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Vitamin D insufficiency as a potential culprit in critical COVID-19 patients

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jmv.26360.

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Short running title: Vitamin D and COVID-19

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Table number: 1 Figures number: 4 Words number: 2109

Abstract

Background: As an immune modulator, vitamin D has been implicated in the Coronavirus disease-2019 (COVID-19) outcome. We aim to systematically explore the association of vitamin D serum levels with COVID-19 severity and prognosis.

Methods: The standardized mean difference (SMD) or odds ratio and 95% confidence interval (CI) were applied to estimate pooled results from seven studies. The prognostic performance of vitamin D serum levels for predicting adverse outcomes with detection of

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the best cutoff threshold was determined by ROC curve analysis. Decision tree analysis by combining vitamin D levels and clinical features was applied to predict severity in COVID-19 patients.

Results: Mean vitamin D serum level of 1,368 patients, was 22.9 nmol/L (21.9-23.8). Significant heterogeneity was found ($I^2 = 99.9\%$, $P < 0.001$). Patients with poor prognosis (N=634) had significantly lower serum levels of vitamin D compared to those with good prognosis (N=669), representing an adjusted standardized mean difference of -5.12 (95% CI= -9.14 to -1.10, $P = 0.012$).

Conclusion: Serum vitamin D levels could be implicated in the COVID-19 prognosis. Diagnosis of vitamin D deficiency could be a helpful adjunct in assessing patients' potential of developing severe COVID-19. Appropriate preventative and/or therapeutic intervention may improve COVID-19 outcomes.

Highlights

- COVID-19 patients with poor prognosis have low serum levels of vitamin D
- Greater levels of vitamin D insufficiency were noted among Asian patients with severe presentations
- Diagnosis of vitamin D deficiency could be a helpful adjunct in assessing patients' potential of developing severe COVID-19.

- Appropriate preventative and/or therapeutic intervention may improve COVID-19 outcomes.

Keywords: COVID-19; meta-analysis; outcome; SARS-CoV-2; vitamin D

Introduction

Coronavirus disease-2019 (COVID-19) is caused by novel strain Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) ¹. SARS-CoV-2 has shown rapid spread worldwide, mediated via respiratory droplets and airborne transmission, close contact, fecal-oral transmission, and even through tears ². Transmissibility and infectivity are higher in SARS-CoV-2 compared to its predecessors SARS-CoV-1 and the Middle East Respiratory Syndrome (MERS)-CoV, but the mortality rate is low ³. SARS-CoV-1 was first identified in China in 2002, and MERS-CoV first identified in Saudi Arabia in 2012 ⁴. Recently, no apparent evidence was implied that the prevalence of COVID-19 pandemic associated with close contacts through human-to-human transmission in the midpoint of December 2019 ^{5,6}. WHO declared an international public health emergency on January 30, and a subsequent pandemic on March 11, 2020 ⁷. The tracking of COVID-19 cases is challenging. The majority (80-85%) of infected people are either asymptomatic or have a self-limiting disease course, with the remainder constituting either mild or severe cases ⁸. Mild cases present with non-specific symptoms, which typically include fever, cough, fatigue, diarrhea, headache, and sore throat ⁹. Asymptomatic or mild COVID-19 commonly affects younger individuals, while severe forms are more likely presented in elderly populations. According to the United States Centers for Disease Control and Prevention (CDC), severe cases of COVID-19 are

suspected in the presence of cyanosis of the lips or face; tachypnea; shortness of breath; chest pain with breathing; paroxysmal nocturnal dyspnea; new-onset confusion; or somnolence ¹⁰.

Additionally, acute respiratory distress syndrome (ARDS) is a feared complication associated with severe manifestations of COVID-19 and commonly leads to death. Consistent with the above, mortality rates vary drastically by age—those less than 60 years old die at a rate of 0.46%, compared to patients greater than 60 years old dying at a rate of 8.8%. Pre-existing conditions (such as diabetes or hypertension), of which elderly populations are more likely predisposed, raise the mortality rate even further ⁷.

Recent work has illustrated the role of vitamin D deficiency—a common condition that affects the elderly, the obese, those with darker skin tones, or those with inadequate exposure to sunlight—as a risk factor for acute respiratory infection (ARI) such as COVID-19 ^{11,12}. At the molecular level, vitamin D and its receptor (VDR) play roles in both the innate and adaptive immune systems, as well as mitigating the antimicrobial and anti-inflammatory processes within the body. Given the relative ease and safety of vitamin D repletion in deficient individuals ^{11,12}, determination of vitamin D's role—if any—in COVID-19 severity is a meaningful endeavor. In this sense, this study focuses on the relationship of varying COVID-19 outcomes with serum vitamin D levels.

