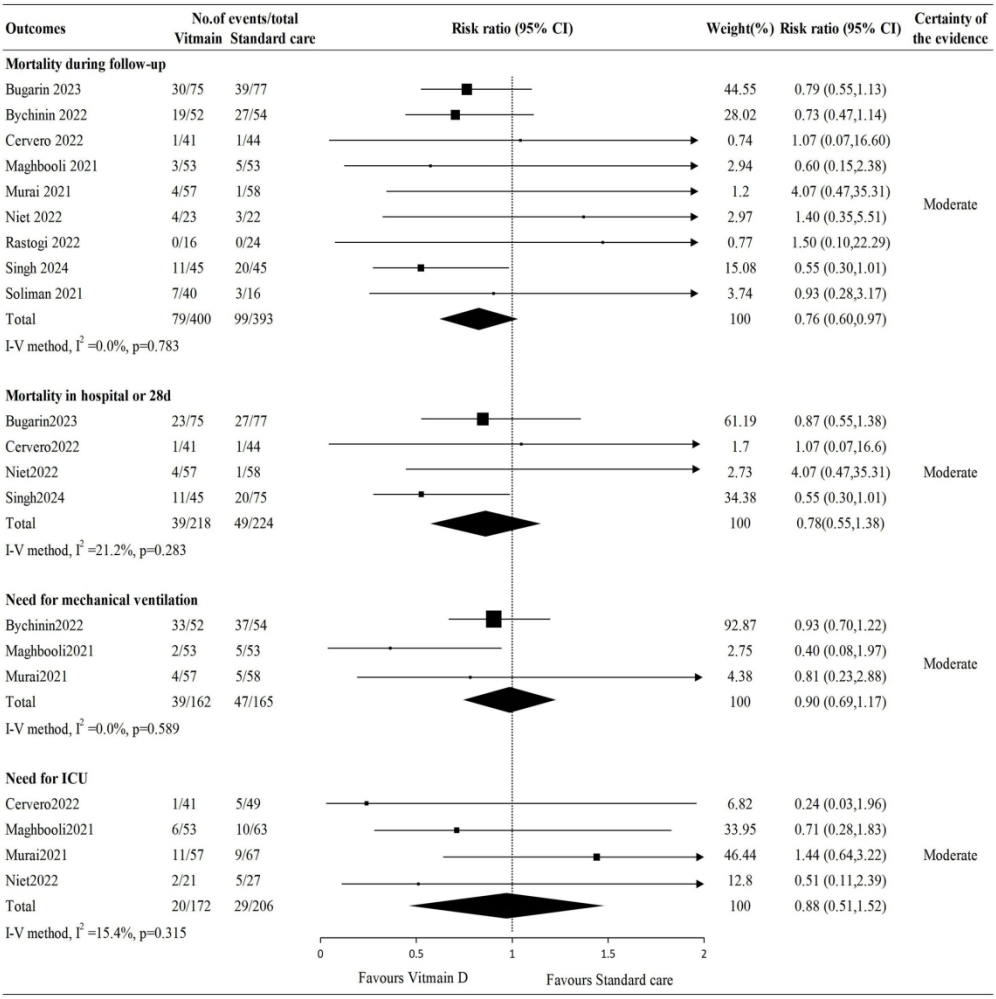

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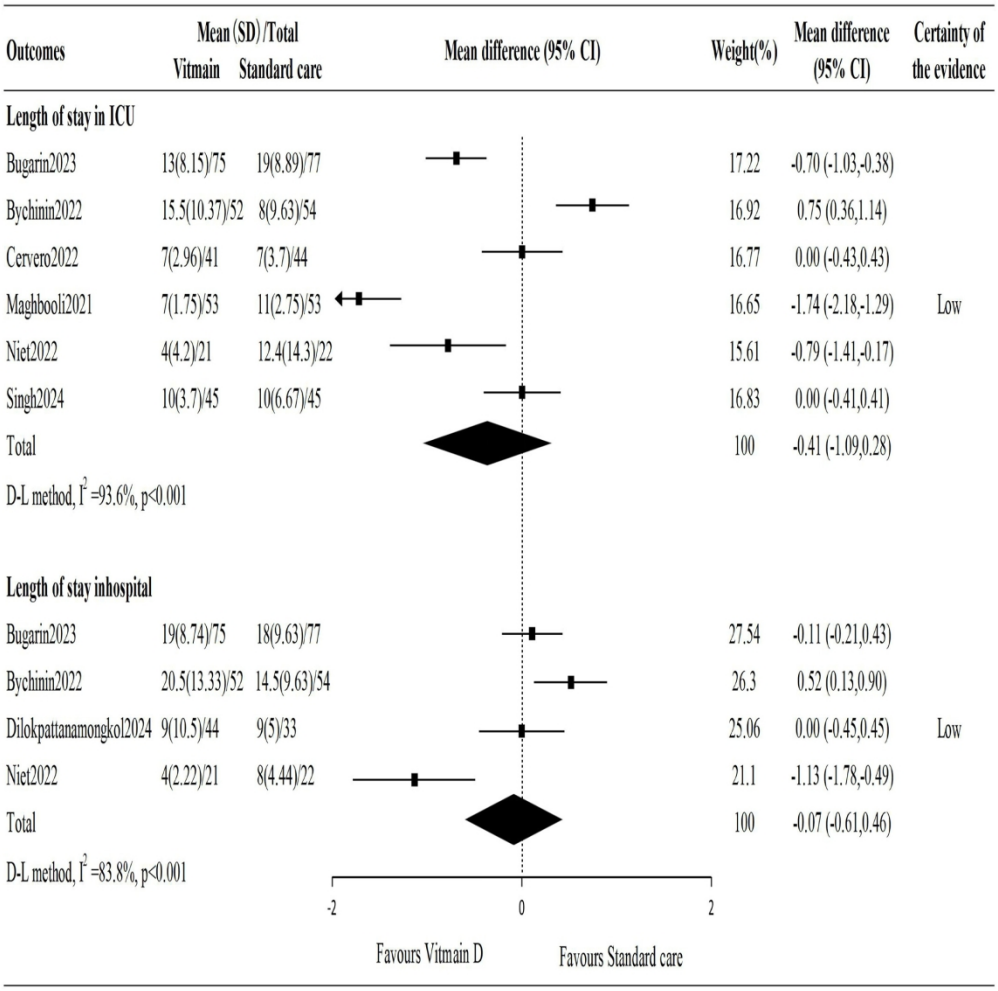

