Effect of a Single High Dose of Vitamin D_3 on Cytokines, Chemokines and Growth Factor in Patients With Moderate to Severe COVID-19

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Abbreviations list: BMI, body mass index; COVID-19, coronavirus disease 2019; GM-CSF, granulocyte-macrophage colony-stimulating factor; ICU, intensive care unit; IFN-γ, interferon *gamma*; IP-10, interferon-inducible protein-10; MIP-1β, macrophage inflammatory protein-1 *beta*; MCP-1, monocyte chemoattractant protein-1; NF-κB, nuclear factor *kappa* B; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TNF-α, tumor necrosis factor *alpha*; UVB, ultraviolet B; VEGF, vascular endothelial growth factor; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D.

Clinical Trial Registration: *ClinicalTrials.gov*, NCT04449718.

Data described in the manuscript, codebook, and analytic code will be made available upon request pending application and approval.

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Abstract

Background: The modulating effect of vitamin D on cytokine levels in severe coronavirus disease 2019 (COVID-19) remains unknown.

Objective: To investigate the effect of a single high-dose of vitamin D_3 on cytokines, chemokines, and growth factor in hospitalized patients with moderate to severe COVID-19.

Methods: This is a post-hoc, ancillary and exploratory analysis from a multicenter, double-blind, placebo-controlled, randomized clinical trial registered in ClinicalTrials.gov, NCT04449718. Patients with moderate to severe COVID-19 were recruited from two hospitals in Sao Paulo, Brazil. Of 240 randomized patients, 200 were assessed in this study and randomly assigned to receive a single oral dose of 200 000 IU of vitamin D_3 (n=101) or placebo (n=99). The primary outcome was hospital length of stay, that has been published in our previous study. The prespecified secondary outcomes were serum levels of interleukin-1β, interleukin-6, interleukin-10, tumor necrosis factor *alpha* (TNF-α) and 25-hydroxyvitamin D. The post-hoc exploratory secondary outcomes were interleukin-4, interleukin-12p70, interleukin-17A, interferon *gamma* (IFN-γ), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-8, interferon-inducible protein-10 (IP-10), macrophage inflammatory protein-1 *beta* (MIP-1β), monocyte chemoattractant protein-1 (MCP-1), growth factor vascular endothelial (VEGF), and leukocytes count. Generalized estimating equations (GEE) for repeated measures, with Bonferroni's adjustment, were used for testing all outcomes.

Results: The study included 200 patients with a mean (SD) age 55.5 (14.3) years and body mass index (BMI) 32.2 (7.1) kg/m^2, of which 109 (54.5%) were male. GM-CSF levels showed a significant group by time interaction effect (*P*=0.04), although

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between-group difference at post-intervention after Bonferroni's adjustment was not significant. No significant effects were observed for the other outcomes.

Conclusions: The findings do not support the use of a single dose of 200 000 IU of vitamin D_3, compared to placebo, for the improvement of cytokines, chemokines, and growth factor in hospitalized patients with moderate to severe COVID-19.

Keywords: Immune response, SARS-CoV-2, inflammation, acute-phase reactants, vitamin D.

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Introduction

Vitamin D has arisen as a mediator of innate(1-3) and adaptive immune response.(4, 5). The active form of vitamin D, 1,25-dihydroxyvitamin D (1,25$[OH]_2$D), could contribute to the induction of viral neutralization and recruitment of neutrophils, monocytes, macrophages, and dendritic cells (6). Furthermore, 1,25$(OH)_2$D may avoid chronic activation of the innate immune response by limiting dendritic cells maturation, inducing immune tolerance, down-regulating toll-like receptors, and adjusting both tumor necrosis factor α (TNF-α) /nuclear factor-κB and interferon-γ (IFN-γ) signaling pathways. By these means, it has been hypothesized that sufficient vitamin D levels could prevent cytokine storm whereas promoting an adequate adaptive immune response in patients with coronavirus disease 2019 (COVID-19) (6-10).