Methods

Search strategy

This systematic review and meta-analysis were reported following the Preferred

Reporting Items for Systemic Review and Meta-Analysis (PRISMA) guidelines¹³. The authors selected relevant studies published up to June 8, 2020, by searching Google Scholar, ScienceDirect, PubMed, Scopus, Web of Science, and MedRxiv search engines. Searches initially used the following strings: "Vitamin D", "25-Hydroxyvitamin D", "Calcitriol", "Low Vitamin D". The results of these searches were combined with sets created with "COVID-19", "SARS CoV-2", "Coronavirus".

Selection criteria

We performed a systematic review of studies that described COVID-19 severity, intensive care unit (ICU) admission, or mortality. Inclusion criteria for eligibility were as follows: (1) study type: retrospective, prospective, observational, case series, descriptive or case-control studies reporting serum vitamin D level; (2) subjects: diagnosed patients with COVID-19; (3) exposure/intervention: data including at least one pairwise comparison, which was defined as severe (defined as acute respiratory distress syndrome and/or mechanical ventilation) *versus* non-severe outcome, ICU admission *versus* floor admission, living *versus* expired cohorts; and (4) outcome indicator: the mean and standard deviation for laboratory test values of vitamin D, the total sample size for demographics, comorbidities, and complications. The following exclusion criteria were considered: (1) case reports, reviews, abstracts, and summaries of discussion; (2) insufficient reported data information; and (3) absence of vitamin D measurement.

Data abstraction

Two investigators separately conducted literature screening, followed by data abstraction

in a predesigned excel sheet by three investigators (RM, RE, NS). Any disagreement was resolved by another investigator (ET). Study characteristics (author name, publication, date, journal name, ethnicity, study design, and sample size) and patients' demographics (age, gender, BMI) were collected.

Statistical analysis

The manipulation of data analysis was conducted utilizing the OpenMeta[Analyst]¹⁴ and the comprehensive meta-analysis (CMA) program version 3¹⁵. The estimated pool results were computed to calculate the Hedge's *g* or odds ratio (OR) with their 95% confidence intervals (CI). First, a single-arm pooled analysis was performed for seven articles (eight datasets). Then, a two-arm meta-analysis was carried out to determine the poor outcomes of COVID-19 patients with vitamin D deficiency. Our team defined poor outcomes of COVID-19 patients as those with severe presentation (acute respiratory distress syndrome and/or mechanical ventilation), intensive care unit (ICU) admission or died. We conducted a three pairwise comparison for severity, ICU admission, and survival, followed by combining all records to compare patients with poor versus good prognosis. A meta-analysis study was processed utilizing the random effect model¹⁶. The estimation of heterogeneity within studies was evaluated using Cochran's Q statistic with Higgin's I^2 statistics. Next, the assessment of publication bias was processed using a funnel plot with Egger's regression test, with a two-tailed *p-value* less than 0.1 suggesting obvious publication bias¹⁷. The trial sequential analysis was employed to compute the statistical trustworthiness of this established meta-analysis by merging the cumulative sample sizes of all records to improve the power of findings¹⁸. In the event that the cumulative Z-

curve accomplished its target and transverses the appropriate boundaries, statistical significance is reached, and no further records are essential. Trial sequential analysis (TSA) software version 0.9.5.10 was applied in this procedure.

Results

Characteristics of the study population

Of 1,351 studies obtained from the literature search, a total of 1,195 were screened for eligibility, and seven articles (eight datasets) were included in this meta-analysis (**Figure 1**). All were retrospective in nature, with only one case series, including different ethnic backgrounds (3 Asian, 3 European, 1 American). Publishing dates ranged from April 9 to May 20, 2020. Characteristics of enrolled studies^{8,16,19-23} are demonstrated in **Table 1**.

Demographic data

Pooled mean age at presentation was 63.8 years (56.5-71.2), with male patients constituting 55.6% (28.4%-82.7%). Pairwise comparison between patients presenting with severe symptomology, ICU admission, or expiration and those with good outcomes revealed an insignificant difference between age (SMD = -0.02, 95%CI = -0.27 to 0.22, $p = 0.82$) or sex (OR = 2.95, 95%CI = 0.74 to 11.6, $p = 0.12$) in both groups.

Comorbidities

One-arm meta-analysis showed low proportions of comorbidities across included studies, with hypertension being the most frequent (proportion = 14.3%, 95%CI = 8% to 20.6%), followed by congestive heart failure (proportion = 3.6%, 95%CI = 1.4% to 5.8%),

diabetes (proportion = 1.2%, 95%CI = 0% to 2.4%), and chronic obstructive pulmonary disease (COPD) (proportion = 0.9%, 95%CI = 0.1% to 1.9%). As depicted in **Figure 2**, pairwise comparison revealed no significant difference between cohorts with poor *versus* good outcomes; for hypertension (OR = 2.51, 95%CI = 0.38-16.6, $p = 0.33$), congestive heart failure (OR = 0.59, 95%CI = 0.29-1.17, $p = 0.13$), diabetes mellitus (OR = 0.84, 95%CI = 0.37-1.89, $p = 0.67$), and COPD (OR = 0.79, 95%CI = 0.34-1.85, $p = 0.59$). No evidence of heterogeneity was found for all comorbidities ($I^2 = 0$, $p > 0.1$), apart of studies reporting hypertension ($I^2 = 72.23\%$, $p = 0.001$) were considerable heterogeneity was found.