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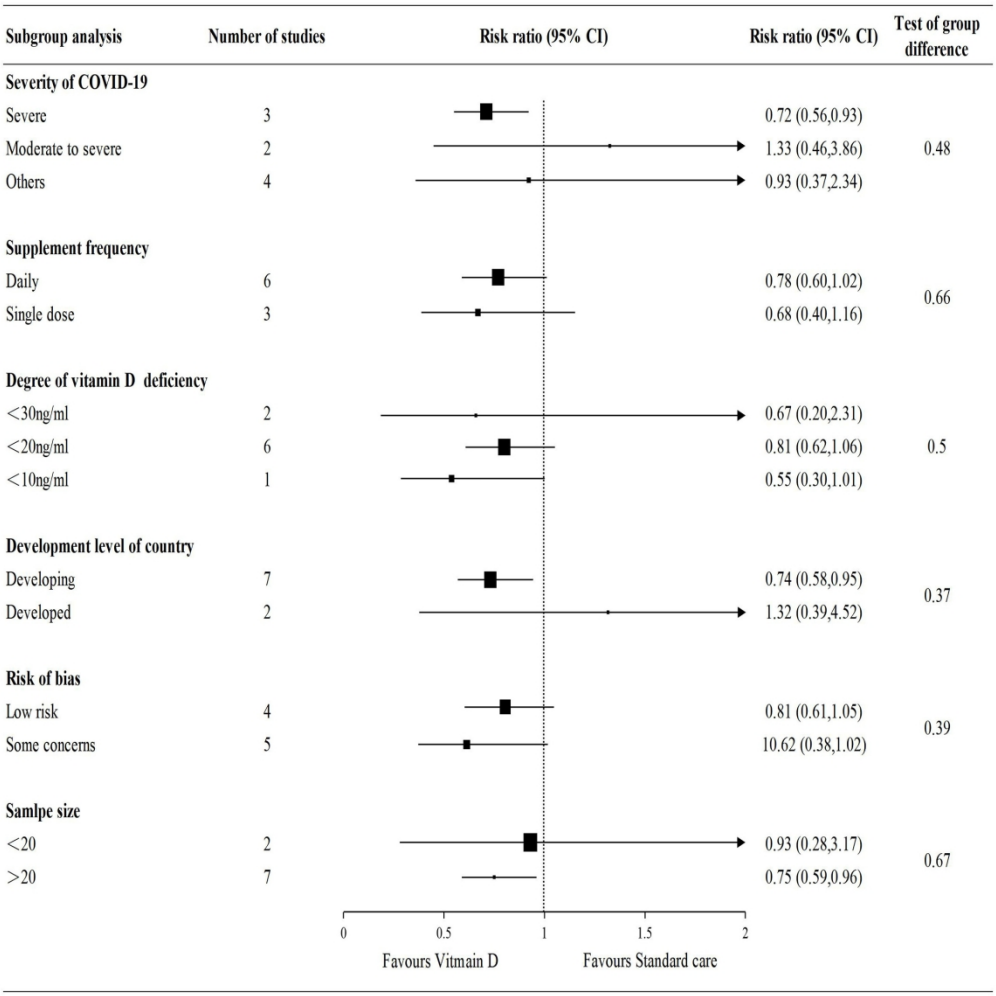

