Supplementary Appendix

COVID-19 and Vitamin D (Co-VIVID Study): a systematic review and meta-analysis of randomized controlled trials

Supplementary Table 1. The study characteristics of all RCTs included in the meta-analysis.

Variable	Castillo ME et al., 2020 [21]	Lakkireddy et al., 2021 [22]	Murai IH et al., 2021 [23]	Rastogi A et al., 2020 [24]	Sabico S et al., 2021 [25]	Sánchez-Zuno GA et al., 2021 [26]
Design	• Parallel pilot randomized open label, double-masked clinical trial • Pilot study of COVIDIOL trial (NCT04366908)	• Randomized prospective open label parallel assignment interventional clinical trial • CTRI/2020/12/030083	• Multicenter double-blind randomized placebo-controlled trial • NCT04449718	• Randomized placebo-controlled trial • NCT04459247	Multicenter randomized clinical trial	Randomized clinical trial
Setting	University hospital setting, Spain	Gandhi Medical College Hospital, India	University of Sao Paulo, Brazil	Post graduate institute of medical education and research, India	All tertiary care hospitals, Riyadh, Saudi Arabia	Universidad de Guadalajara, Mexico
Participants	• Total (76) • M/F (45/31) • Mean age (53y)	• Total (130) • Study completed (87) • M/F (65/22) • Mean age (45y)	• Total (240) • Randomized (237) • M/F (133/104) • Mean age (56.2y)	• Total (40) • M/F (20/20) • Age range (36-51y)	• Total (77) • Randomized (73) • M/F (34/35) • Mean age (49.8y)	• Total (42) • M/F (20/22) • Mean age (43y)
Groups	• Experimental: Calcifediol treatment	• Experimental: Vitamin D treatment	• Experimental: Vitamin D3 treatment	• Experimental: cholecalciferol treatment	• Experimental: 5000 IU cholecalcifero	• Experimental: vitamin D3 (n=22)

	(n=50) • Comparator: no-calcifediol (n=26) groups	(n=44) • Comparator: non-vitamin D (n=43) groups	(n=119) • Comparator: Placebo (n=118) groups	(n=16) • Comparator Placebo (n=24) groups	l (n=36) • Comparator: 1000 IU cholecalciferol (n=33) groups	• Comparator: no-vitamin D3 (n=20) control groups
Matching	Age, sex, comorbidities (but HT), baseline oxygen saturation, CRP, LDH, D-D, lymphocytes, Ferritin, IL-6	Age, BMI, duration of symptoms, comorbidities, DBP, SBP, HR, SpO_2, mean hospital stay	Age, sex, BMI, race, time form symptom onset to enrollment, comorbidities, treatments, mean hospital stay, duration of MV, baseline vitamin D3 level, CRP, D-D	Age, baseline vitamin D3 level, fibrinogen, D-D, procalcitonin, CRP, phosphorus	All anthropometries (but age, BMI), comorbidities, vital signs, symptoms, vitamin D levels	Anthropometries (age, sex, BMI), comorbidities, number of symptoms, treatment, baseline vitamin D level & sufficiency
Sufficiency	• Baseline vitamin D levels not available	• Patients with hypovitaminosis D (<30 ng/mL) were included	• Deficiency <20 ng/mL (115) • Sufficiency ≥30 ng/mL	• Deficiency <20 ng/mL (40)	• Suboptimal vitamin D status • Mild deficiency <50 nmol/L (40) • Insufficiency (rest of the cases) • Sufficiency ≥75 nmol/L	• Sufficiency ≥30 ng/mL (8) • Insufficiency<30 ng/mL (34)
Vitamin-D treatment	• Oral Calcifediol (Faes-Farma, Lejona, Spain) • On admission day (0.532 mg) • On day 3 & 7	• Pulse D therapy in the form of aqueol nano solution (Deksel)	• Single oral dose of vitamin D3 (200000 IU dissolved in 10mL peanut oil solution) for	• Oral cholecalciferol (nano-liquid droplets) 60000 IU daily for 7	• 125 µg cholecalciferol orally daily for 2w (5000 IU group) • 25 µg	• 10000 IU cholecalciferol orally for 14 days (Soft capsule)