Vitamin D serum level

Across seven studies with 1,368 COVID-19 patients, the mean vitamin D level was 22.9 nmol/L (95%CI = 21.9-23.8). Significant heterogeneity was found ($I^2 = 99.9\%$, $p < 0.001$). Subgroup analysis revealed the highest vitamin D serum levels among Americans (mean = 35.9 nmol/L, 95%CI = 34.4-37.3) and the least among Europeans (19.3 nmol/L, 95%CI = 18.9-19.6). Apart from Asian studies, heterogeneity was otherwise resolved (**Figure 3A**).

Patients with poor prognosis (N=634) had significantly lower levels of vitamin D compared to those with good prognosis (N=669), representing an adjusted standardized mean difference of -5.12 (95%CI = -9.14 to -1.10, $p = 0.012$). Major differences with greater levels of vitamin D insufficiency were noted among Asian patients with severe presentations (SMD = -11.15, 95%CI = -21.4 to -0.84, $p = 0.034$) (**Figure 3A**). Subgroup analysis also showed large differences between expired and survived cohorts (SMD = -

14.6, 95%CI = -15.3 to -13.8) (**Figure 3B**). The temporal analysis demonstrated that studies published in April had the lowest levels of vitamin D among patients with poor outcomes (SMD = -11.15, 95%CI = -21.4 to -0.84) (**Figure 3C**). No evidence of publication bias was found (Egger's p-value = 0.21). The trial sequential analysis illustrated the cumulative "Z-curve" extended over the trial monitoring boundary before reaching the required sample size (N=1,303), indicating that the accumulative proof was satisfactory with no further records needed to substantiate these conclusions (**Figure 4**).

Discussion

In this meta-analysis with a total of 1,368 COVID-19 positive patients, low vitamin D level (i.e. deficiency) was significantly associated with poorer patient outcomes and prognosis. Remaining unaffected by possible "confounders" such as age, gender, or comorbid conditions, vitamin D deficiency plays an independent causative role in the manifestation of disease severity. Of note was the slightly pronounced difference in vitamin D levels between patients who survived versus expired due to COVID-19. The possible cause of this observation may be somewhat explained by vitamin D's role in a variety of the body's immune responses. 1,25 dihydroxy vitamin D, otherwise known as calcitriol, is the active form of vitamin D. Upon interacting with the VDR which is found on immune (B-cells, T-cells, and antigen-presenting cells) and pulmonary epithelial cells, the calcitriol-VDR complex induces transcriptional expression of antimicrobial peptides such as cathelicidins and defensins. Cathelicidins serve to disrupt the bacterial cell membranes, as well the enveloped viruses such as SARS-CoV-2, while defensins promote chemotaxis of inflammatory cells via increased capillary permeability ²⁴.

Despite vitamin D promoting the expression of several inflammatory cytokines via T-cell inactivation and interferon-gamma activation²⁵, it simultaneously down-regulates pro-inflammatory markers interleukin-6 and tumor necrosis factor-alpha the two key cytokines involved with the development of the "cytokine storm" that precedes ARDS²⁶. Given this understanding at the molecular level, one can reasonably imply that patients with vitamin D deficiency are at an increased risk of developing more severe manifestations of COVID-19 and/or worse prognosis²⁷. The importance of vitamin D and immunity is echoed by the fact that Bacillus Calmette–Guérin (BCG) vaccination in COVID-19 patients appears to have a protective effect via the upregulation of the same pathways mentioned above²⁵.

Differences noted in subgroup analyses could be attributed to various geographic, cultural, and/or behavioral differences at the individual and group level. Vitamin D is primarily biosynthesized by the skin upon exposure to sunlight; additional sources of vitamin D can be incurred through the diet, including fish, eggs, leafy greens, or fortified foods. Temporal subgroup analysis yielded the lowest vitamin D levels in April—a highpoint of the pandemic's spread and a time when many were quarantining and likely limiting their time outdoors. COVID-19's initial presentation during "cold season" is also a time of limited sun exposure¹². Within the same vein, Americans having the highest vitamin D levels amongst the ethnic groups could be in part due to the "sun-happy" culture, where tans are viewed as desirable or even healthy. This is in direct contrast to many Eastern cultures, in which sun exposure is strictly limited. Geography is also important, as certain areas of the world simply receive less sunlight than others. Lastly, supplementation via diet may be impacted based on individual tastes and preferences, or

socioeconomic factors, such as limited access to healthy, nutritionally complete foods.

Regardless of etiology, vitamin D deficiency is both easily and inexpensively diagnosable and treatable. A recent meta-analysis yielded measurable benefits in protection against ARIs when providing daily vitamin D supplementation; these effects were even more pronounced in vitamin D-deficient patients²⁸. Given that vitamin D is generally safe, affordable, and accessible, timely preventative and therapeutic measures may prevent poor outcomes in at-risk populations.