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Supplement

Search strategy

PubMed

1. “COVID-19”[Mesh] OR "COVID-19"[tiab] OR “COVID 19” [tiab] OR “2019-nCoV Infection” [tiab] OR “2019 nCoV Infection” [tiab] OR “2019-nCoV Infections” [tiab] OR “Infection, 2019-nCoV” [tiab] OR “SARS-CoV-2 Infection” [tiab] OR “Infection, SARS-CoV-2” [tiab] OR “SARS CoV 2 Infection” [tiab] OR “SARS-CoV-2 Infections” [tiab] OR “2019 Novel Coronavirus Disease” [tiab] OR “2019 Novel Coronavirus Infection” [tiab] OR “COVID-19 Virus Infection” [tiab] OR “COVID 19 Virus Infection” [tiab] OR “COVID-19 Virus Infections” [tiab] OR “Infection, COVID-19 Virus” [tiab] OR “Virus Infection, COVID-19” [tiab] OR “COVID19” [tiab] OR “Coronavirus Disease 2019” [tiab] OR “Disease 2019, Coronavirus” [tiab] OR “Coronavirus Disease-19” [tiab] OR “Coronavirus Disease 19” [tiab] OR “Severe Acute Respiratory Syndrome Coronavirus 2 Infection” [tiab] OR “COVID-19 Virus Disease” [tiab] OR “COVID 19 Virus Disease” [tiab] OR “COVID-19 Virus Diseases” [tiab] OR “Disease, COVID-19 Virus” [tiab] OR “Virus Disease, COVID-19” [tiab] OR “SARS Coronavirus 2 Infection” [tiab] OR “2019-nCoV Disease” [tiab] OR “2019 nCoV Disease” [tiab] OR “2019-nCoV Diseases” [tiab] OR “Disease, 2019-nCoV” [tiab] OR “COVID-19 Pandemic” [tiab] OR “COVID 19 Pandemic” [tiab] OR “Pandemic, COVID-19” [tiab] OR “COVID-19 Pandemics” [tiab]
2. "Vitamin D"[Mesh] OR "vitamin D"[tiab] OR "vitamin D3"[tiab] OR "vit D"[tiab] OR "vit D3"[tiab] OR "calciferol"[tiab] OR "cholecalciferol"[tiab] OR "calcidiol"[tiab] OR "calcitriol"[tiab] OR "25 hydroxyvitamin d"[tiab] OR "25 hydroxyvitamin D3"[tiab] OR "25 hydroxycalciferol"[tiab] OR "1,25 dihydroxyvitamin D"[tiab] OR "1,25 dihydroxyvitamin D3"[tiab] OR "calcifediol"[tiab]
3. Deficiency[tiab] OR Deficient[tiab] OR Deficiencies[tiab] OR Insufficiency[tiab] OR Insufficient[tiab] OR Inadequacy[tiab] OR Inadequate[tiab] OR Depletion[tiab] OR Depleted[tiab]
4. “Mortality”[tiab] OR “Mechanical ventilation”[tiab] OR “Intensive care unit”[tiab] OR “Length of stay”[tiab]
5. ((compar*[tiab]) OR ((singl*[tiab] or doubl*[tiab] or tripl*[tiab]) and (mask*[tiab] or blind*[tiab]))) OR (random*[tiab] or placebo[tiab] or controlled[tiab] or trial*[tiab])
6. #1 AND #2 AND #3 AND #4 AND $5

Cochrane Library

1. MeSH descriptor: [COVID-19] explode all trees
2. (COVID-19 OR COVID 19 OR 2019 nCoV Infection OR SARS-CoV-2 Infection OR Infection, SARS-CoV-2 OR SARS CoV 2 Infection OR SARS-CoV-2 Infections OR 2019 Novel Coronavirus Disease OR 2019 Novel Coronavirus Infection OR COVID-19 Virus Infection OR COVID 19 Virus Infection OR COVID-19 Virus Infections OR Infection, COVID-19 Virus OR Virus Infection, COVID-19 OR COVID19 OR Coronavirus Disease 2019 OR

Disease 2019, Coronavirus OR Coronavirus Disease-19 OR Coronavirus Disease 19 OR Severe Acute Respiratory Syndrome Coronavirus 2 Infection OR COVID-19 Virus Disease OR COVID 19 Virus Disease OR COVID-19 Virus Diseases OR Disease, COVID-19 Virus OR Virus Disease, COVID-19 OR SARS Coronavirus 2 Infection OR 2019 nCoV Disease OR COVID-19 Pandemic OR COVID 19 Pandemic OR Pandemic, COVID-19 OR COVID-19 Pandemics):ti,ab

3. #1 OR #2
4. MeSH descriptor: [Vitamin D] explode all trees
5. (vitamin D OR vitamin D3 OR vit D OR vit D3 OR calciferol OR cholecalciferol OR calcidiol OR calcitriol OR 25 hydroxyvitamin d OR 25 hydroxyvitamin D3 OR 25 hydroxycalciferol OR 1,25 dihydroxyvitamin D OR 1,25 dihydroxyvitamin D3 OR calcifediol):ti,ab
6. #4 OR #5
7. (Deficiency OR Deficient OR Deficiencies OR Insufficiency OR Insufficient OR Inadequacy OR Inadequate OR Depletion OR Depleted):ti,ab
8. (mortality or mechanical ventilation or intensive care unit):ti,ab
9. ((compar*) OR ((singl* or doubl* or tripl*) and (mask* or blind*))) OR (random* or placebo or controlled or trial*):ti,ab
10. #3 AND #6 AND #7 AND #8 AND #9