Evidence suggests that COVID-19 may promote hyperactivation of neutrophils, monocytes, and macrophages resulting in dysregulated immune inflammatory response and possible cytokine storm (11). These changes has been associated with increased levels of interleukin-1β, interleukin-6, interferon-inducible protein-10 (IP-10), TNF, IFN-γ, macrophage inflammatory protein-1 *beta* (MIP1β), vascular endothelial growth factor (VEGF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) in patients with COVID-19 (12, 13). In a comprehensive review, Christakos et al. (14) presented important mechanisms of action of vitamin D in the immune system, such as regulation of activated T cells, inhibition of the adaptative immune response, promotion of the innate immune response, immunosuppressive effect associated with the decrease of inflammatory cytokines (for example, IL-2 and IFN-γ), production of IL-4 and IL-10. It also targets dendritic cells and interacts with multiple cell types and activation states related to the immune cascade.

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In view of the possible anti-inflammatory effect of vitamin D regulating the innate and adaptative immune responses, vitamin D_3 supplementation could be a relevant therapeutic strategy to manage hyperinflammation in patients with COVID-19. However, the presumed benefit of vitamin D in improving the cytokine storm remains supported by review (6-8, 15-17) and few observational (18-20) studies. Herein, we report on a post-hoc, ancillary and exploratory analysis from our randomized clinical trial (21) to investigate the effect of a single high dose of vitamin D_3 on systemic inflammatory cytokines, chemokines, and growth factor in hospitalized patients with moderate to severe COVID-19.

Methods

Study design and participants

This is a post-hoc, ancillary and exploratory analysis of secondary outcomes from a multicenter, double-blind, placebo-controlled, randomized clinical trial. The trial is registered in ClinicalTrials.gov, NCT04449718. The study was approved by the ethical committees of both Clinical Hospital (School of Medicine of the University of Sao Paulo) and Ibirapuera Field Hospital (Ethics Committee Approval Number 30959620.4.0000.0068), in accordance with the Good Clinical Practice guidelines and the Declaration of Helsinki. All patients provided written informed consent before being enrolled in the study. The trial protocol and statistical analysis plan were previously published (21).

Hospitalized patients were recruited from the Clinical Hospital of the School of Medicine of the University of Sao Paulo, and Ibirapuera Field Hospital. Patients were enrolled from June 2, 2020, to August 27, 2020. The screening criteria assumed were identical for both centers and the final follow-up occurred on October 7, 2020. All

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patients had positive COVID-19 diagnosis confirmed by polymerase chain reaction (PCR) testing at time of randomization or by serology assay (ELISA) to detect IgG against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) throughout the study.

Patients were eligible for enrollment if they were age 18 years or older and had positive SARS-CoV-2 infection diagnosis by either nasopharyngeal swab PCR or chest computed tomography scan with compatible findings (bilateral multifocal ground-glass opacities with at least 50% lung involvement). Patients should have diagnosis of flu syndrome with hospitalization criteria on hospital admission and should present a respiratory rate greater than 24 breaths/minute, oxygen saturation lower than 93% on room air or risk factors for complications (e.g., heart disease, diabetes, systemic arterial hypertension, neoplasms, immunosuppression, pulmonary tuberculosis, obesity), followed by COVID-19 confirmation. Patients who met these criteria were considered to have moderate to severe COVID-19. Patients were excluded if they were unable to read and sign the written informed consent; they had already been admitted under invasive mechanical ventilation; they have recently received a vitamin D_3 supplementation (greater than 1000 IU/d or weekly equivalent); they had renal failure requiring dialysis or creatinine levels above 2.0 mg/dL; they had hypercalcemia defined by total calcium greater than 10.5 mg/dL; they were pregnant or lactating women; or if they are expecting hospital discharge in less than 24 hours. The criteria used for hospital discharge were absence of fever in the previous 72 hours, no need for supplemental oxygen in the previous 48 hours, and oxygen saturation greater than 93% on room air without respiratory distress.