Although this comprehensive meta-analysis limited by the small number of the enrolled eligible studies and the observed publication bias and heterogeneity between studies, the trial sequential analysis proved that the cumulative Z-curve crossed the monitoring boundary before reaching the required sample size, suggesting that the cumulative proof was reliable, and no additional trials are necessary to achieve the significances.

In conclusion, serum vitamin D levels could have a role in the COVID-19 outcome. Diagnosis of vitamin D deficiency could be a helpful adjunct in assessing patients' potential of developing severe COVID-19.

Funding

None.

Conflicts of interest

All authors declare no conflict of interest.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article.

Authors' contribution

EAT: study design; RM, MHH, RE, NS: study identification and data extraction; MHH, RE, EAT: statistical analysis; MHH, RE, EAR, MSF: data interpretation; RM, MHH, RE, CJ, NS, EAT, MSF: original draft preparation. All authors revised and approved the final version of the manuscript.

Abbreviations

ARDS: acute respiratory distress syndrome; ARI: acute respiratory infection; BCG: Bacillus Calmette–Guérin; CI: confidence intervals; COVID-19: Coronavirus Disease 2019; ICU: intensive care unit; MERS: Middle East Respiratory Syndrome; OR: odds ratio; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; ROC: Receiver Operating Characteristic; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; SMD: standardized mean difference; TSA: Trial sequential analysis; VDR: vitamin D receptor.

References

1. Toraih EA, Elshazli RM, Hussein MH, et al. Association of cardiac biomarkers and comorbidities with increased mortality, severity, and cardiac injury in COVID-19 patients: A meta-regression and decision tree analysis. *J Med Virol.* 2020.

2. Guo YR, Cao QD, Hong ZS, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak - an update on the status. *Mil Med Res.* 2020;7(1):11.
3. Wang L, Wang Y, Ye D, Liu Q. Review of the 2019 novel coronavirus (SARS-CoV-2) based on current evidence. *International Journal of Antimicrobial Agents.* 2020:105948.
4. Tsatsakis A, Petrakis D, Nikolouzakis TK, et al. COVID-19, an opportunity to reevaluate the correlation between long-term effects of anthropogenic pollutants on viral epidemic/pandemic events and prevalence. *Food and Chemical Toxicology.* 2020;141:111418.
5. Li Q, Guan X, Wu P, et al. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. *New England Journal of Medicine.* 2020;382(13):1199-1207.
6. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet.* 2020;395(10223):497-506.
7. Kakodkar P, Kaka N, Baig MN. A Comprehensive Literature Review on the Clinical Presentation, and Management of the Pandemic Coronavirus Disease 2019 (COVID-19). *Cureus.* 2020;12(4):e7560.
8. Lau FH, Majumder R, Torabi R, et al. Vitamin D Insufficiency is Prevalent in Severe COVID-19. *medRxiv.* 2020:2020.2004.2024.20075838.
9. Zhang B, Zhou X, Qiu Y, et al. Clinical characteristics of 82 death cases with COVID-19. *medRxiv.* 2020:2020.2002.2026.20028191.
10. Team CC-R. Severe Outcomes Among Patients with Coronavirus Disease 2019 (COVID-19) - United States, February 12-March 16, 2020. *MMWR Morb Mortal Wkly Rep.* 2020;69(12):343-346.

11. Kennel KA, Drake MT, Hurley DL. Vitamin D deficiency in adults: when to test and how to treat. *Mayo Clin Proc.* 2010;85(8):752-758.
12. Rhodes JM, Subramanian S, Laird E, Griffin G, Kenny RA. Perspective: Vitamin D deficiency and COVID-19 severity - plausibly linked by latitude, ethnicity, impacts on cytokines, ACE2, and thrombosis (R1). *J Intern Med.* 2020.
13. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ.* 2009;339:b2535.
14. Wallace BC, Dahabreh IJ, Trikalinos TA, Lau J, Trow P, Schmid CH. Closing the Gap between Methodologists and End-Users: Ras a Computational Back-End. *Journal of Statistical Software.* 2012;49(5):15.
15. Pierce CA. Software Review: Borenstein, M., Hedges, L. V., Higgins, J. P. T., & Rothstein, H. R. (2006). *Comprehensive Meta-Analysis (Version 2.2.027)* [Computer software]. Englewood, NJ: Biostat. *Organizational Research Methods.* 2008;11(1):188-191.
16. De Smet D, De Smet K, Herroelen P, Gryspeerdt S, Martens GA. Vitamin D deficiency as risk factor for severe COVID-19: a convergence of two pandemics. *medRxiv.* 2020:2020.2005.2001.20079376.
17. Lin L, Chu H. Quantifying publication bias in meta-analysis. *Biometrics.* 2018;74(3):785-794.
18. Elshazli RM, Toraih EA, Elgaml A, Kandil E, Fawzy MS. Genetic polymorphisms of TP53 (rs1042522) and MDM2 (rs2279744) and colorectal cancer risk: An updated meta-analysis based on 59 case-control studies. *Gene.* 2020;734:144391.
19. Alipio M. Vitamin D Supplementation Could Possibly Improve Clinical Outcomes of Patients Infected with Coronavirus-2019 (COVID-19). *SSRN.* 2020.