Embase

1. 'coronavirus disease 2019'/exp
2. ((Covid-19) OR (Covid 19) OR (2019-nCoV Infection) OR (SARS-CoV-2 Infections)):ti,ab
3. #1 OR #2
4. 'vitamin d'/exp
5. ((vitamin D) OR (vitamin D3) OR (25 hydroxycalciferol) OR (1,25 dihydroxyvitamin D3)):ti,ab
6. #4 OR #5
7. (Deficiency OR Deficient OR Deficiencies OR Insufficiency OR Insufficient OR Inadequacy OR Inadequate OR Depletion OR Depleted):ti,ab
8. (mortality or mechanical ventilation or intensive care unit):ti,ab
9. compar* OR ((singl* OR doubl* OR tripl*) AND (mask* OR blind*)) OR random*:ti,ab OR placebo:ti,ab OR controlled:ti,ab OR trial*:ti,ab

10. #3 AND #6 AND #7 AND #8 AND #9

Web of Science

1. TS=(COVID-19 OR COVID 19 OR 2019-nCoV Infection OR 2019 nCoV Infection OR 2019-nCoV Infections OR Infection, 2019-nCoV OR SARS-CoV-2 Infection OR Infection, SARS-CoV-2 OR SARS CoV 2 Infection OR SARS-CoV-2 Infections OR 2019 Novel Coronavirus Disease OR 2019 Novel Coronavirus Infection OR COVID-19 Virus Infection OR COVID 19 Virus Infection OR COVID-19 Virus Infections OR Infection, COVID-19 Virus OR Virus Infection, COVID-19 OR COVID19 OR Coronavirus Disease 2019 OR Disease 2019, Coronavirus OR Coronavirus Disease-19 OR Coronavirus Disease 19 OR Severe Acute Respiratory Syndrome Coronavirus 2 Infection OR COVID-19 Virus Disease OR COVID 19 Virus Disease OR COVID-19 Virus Diseases OR Disease, COVID-19 Virus OR Virus Disease, COVID-19 OR SARS Coronavirus 2 Infection OR 2019-nCoV Disease OR 2019 nCoV Disease OR 2019-nCoV Diseases OR Disease, 2019-nCoV OR COVID-19 Pandemic OR COVID 19 Pandemic OR Pandemic, COVID-19 OR COVID-19 Pandemics)
2. TS= (vitamin D OR vitamin D3 OR vit D OR vit D3 OR calciferol OR cholecalciferol OR calcidiol OR calcitriol OR 25 hydroxyvitamin d OR 25 hydroxyvitamin D3 OR 25 hydroxycalciferol OR 1,25 dihydroxyvitamin D OR 1,25 dihydroxyvitamin D3 OR calcifediol)
3. TS= (Deficiency OR Deficient OR Deficiencies OR Insufficiency OR Insufficient OR Inadequacy OR Inadequate OR Depletion OR Depleted)
4. TS= (mortality or mechanical ventilation or intensive care unit)
5. TS=(((compar*) OR ((singl* or doubl* or tripl*) and (mask* or blind*))) OR (random* or placebo or controlled or trial*))
6. #1 AND #2 AND #3 AND #4 AND #5

eTable1. Risk of bias of included studies

Outcome	D1	D2	D3	D4	D5	Overall bias
Study: Bugarin2023						
Mortality during follow up	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
28-day mortality	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Length of stay in ICU	Low risk	Some concerns	Low risk	Low risk	Low risk	Some concerns
Length of stay in hospital	Low risk	Some concerns	Low risk	Low risk	Low risk	Some concerns
Study: Bychinin2022						
Mortality during follow up	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Need for mechanical ventilation	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Length of stay in ICU	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Study: Cervero2022						
Mortality during follow up	Low risk	Low risk	Low risk	Low risk	Some concerns	Some concerns
28-day mortality	Low risk	Low risk	Low risk	Low risk	Some concerns	Some concerns
Length of stay in ICU	Low risk	Some concerns	Low risk	Low risk	Some concerns	Some concerns
Study: Dilokpattanamongkol2024						
Length of stay in hospital	Low risk	Some concerns	Low risk	Low risk	Low risk	Some concerns
Study: Maghbooli2021						
Mortality during follow up	Low risk	Low risk	Low risk	Low risk	Some concerns	Some concerns
Need for mechanical ventilation	Low risk	Low risk	Low risk	Low risk	Some concerns	Some concerns
Need for ICU admission	Low risk	Low risk	Low risk	Low risk	Some concerns	Some concerns