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Randomization and masking

Eligible patients were assigned in a 1:1 ratio into either the vitamin D_3 group or the placebo group. The randomization list was created using a computer-generated code, in bloc sizes of 20 participants, which was managed by a staff member who had no role in the study.

The vitamin D_3 group received on the same day of randomization a single oral dose of 200 000 IU of vitamin D_3 diluted in vehicle (10 mL of a peanut oil solution). This selected dose is within the indicated range to effectively increase serum/plasma 25-hydroxyvitamin D concentrations in several vitamin D-sufficient and -deficient populations (22). Patients enrolled in the placebo group received only vehicle. The vitamin D_3 and placebo solutions were identical (in color, taste, smell, consistency, and container), and have been prepared by the pharmacy unit of the Clinical Hospital. Both were labeled by a staff member who did not participate in the study, and allocation blindness was kept until the final statistical analysis.

Procedures

Self-reported anthropometric characteristics (weight and height) and coexisting chronic diseases, acute COVID-19 symptoms, patients' concomitant medications during hospitalization, oxygen supplementation requirement, and imaging features were assessed upon hospital admission. Subsequently, self-reported coexisting chronic diseases and previous medications were checked according to the medical records for each patient. To provide a comprehensive demographic characterization, self-reported race/ethnicity data were also collected based on the following fixed categories: White,

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Black, Asian, and Pardo (the latter refers to people of mixed race/ethnicities, according to the Brazilian Institute of Geography and Statistics – IBGE).

Serum levels of 25-hydroxyvitamin D was assessed by a chemiluminescent immunoassay (ARCHITECT 25-OH Vitamin D 5P02; Abbott Diagnostics). All cytokines, chemokines and growth factor were analyzed by the Luminex® xMAP (Multiple Analyte Profiling) assay, a Multiplex technique using commercial Milliplex MAP kit (Millipore Corp., Billerica, MA, USA), at the same time by a blinded technician, following the manufacturer's recommendations. Leucocyte count was assessed by automated assay. All the assessments described were performed on the day of randomization and on hospital discharge. Importantly, only patients who had blood sample collected on the day of randomization and on hospital discharge were assessed in this study. Therefore, patients who died during follow-up were not included due to the absence of blood samples.

Outcomes

The primary outcome, hospital length of stay, was not significantly different between the vitamin D_3 and placebo group as previously published (21). The prespecified secondary outcomes were serum levels of interleukin-1β, interleukin-6, interleukin-10, TNF-α and 25-hydroxyvitamin D.

In order to provide a broader understanding of vitamin D_3 effects on COVID-19-related hyperinflammation and immunomodulation, the following exploratory secondary outcomes were included as post-hoc analyses: serum levels of cytokines (interleukin-4, interleukin-12p70, interleukin-17A, IFN-γ, and granulocyte-macrophage colony-stimulating factor [GM-CSF]), chemokines (interleukin-8, interferon-inducible protein-10 [IP-10], macrophage inflammatory protein-1β [MIP-1β], and monocyte

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chemoattractant protein-1 [MCP-1]), growth factor (vascular endothelial growth factor [VEGF]), and white blood cell (leukocytes count).

The intra-assay and inter-assay coefficient of variation (CV) for interleukin-1β (2.3% and 6.7%), interleukin-6 (2.0% and 18.3%), interleukin-10 (1.6% and 16.8%), TNF-α (2.6% and 13.0%), interleukin-4 (2.9% and 14.2%), interleukin-12p70 (2.2% and 16.7%), interleukin-17A (2.2% and 7.9%), IFN-γ (1.6% and 12.0%), and GM-CSF (3.1% and 10.1%), interleukin-8 (1.9% and 3.5%), IP-10 (2.6% and 15.3%), MIP-1β (2.4% and 8.8%), MCP-1 (1.5% and 7.9%), and VEGF (3.7% and 10.4%) according to the manufacturer's specifications.