	(0.266 mg) • Weekly until discharge/ICU admission (0.266 mg)	• 60,000 IUs of vitamin D daily for 8 days in case of BMI (18-25) or 10 days in case of BMI (>25) in addition to standard treatment	experimental group • 10 mL of peanut oil solution for placebo group	days initially with a therapeutic target of >50 ng/mL. • Supplementation was continued (n=6) when vitamin D level <50 ng/mL in the intervention arm for another 7 days until day-14. • 5 mL of distilled water for placebo control group	cholecalciferol orally daily for 2w (1000 IU group, Synergy Pharma, UAE)	
Guidelines	WHO, CONSORT	WHO, CONSORT, ICMR, DGHS (GoI)	PCR, ELISA for IgG, computed tomography	ICMR, CONSORT	MoH-SA, GCC, CONSORT	NA
Inclusion criteria	• Age ≥18y • Radiographic pattern of viral pneumonia • Positive SARS-CoV-2 PCR with CURB65 severity scale	• Age >18y • Hypovitaminosis D (<30 ng/mL) • Mild-moderate illness (SpO_2>90%	• Age ≥18y • Moderate-severe COVID-19 diagnosed by PCR, ELISA (IgG) or computed tomography (bilateral	• Age >18y • Mildly symptomatic or symptomatic COVID-19 diagnosed by RT-PCR	• Age 20-75y • RT-PCR confirmed mild-moderate SARS-CoV-2 cases	Age >18y Mild disease diagnosed by RT-PCR

		)	multifocal ground-glass opacity ≥50%) or respiratory rate >24/min or saturation <93%			
Exclusion criteria	• Age <18 y • Pregnant women	• Severe illness • High dose vitamin D (60,000 IUs) in last 3m • Active malignancy • CKD • HIV • Pregnant and breast feeding women	• Already admitted and receiving invasive mechanical ventilation • Received previous vitamin D3 supplementation • Kidney failure • Pregnant or lactating women	• Patients requiring invasive ventilation • Uncontrolled hyperglycemia or hypertension • Vitamin D >20 ng/mL	• Severe COVID-19 cases • Asymptomatic cases • Children and pregnant women • Vitamin D >75 nmol/L	• Age <18 y • Previous vitamin D3 supplementation
Comorbidities/other risk factors	• Age ≥60 y (19) • Lung disease (6) • CKD (0) • DM (8) • HT (26) • CVD (3) • IST (7) • AC (40)	DM or HT (34)	HT (125) DM (84) CVD (32) Rheumatic (23) Asthma (14) COPD (12) CKD (2)	HT DM CKD CLD COPD (Exact number of patients with comorbidities not available)	HT (38) DM (35) OB (23) HL (9) CKD (5) CVD (4) Asthma (3) Rheumatoid (2) Thyroid (2) Epilepsy (1)	HT (7) DM (2) Asthma (1) Smoke (4)
Treatments	• Standard care (hospital	• Routine standard	Supplemental oxygen therapy at baseline	Standard care for SARS-CoV-2 and for	Vitamin C (34)	Analgesic (22), Antipyretic (17),