Length of stay in ICU	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Study: Murai2021						
Mortality during follow up	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Need for mechanical ventilation	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Need for ICU admission	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Study: Niet2022						
Mortality during follow up	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
28-day mortality	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Need for ICU admission	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Length of stay in ICU	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Length of stay in hospital	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Study: Rastogi2022						
Mortality during follow up	Low risk	Low risk	Low risk	Some concerns	Low risk	Some concerns
Study: Singh2024						
Mortality during follow up	Low risk	Low risk	Low risk	Low risk	Some concerns	Some concerns
28-day mortality	Low risk	Low risk	Low risk	Low risk	Some concerns	Some concerns
Length of stay in ICU	Low risk	Low risk	Low risk	Low risk	Some concerns	Some concerns
Study: Soliman2021						
Mortality during follow up	Some concerns	Low risk	Low risk	Low risk	Some concerns	Some concerns

D1: Randomisation process
D2: Deviations from the intended interventions
D3: Missing outcome data

D4: Measurement of the outcome
D5: Selection of the reported result
eTable2.

Outcomes	No. of participants (No. of trials)	Risk ratio (95%CI)	Mean difference (95%CI)	Risk of bias[a]	Inconsistency[b]	Imprecision[c]	Small study effects[d]	Certainty of evidence
Mortality during follow-up	737 (8)	0.76 (0.59,0.96)		Not Down graded	Not down graded	Down graded	Not down graded	Moderate
28-day mortality	442 (4)	0.79 (0.49,1.26)		Not Down graded	Not down graded	Down graded	Not down graded	Moderate
Need for mechanical ventilation	327 (3)	0.90 (0.69,1.17)		Not Down graded	Not down graded	Down graded	Not down graded	Moderate
Need for ICU admission	349 (4)	0.84 (0.45,1.56)		Not down graded	Not down graded	Down graded	Not down graded	Moderate
Length of stay in ICU	582 (6)		-0.41 (-1.09,0.28)	Not Down graded	Down graded	Down graded	Not down graded	Low
Length of stay in hospital	378 (4)		-0.07 (-0.61,0.46)	Not down graded	Down graded	Down graded	Not down graded	Low

ICU, intensive care unit

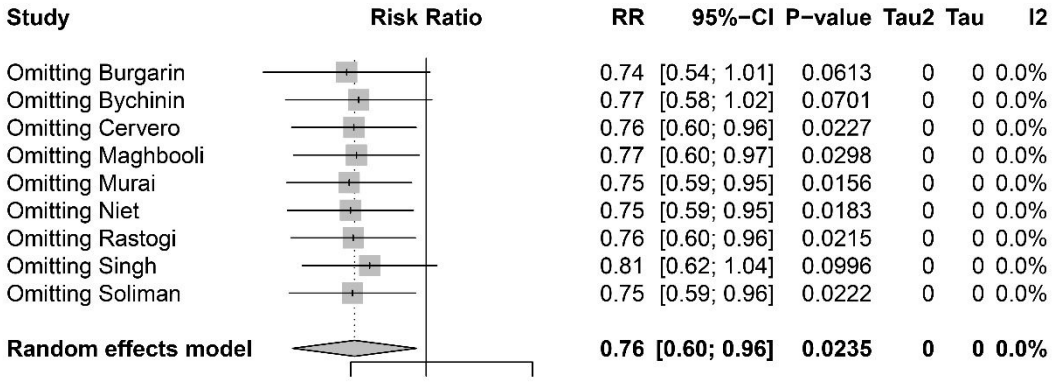
[a] Downgraded by one level because >25% of participants in this comparison were from studies at high risk of bias.

[b] Downgraded by one level because heterogeneity (I^2) >50%.

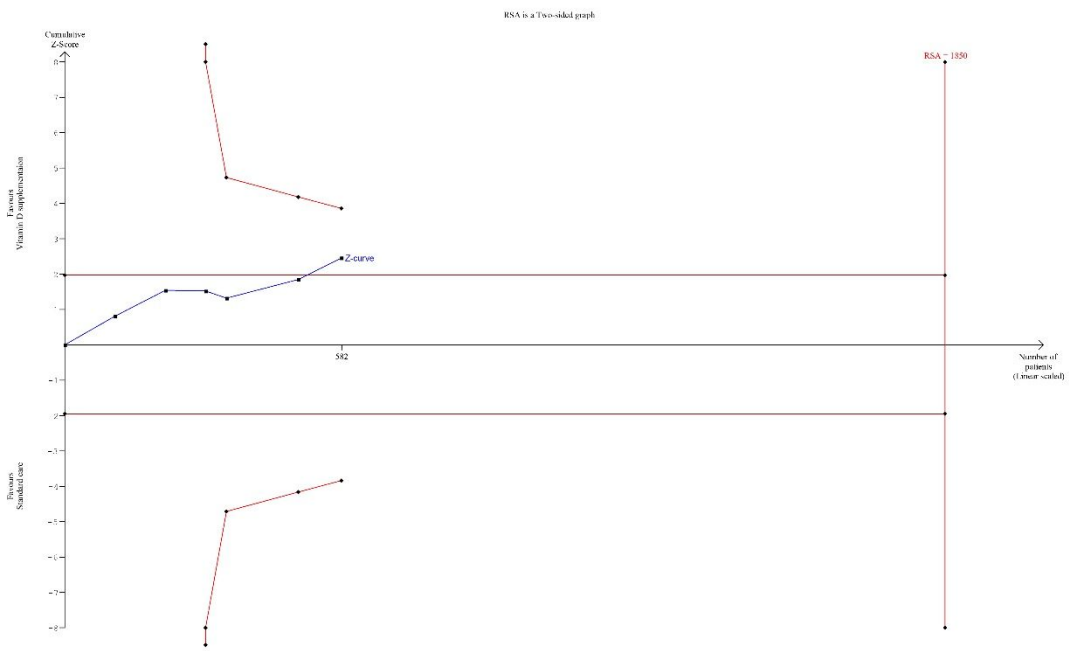
[c] Downgraded by one level because the limits of the 95% confidence interval were 20% different to the point estimates.