Statistical analysis

Sample size was chosen based on feasibility and resources, as described in detail in a previous study (21).Our study was powered considering a repeated measure ANOVA, within-between interaction, at two-sided significance level of 5% ($\alpha = 0.05$), an assumed Partial Eta Squared ($\eta 2 = 0.04$), Effect Size ($f = 0.20$), and total sample of 200 patients, achieving the post-hoc power ($1 - ß$) > 99% performed with G*Power software, version 3.1.9.4.Generalized estimating equations (GEE) for repeated measures were used for testing possible differences in all outcomes assuming group and time as fixed factors, with marginal distribution, and a first-order autoregressive correlation matrix to test the main and interaction effects. Bonferroni's adjustment was performed in GEE analyses to maintain a family-wise two-sided significance threshold of 0.05, considering 6 pairwise comparisons for all outcomes. Proportions were compared between groups using χ^2 and Fisher's exact tests. In order to handle potential confounders, GEE was adjusted by 4 models: cumulative glucocorticoid doses; cumulative glucocorticoid doses and time from symptom onset to randomization;

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cumulative glucocorticoid doses and baseline 25-hydroxyvitamin D levels; and cumulative glucocorticoid doses and hospitals from which patients were recruited, using a per protocol approach.

There were no missing data for cytokines, chemokines, growth factor, and serum 25-hydroxyvitamin D. Missingness for leukocytes count (2 patients in the vitamin D_3 group) was at random and handled by GEE models, with no imputation for missing data. Statistical analyses were performed with IBM-SPSS software, version 20.0. The significance level was set at two-sided P value ≤ 0.05.

Results

Of the 1240 patients assessed for eligibility, 240 underwent randomization: 122 patients from the Clinical Hospital of the School of Medicine and 118 patients from the Ibirapuera Field Hospital. Of the 120 patients assigned to the vitamin D_3 group, 1 withdrew the consent, 9 were excluded due to lack of blood sample, and 9 died throughout the follow-up. Of the 120 patients assigned to the placebo group, 2 withdrew consent, 13 were excluded due to lack of blood sample, and 6 died throughout the follow-up (**Figure 1**). The mean (SD) age was 55.5 (14.3) years, the mean (SD) body mass index was 32.2 (7.1) kg/m^2, and 109 (54.5%) were male. Regarding ethnicity, 111 (55.5%) patients were White, 62 (31.0%) were Pardo, 26 (13.0%) were Black, and 1 (0.5%) was Asian (**Table 1**).

Serum GM-CSF levels demonstrated a significant group by time interaction ($P <$ 0.05 for all models) with a decrease from baseline to post after a single dose of vitamin D_3 (from 3.4 [5.2] pg/mL to 2.9 [4.8] pg/mL) compared to an increase in the placebo group (from 3.0 [4.1] pg/mL to 4.4 [9.7] pg/mL), although no significant difference after Bonferroni's adjustment was observed (between-group difference at post-

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intervention, -1.5 pg/mL [95% CI, -4.4, -1.3]; $P > 0.05$ for all models) (**Table 2**). The mean (SD) 25-hydroxyvitamin D levels significantly increased from baseline after a single high-dose of vitamin D_3 (from 21.1 [10.1] ng/mL to 44.6 [14.7] ng/mL) compared to placebo (from 20.2 [8.1] ng/mL to 19.8 [10.5] ng/mL) (between-group difference at post-intervention, 24.9 ng/mL [95% CI, 20.2, 29.6]; $P < 0.001$ for all models) (Table 2). No significant differences between the vitamin D_3 and placebo groups for serum levels of interleukin-1β, interleukin-4, interleukin-6, interleukin-10, interleukin-12p70, interleukin-17A, IFN-γ, TNF-α, interleukin-8, IP-10, MIP-1β, MCP-1, VEGF, and leucocytes counts were observed (Table 2).