		protocol) • HCQ (400 mg every 12h on 1st day & 200 mg every 12h for following 5 days) • AZM (500 mg for 5 days) • CRO (2g IV every 24h for 5 days for pneumonia patients with NEWS score ≥5)	treatment for COVID-19 • Remdesivir, Favipiravir, Ivermectin or Dexamethasone (n=57)	(181) Noninvasive ventilation (31) Anticoagulant (210) Antibiotic (204) Corticosteroids (150) Antihypertensive (124) Proton-pump inhibitor (96) Antiemetic (99) Analgesic (97) Hypoglycemic (50) Hypolipidemic (33) Thyroid (20) Antiviral (8)	pre-existing comorbidities as per institute protocol		Antibiotic (8), Antihistamine (6), Anticoagulant (5), Other drugs (10)
Adverse events related to Vit. D treatment	NA		None	None (Except for 1 patient who vomited)	None	None	None
Randomization, Allocation	• Electronic randomization • 2:1 • Homogenous distribution has not been achieved for all variables between comparison groups		• Alternatively as per pre-allotted serial numbers • 1:1	• Computer-generated code with block sizes of 20 • 1:1	• Randomized into interventional and placebo-control groups • 1:1.5	• Computer generated using permuted blocks • 1:1	• Randomized into interventional and control groups • 1:1
Blinding	• Not double-		• NA	• Double-blind	• NA	• Not double-	• Not double-

	blinded • Observation bias was minimized by blind access to technical data collectors and the statistician who carried out the study.		• Patients and investigators remained blinded to randomization until the final analysis		blinded • Risk of bias was minimized by blinded data collection	blinded
Follow-up/Study duration/lost to follow-up	Until admission to ICU, hospital discharge or death	Analysis on 9th or 11th day, deaths till 21 days on enrolment	June 2 to Aug 27, 2020 Final follow-up on Oct 7, 2020	• Days-7, 14 and until day-21 or virus negativity	• 29 July – 22 Sep 2020 • Followed-up on Day 7 or on discharge day and 30 days after discharge and/or the last vitamin dose	Form the day of recruitment to 14 days
Information on follow-up loss/withdrawal	Mentioned in the flow diagram	Mentioned in the flow diagram	Mentioned in the flow diagram	Mentioned in the flow diagram	Mentioned in the flow diagram	NA
Outcomes studied	• ICU admission • Deaths	• Inflammatory markers (CRP, LDH, IL6, Ferritin, N/L ratio) • ICU care • Deaths	• Length of hospital stay/discharge probability (date of randomization to date of discharge)	• SARS-CoV-2 RT-PCR negativity before day-21 and change in inflammatory markers	• Days to resolve symptoms/discharge • Metabolic profile (CBC, lipids, CRP, D-D, LDL,	Severity RT-PCR positivity Seropositivity

			• ICU care • MV • Duration of MV • Deaths		IL6, Ferritin) • ICU admission • Deaths	
Statistical analysis	Univariate and multivariate logistic regression	Number of events for ICU care and deaths	Number of events for ICU care, MV, deaths and discharge probability. Kaplan-Meier curves and Cox regression models for length of hospital stay/discharge	Number of events for SARS-CoV-2 RT-PCR negativity	Number of events for ICU care and deaths	Number of patients with severity (>3 symptoms), RT-PCR and seropositivity in experimental and comparator groups
Adjustment for confounders	DM, HT	To overcome the non responder's bias, sample size was adjusted by assuming an expected response proportion of 50%	Joint pain, sore throat, HT, DM, PTH and creatinine	NA	Age, sex, baseline BMI, D-D	NA
Effect sizes	Univariate and multivariate odds ratio	Univariate odds ratio	Univariate odds ratios for ICU care, MV and Deaths Uni and multivariate hazard ratios for hospital discharge	Univariate odds ratio	Univariate odds ratio	Univariate odds ratio
Results	Vitamin D treatment resulted in significantly less probability of ICU admission. The statistical significance was retained after adjusting for HT and	Improvement of serum vitamin D level to 80–100 ng/mL has significantly reduced the inflammatory	The length of hospital stay, ICU care, MV and mortality was not significant between groups. Serum vitamin D3 level significantly increased after a single	Significant increase in serum vitamin D with a significant decrease in fibrinogen in the intervention group. Significant difference	5000 IU vitamin D group had shorter time to recovery symptoms like cough and ageusia	A vitamin D3 dose of 10000IU daily for 14 days sufficiently raises serum vitamin D levels