[D] Downgraded by one level owing to small study bias.

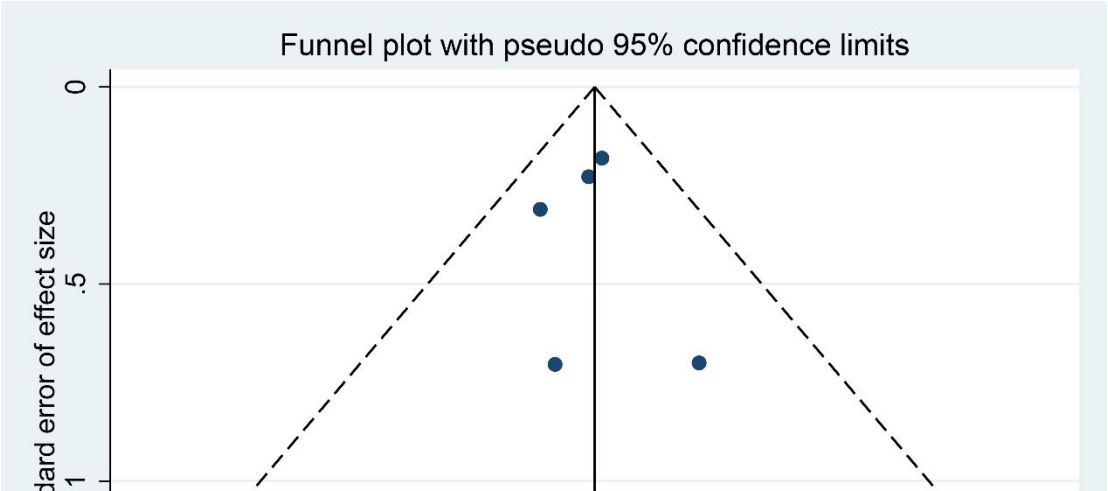
eFigure1. Leave-one-out on mortality during follow-up



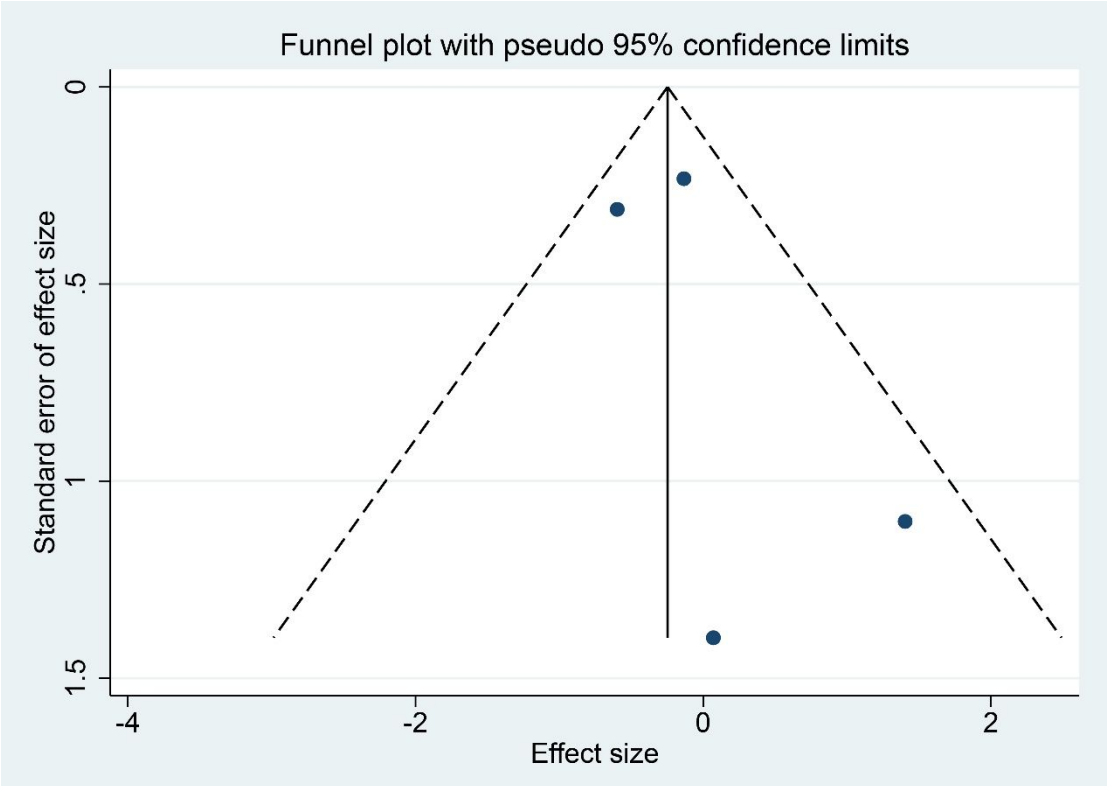
eFigure2. Trial sequential analysis on mortality during follow-up