Discussion

In this post-hoc, ancillary and exploratory analysis from a multicenter, double-blind, placebo-controlled, randomized clinical trial, a single high-dose of vitamin D_3 did not significantly change systemic inflammatory cytokines, chemokines, and growth factor, compared to placebo, among hospitalized patients with moderate to severe COVID-19. To our knowledge, this is the first randomized clinical trial to report the effects of single high-dose of vitamin D_3 on cytokine-related inflammation in this population.

To gather knowledge on the effects of a single high dose of vitamin D_3 on systemic inflammation, we broadly assessed serum levels of cytokines, chemokines, and growth factor. However, the current trial demonstrated that administration of a single dose of 200 000 IU of vitamin D_3 did not result in any effect on these inflammatory markers, except for an interaction effect on GM-CSF.

GM-CSF is immunoregulatory cytokine with pivotal role in inflammation, and its overexpression may be associated with cytokine storm in severe COVID-19 (13). GM-

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CSF-blockade therapies have been proposed to mitigate hyperimmunoinflammation in severe COVID-19 (23). The present findings suggest that vitamin D could have a slight effect in GM-CSF in COVID-19 patients, although no significant differences between vitamin D_3 and placebo were observed.

Severe inflammatory states of COVID-19 are associated with GM-CSF overexpression by autocrine response and positive feedback loop (23). In this sense, the presumed therapeutic effect of vitamin D could modulate an adequate innate immune response while decreasing the GM-CSF upregulation, although, to date, it has not yet been reported. This study found reduced levels of GM-CSF from baseline to post in the vitamin D_3 group compared to the increased level in the placebo group, although it is not ruled out the possibility that glucocorticoid use influences no significant difference in the multiple comparison test (24).

Regarding the timing, recent study of Liu et al., (25) suggest that the most critical patients with COVID-19 demonstrated late immune response with mild and delayed performance of the immediate first line of defense to suppress viral replication/spread, and peak in proinflammatory cytokine. In these more critical patients, an inflammatory peak that characterizes a second wave in the cytokine storm is expected by days 17-23 from symptom onset.

In our study, the median time of 18 days from symptoms onset to hospital discharge suggests that cytokine assessments were clinically timely to detect changes in the target outcomes if they had occurred. Noteworthy, the relatively long time from symptom onset and vitamin D_3 administration (i.e., median of 10 days), in addition to the time required for the active form of vitamin D to act on the expected immune function, may have blunted the effect of vitamin D_3 on clinical and biochemical outcomes.

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Glucocorticoid, such as dexamethasone, has been adopted to attenuate COVID-19-related inflammatory injury (26) and reduce mortality (27). In the present findings, 65.0% of patients (67 in the vitamin D_3 and 63 in the placebo group) received glucocorticoids with a mean dexamethasone cumulative dose of 39.7 mg, leading to assumption a possible mitigating effect on acute-phase reactants (28) such as cytokines, chemokines and growth factor that overlaps the purported immunomodulatory effect of high-dose vitamin D_3 in the presence (29, 30) or absence (31, 32) of acute inflammation. However, we observed that C-reactive protein levels, an important systemic inflammatory marker, remained elevated after a mean of 7 days of glucocorticoid treatment, suggesting that vitamin D_3 supplementation could plays a role as an additional therapeutic agent in this disease (33-35).

It is important to note that circulating concentration of 25-hydroxyvitamin D is the best clinical indicator of vitamin D nutritional status, and its greater amount comes from dermal production in response to ultraviolet B (UVB) sunlight exposure while the least part comes from diet (36). Regarding diagnosis, there is a lack of international consensus on the definition of vitamin D deficiency and sufficiency (37), which hinders to classify sufficiency status as greater than 20 ng/mL (37, 38) or 30 ng/mL(39). The European Calcified Tissue Society position statement estimates that vitamin D deficiency (serum 25-hydroxyvitamin D < 20 ng/mL) occurs in up to 20% of the population in Northern Europe, between 30 and 60% in Western, Southern Europe and Eastern, and up to 80% in Middle Eastern countries (37), a difference proportional to the seasonality of exposure to sunlight in these regions (40). Since that the best response to sun exposure is elevation of vitamin D status and patients hospitalized with COVID-19 are deprived of it, a higher prevalence of vitamin D deficiency (< 50 nmol/L or 20

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ng/mL) is observed while increase the chance of hospitalization and death in patients with COVID-19 (41).