	DM.	markers without any side effects.	dose supplementation	in the number of SARS-CoV-2 RT-PCR negativity between two groups.		
Conclusions	Calcifediol supplementation may improve the clinical outcome of subjects requiring hospitalization for COVID-19	Adjunctive Pulse D therapy can be added safely to the existing treatment protocols of COVID-19	A single high dose of vitamin D3 did not significantly reduce hospital length of stay	High dose cholecalciferol supplementation resulted in a greater proportion of SARS-CoV-2 RT-PCR negativity	The beneficial effects of 5000 IU vitamin D as an adjuvant therapy for COVID-19 patients with suboptimal vitamin D status	Supplementation of vitamin D have significant benefits in COVID-19 due to immunomodulatory effects
Limitations	• Pilot study and not double-blind placebo controlled • Role of BMI/obesity not considered • Serum 25OHD levels not available	Single centre study	• Low sample size • Coexisting diseases • Sample and treatment heterogeneity • A single high dose vitamin D3 supplementation after a mean duration of 10.3days from symptom onset to randomization	• Only mildly symptomatic or asymptomatic cases • High-dose of cholecalciferol • Water supplement for placebo	• Open-label design (risk of bias has been minimized by blinded data collection) • Only mild-moderate cases	No double blind design Only mild cases
IEC approval	Reina Sofia University Hospital, Corodoba, Spain	Gandhi Medical College, Hyderabad, India	Clinical Hospital, School of Medicine, University of Sao Paulo	Post graduate institute of medical education and research, India	King Fahad Medical City, Saudi Arabia	Universidad de Guadalajara, Faculty of Medicine, Mexico

AC: any comrobidity, BMI: body mass index, CKD: chronic kidney disease, CLD: chrnic liver disease, CONSORT: Consolidated Standards of Reporting Trials, COPD: chronic obstructive pulmonary diasese, CRP: c reactive protein, CVD: cardiovascular disease,

DBP: diastolic blood pressure, D-D: D-dimer, DGHS: Directorate General of Health Services, DM: diabetes mellitus, ELISA: enzyme-linked immunoassay, F: female, GCC: Gulf Cooperation Council, GoI: Government of India,HIV: Human immunodeficiency virus, HL: hyperlipidemia, HR: heart rate, HT: hypertension, ICMR: Indian council of medical research, ICU: intensive care unit, Ig: immunoglobulin, IL-6: interleukin 6, IST: Immunosuppressed and transplanted, IU: international units, LDH: lactate dehydrogenase, M: male, MoH-SA: Ministry of Health-Saudi Arabia, MV: mechanical ventilation, NA: not available, OB: obesity, OR: odds ratio, RT-PCR: reverse transcription–polymerase chain reaction, SARS-CoV-2: severe acute respiratory syndrome associated with coronavirus-2, SBP: systolic blood pressure, SpO2: oxygen saturation, Vitamin D3: cholecalciferol, WHO: world health organization, y: years. The numerical values in ( ) indicate the number of participants.