20. D'Avolio A, Avataneo V, Manca A, et al. 25-Hydroxyvitamin D Concentrations Are Lower in Patients with Positive PCR for SARS-CoV-2. *Nutrients*. 2020;12(5).
21. Faul JL, Kerley CP, Love B, et al. Vitamin D Deficiency and ARDS after SARS-CoV-2 Infection. *Irish Medical Journal*. 2020;113(5):84.
22. Raharusuna P, Priambada S, Budiarti C, Agung E, Budi C. Patterns of COVID-19 Mortality and Vitamin D: An Indonesian Study. *SSRN*. 2020.
23. Tian Y, Rong L. Letter: does vitamin D have a potential role against COVID-19? Authors' reply. *Aliment Pharmacol Ther*. 2020.
24. Gombart AF. The vitamin D-antimicrobial peptide pathway and its role in protection against infection. *Future Microbiol*. 2009;4(9):1151-1165.
25. Toraih EA, Sedhom JA, Dokunmu TM, et al. Hidden in plain sight: The effects of BCG vaccination in COVID-19 pandemic. *bioRxiv*. 2020:2020.2006.2009.142760.
26. Coperchini F, Chiovato L, Croce L, Magri F, Rotondi M. The cytokine storm in COVID-19: An overview of the involvement of the chemokine/chemokine-receptor system. *Cytokine & Growth Factor Reviews*. 2020;53:25-32.
27. Panagiotou G, Tee SA, Ihsan Y, et al. Low serum 25-hydroxyvitamin D (25 [OH] D) levels in patients hospitalised with COVID-19 are associated with greater disease severity. *Clinical endocrinology*. 2020; doi:10.1111/cen.14276.
28. Grant WB, Lahore H, McDonnell SL, et al. Evidence that Vitamin D Supplementation Could Reduce Risk of Influenza and COVID-19 Infections and Deaths. *Nutrients*. 2020;12(4).

Figure legends

FIGURE 1. The workflow of the study selection process.