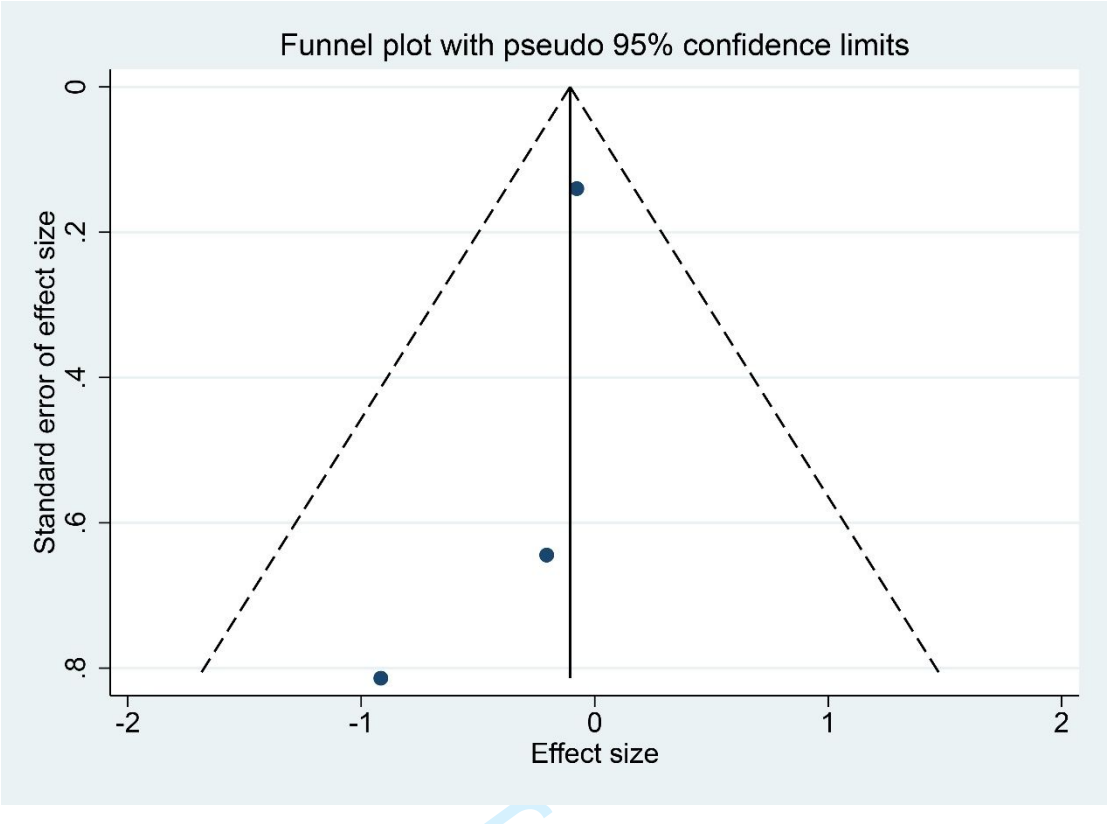
eFigure3. Funnel plot of mortality during follow-up