Supplementary Table 2. The results of subgroup analysis

Outcome variable	No. of studies	RR (95% CI)	Z	p-value	I^2%
All RCTs					
All outcomes cumulative: (severity, ICU, MV, mortality, sero and RT-PCR positivity)	6	0.60 (0.40-0.92)	2.33	0.02	48
Symptom severity	1	0.10 (0.01-1.77)	1.57	0.12	NA
ICU Care	4	0.44 (0.15-1.30)	1.48	0.14	66
Mechanical ventilation	1	0.52 (0.24-1.13)	1.65	0.10	NA
Mortality	4	0.78 (0.25-2.40)	0.44	0.66	33
Seropositivity	1	0.97 (0.68-1.39)	0.17	0.87	NA
RT-PCR positivity	2	0.46 (0.24-0.89)	2.31	0.02	0
Single center studies					
ICU	2	0.19 (0.01-4.23)	1.04	0.30	86
Mortality	2	0.29 (0.07-1.19)	1.71	0.09	0
Multicentre studies					
ICU	2	0.74 (0.44-1.24)	1.15	0.25	0
Mortality	2	1.57 (0.61-4.09)	0.93	0.35	0
Cholecalciferol					
ICU	3	0.75 (0.46-1.20)	1.21	0.23	0
Mortality	3	1.05 (0.41-2.69)	0.10	0.92	13
Vitamin D suboptimal status					
All outcomes cumulative: (severity, ICU, MV, mortality, sero and RT-PCR positivity)	4	0.70 (0.50-1.15)	1.31	0.19	3
ICU	3	1.01 (0.54-1.89)	0.02	0.98	0
Mortality	3	1.28 (0.25-6.62)	0.29	0.77	41
Other Outcomes					
Discharge	2	1.04 (0.97-1.12)	1.16	0.25	0

ICU: intensive care unit, MV: mechanical ventilation, NA: not available, RR: relative risk/risk ratio, RT-PCR: reverse transcription–polymerase chain reaction,

Risk of bias assessment

Risk of bias assessment was done using the Cochrane risk of bias tool.

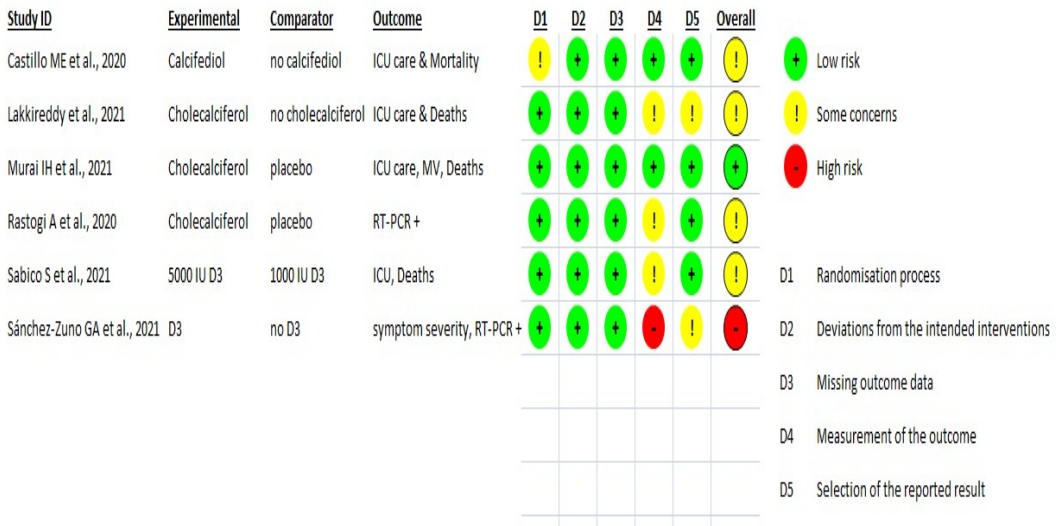
Reference:

RoB 2: a revised tool for assessing risk of bias in randomised trials. DOI: 10.1136/bmj.l4898

https://pubmed.ncbi.nlm.nih.gov/31462531/

The risk of bias was assessed at five domains (D1-D5 as stated in the below figure) for all the included RCTs.

Our assessment indicated some concerns of bias in all the studies. Although we could not judge any bias/concerns in a study by Murai et al. This interpretation should be cautioned to the use of single high dose of vitamin D3 supplementation in the concerned study.



The below figure indicates domain level bias for all the included studies along with the overall bias. As seen in the figure, the overall bias of the included studies could be judged as having “some concerns” related to randomization, blinding, outcome analysis and reporting of results.