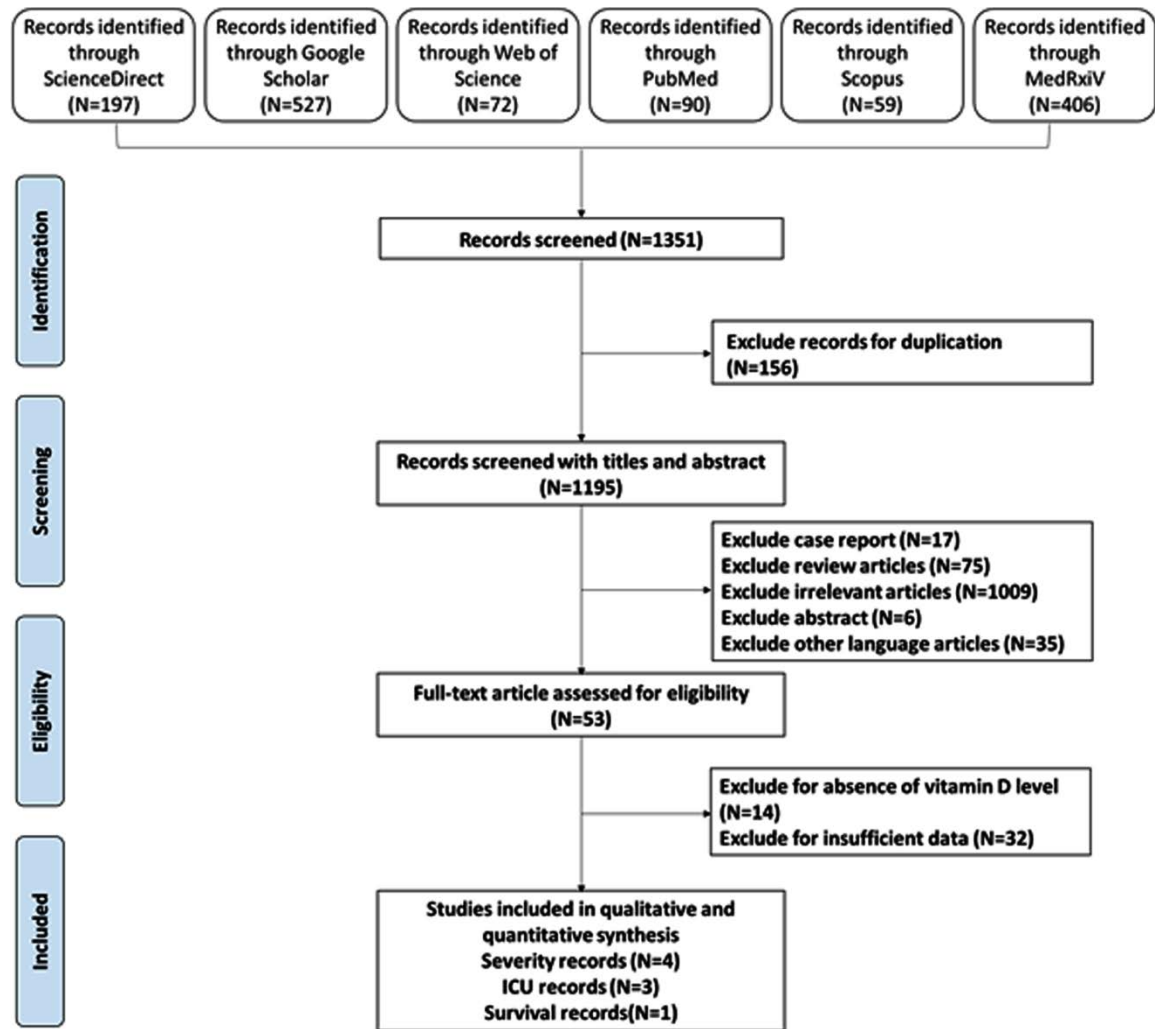


FIGURE 2. Forest plot showing pooled summary for the proportion of comorbidities.

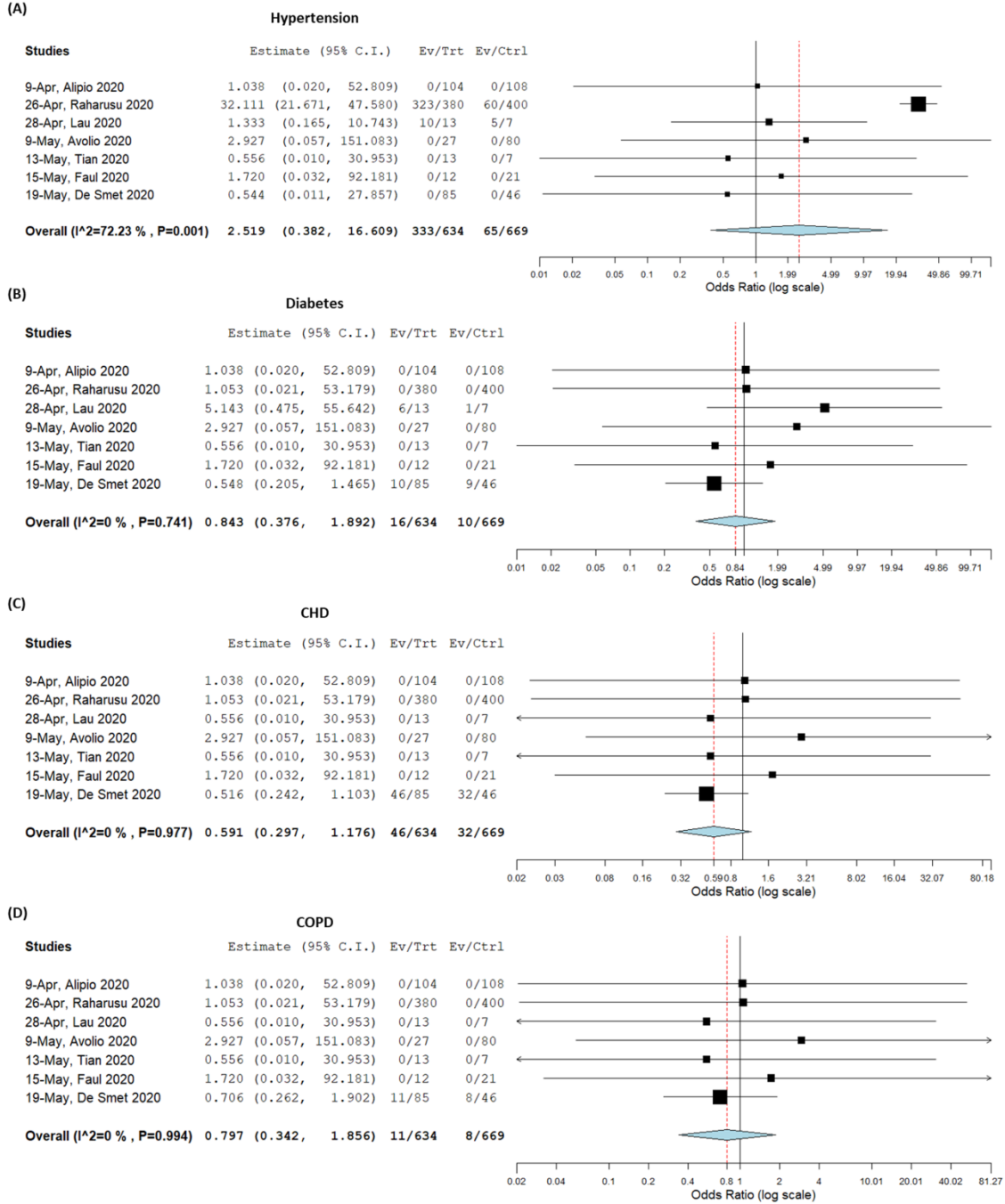


FIGURE 3. Subgroup analysis for vitamin D measurements in COVID-19 patients.