Aside from experimental design, the strengths of this trial include the enrollment of hospitalized patients with moderate to severe COVID-19; and the adequate timing of collection of diverse acute-phase reactants and cell-signaling molecules regarding the peak of immune-cell and hyperinflammation. This study has limitations. First, patients showed heterogeneous pre-existing diseases medication regimens which may have contributed to the results. Second, the study may have had an inadequate power to detect small between-group differences, particularly considering the known variability inherent to the reactants. This study does not rule out the possibility that early vitamin D treatment could improve clinical status and cytokine-related inflammation in patients with less severe COVID-19, so further randomized clinical trials that consider this perspective would be critical.

In summary, a single high-dose of 200 000 IU of vitamin D_3 compared to placebo did not elicit meaningful changes in systemic inflammatory cytokines, chemokines, and growth factor among hospitalized patients with COVID-19 who have already had a sufficient vitamin D. The findings do not support the use of high-dose vitamin D_3 in the modulation of cytokine-related inflammation of moderate to severe COVID-19 in advanced symptoms onset.

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Author Contributions

Dr. R M R Pereira had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: A L Fernandes, I H Murai, A J Pinto, K F Goessler, B Gualano, R M R Pereira. Acquisition, analysis, and interpretation: All authors. Drafting of the manuscript: A L Fernandes, I H Murai, B Z Reis, B Gualano, R M R Pereira. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: A L Fernandes, I H Murai, B Z Reis, A J Pinto, B Gualano, R M R Pereira. Obtained funding: B Gualano, R M R Pereira. Supervision: B Gualano, R M R Pereira. Administrative, technical, or material support: L P Sales, M D Santos, L Antonangelo, V F Caparbo. All authors declare no competing interests.

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Disclosure Summary

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Data Availability

Deidentified participant data of this study must be requested from the corresponding author upon publication (sent to rosamariarp@yahoo.com). The codebook of this study will be made available upon request by qualified clinical researchers for specified purposes dependent on the nature of the request and the intention use of the data, with investigator support. The request must include a statistician.

The lead author (Dr R M R Pereira) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as originally planned (and, if relevant, registered) have been explained.