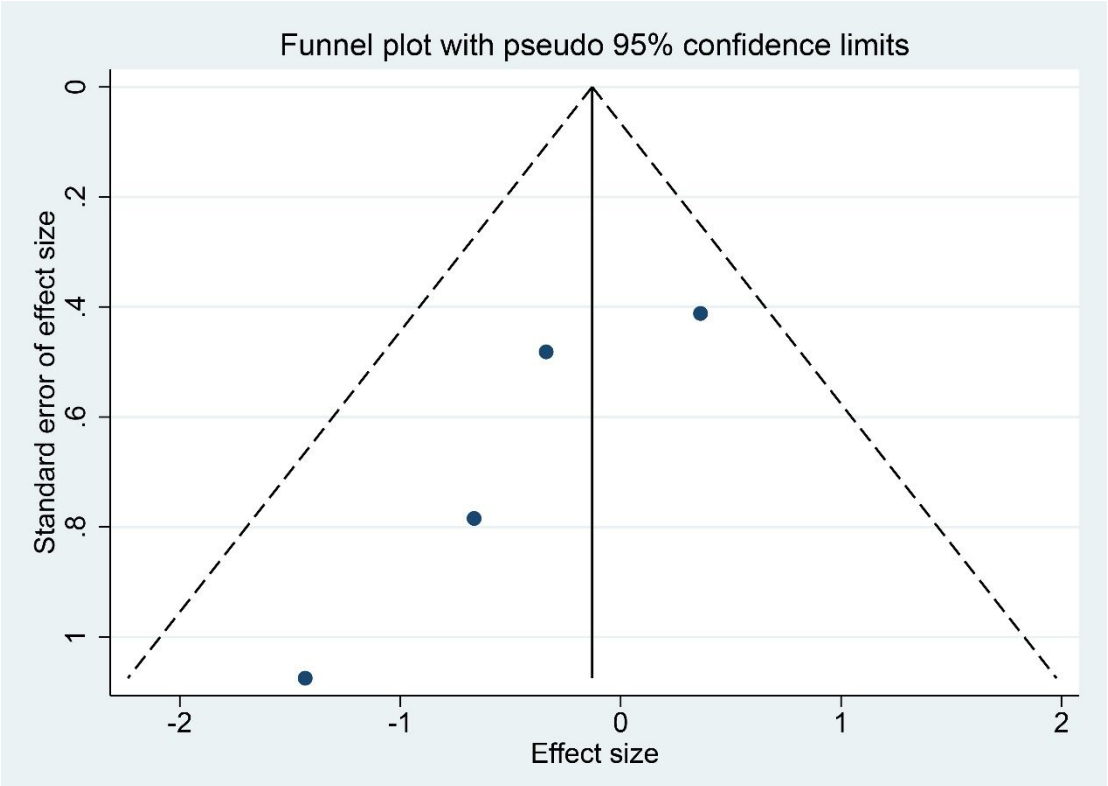
eFigure4. Funnel plot of 28day-mortality



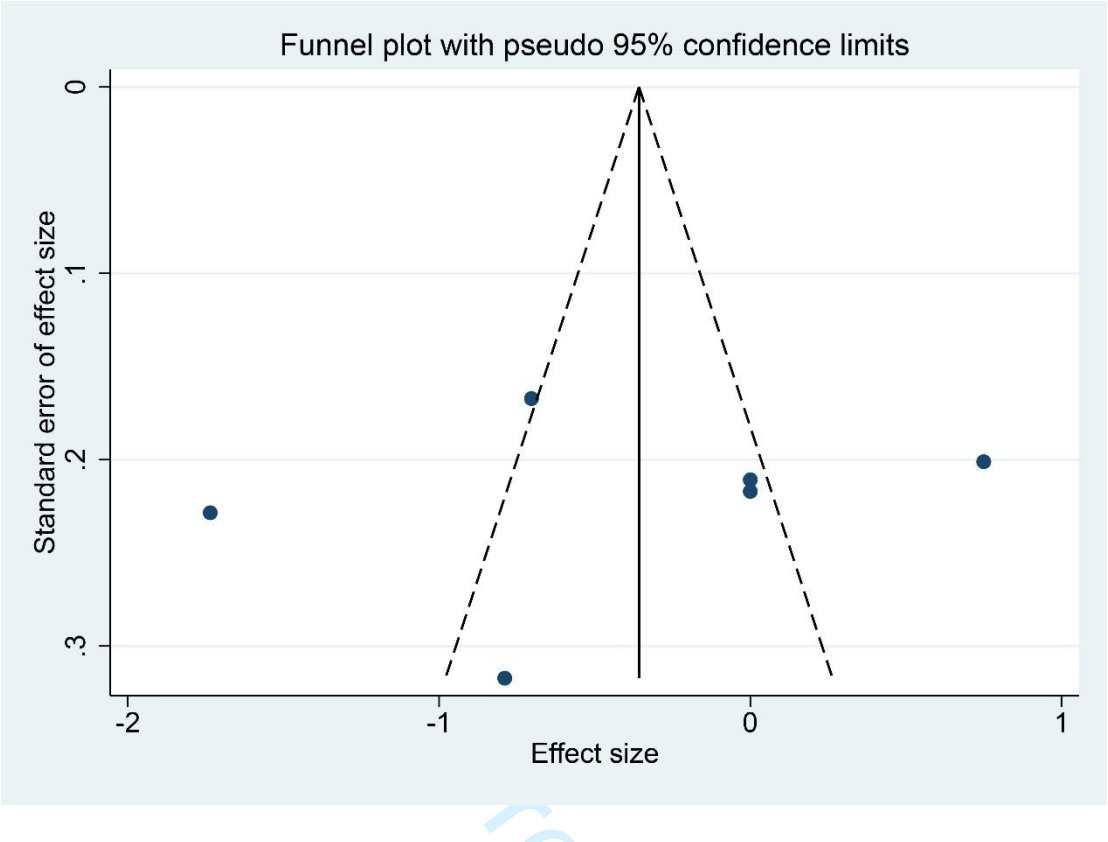
eFigure5. Funnel plot of need for mechanical ventilation



eFigure6. Funnel plot of need for ICU admission



eFigure7. Funnel plot of length of stay in ICU



eFigure8. Funnel plot of length of stay in hospital