Comparison between patients with poor versus good outcomes, stratified by (A) Ethnicity, (B) Comparison group, (C) Date of publication.

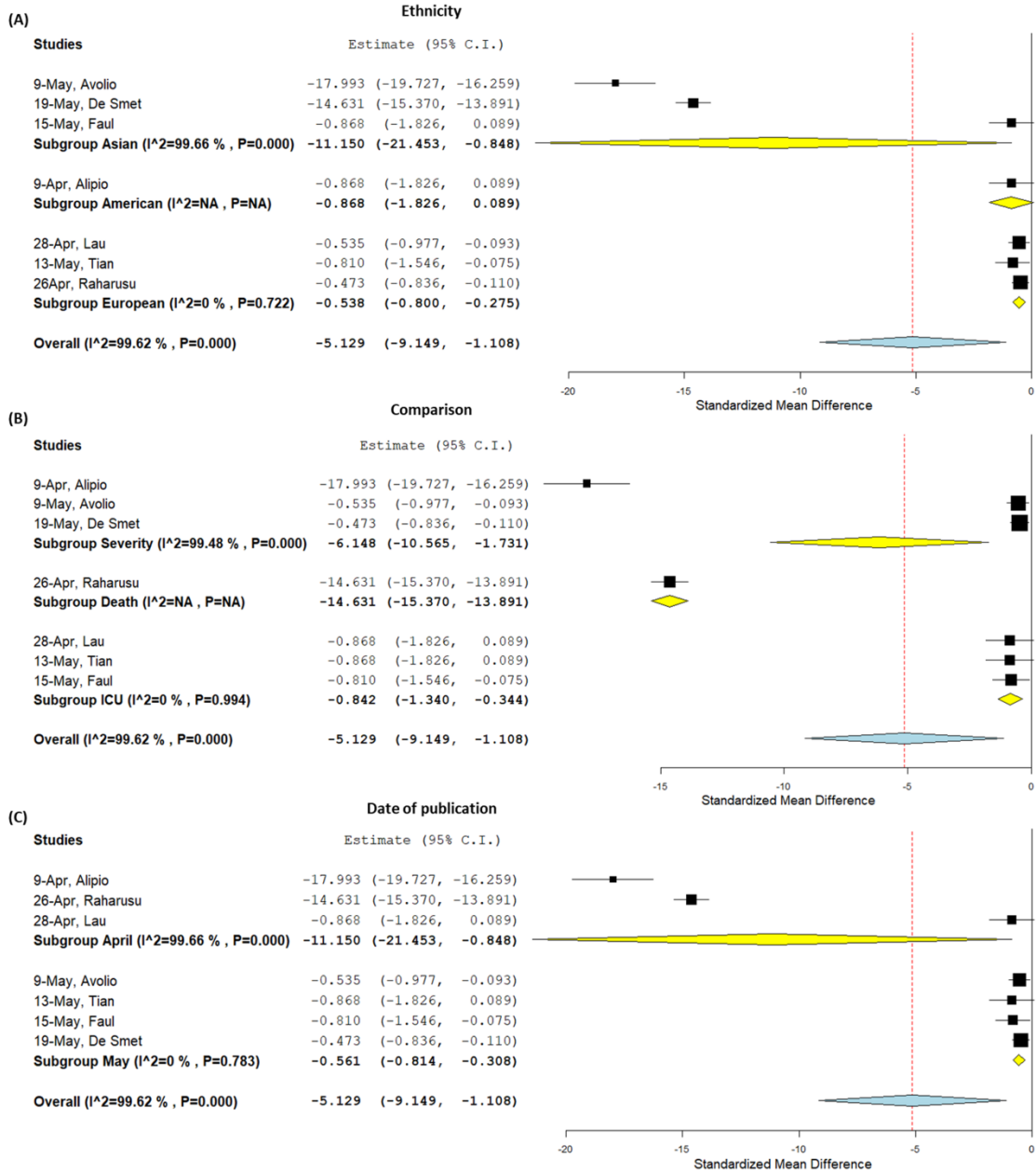


FIGURE 4. Pooled analysis of vitamin D measurements in COVID-19 patients. (A) Pooled estimate comparing patients with poor versus good prognosis. Hedge's g and confidence interval (CI) were estimated for pooled analysis. **(B)** Funnel plot for assessment of publication bias. Egger's regression test was used, showing no evidence of bias. **(C)** The trial sequential analysis was employed to test for the merging sample sizes are enough to substantiate a significant conclusion, and showed that the cumulative "Z-curve" crossed the trial monitoring boundary before reaching the required sample size (N=1303), indicating that the accumulative proof was satisfactory and no further records were required.

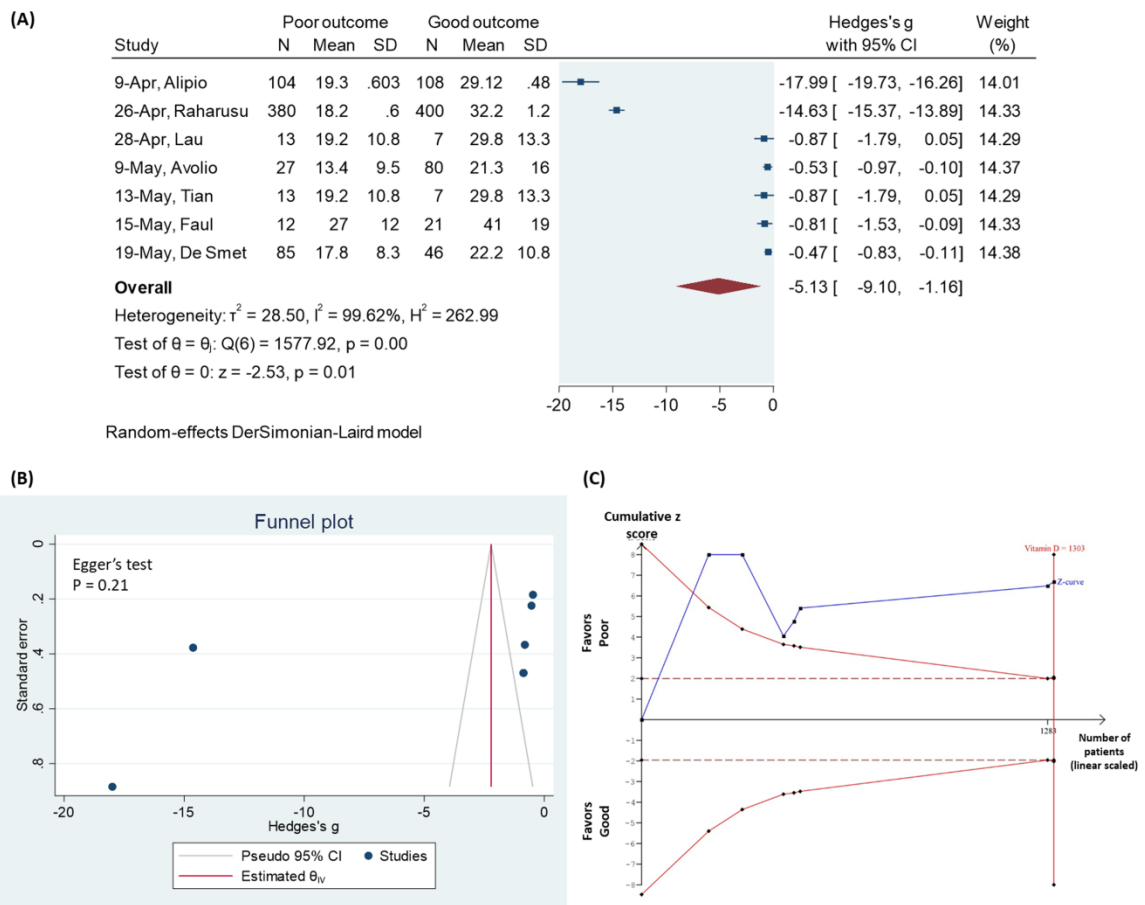


Table 1. Characteristics of included studies

First author ^{Ref.}	Publication date	Country of authors	Study design	Sample size			Mean age	Malae %	Vitamin D level	
				Total	Poor	Good			Mean	SD
Alipio ¹⁵	9-Apr	Philippines	Retrospective	212	104	108	N/A	N/A	23.80	0.24
			Case series	10	N/A	N/A	49.6	50.0	10.97	3.75
Raharusun ^{a16}	26-Apr	Indonesia	Retrospective	780	380	400	55.507	41.0	25.38	0.29
Lau ¹⁷	28-Apr	USA	Retrospective	20	13	7	65.175	45.0	22.91	7.20
Avolio ¹⁸	9-May	Switzerland	Retrospective	107	27	80	72.328	54.2	19.31	2.35
Tian ¹⁹	13-May	China	Retrospective	20	13	7		N/A	22.91	7.20
Faul ²⁰	15-May	Ireland	Retrospective	33	12	21	57.454	100.0	35.91	4.26

De Smet ²¹	19-May	Belgium	Retr o	186	85	46	69.2	42.5	19.3 5	5.4 0
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All papers were published in the year 2020. Retro: retrospective, CS: case series, N/A: not applicable. The sample size includes patients with poor and good prognosis. Poor prognosis was defined as having a severe presentation, intensive care unit (ICU) admission, or expiration.