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Figure 1

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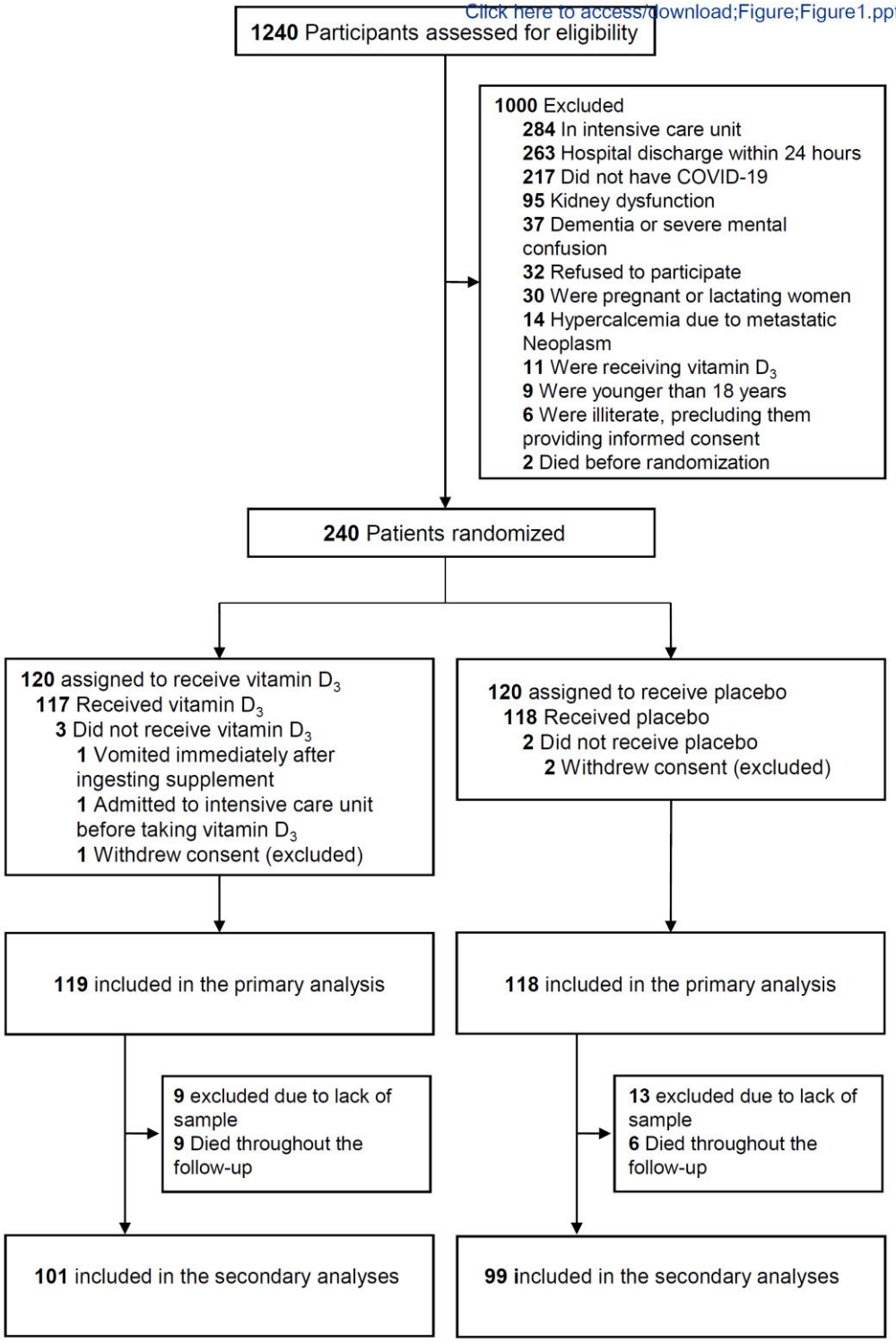

Figure 1. Trial CONSORT diagram. All analyses were performed according to the patient's randomization group using intention-to-treat approach. There were no missing

data for cytokines, chemokines, growth factor, and serum 25-hydroxyvitamin D. Missingness for leukocytes count (2 patients in the vitamin D_3 group) was random and handled by generalized estimating equations (GEE) models. For patients who died throughout the follow-up, blood samples were not collected at the post-intervention. 25(OH)D denotes serum 25-hydroxyvitamin D levels.

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Table 1. Baseline Demographic and Clinical Characteristics[1]

Characteristic	Vitamin D_3 group (n = 101)	Placebo group (n = 99)
Age, years	55.3 ± 14.2	55.7 ± 14.5
Sex, n (%)		
Male	58 (57.4)	51 (51.5)
Female	43 (42.6)	48 (48.5)
Race or ethnicity, n (%)		
White	52 (51.5)	59 (59.6)
Pardo [2]	32 (31.7)	30 (30.3)
Black	16 (15.8)	10 (10.1)
Asian	1 (1.0)	0 (0)
Time from symptom onset to randomization, days	10.0 (7.0-12.5)	10.0 (7.0-14.0)
Time from symptom onset to hospital discharge, days	17.0 (13.0-21.0)	18.0 (15.0-22.0)
Time from hospital admission to randomization, days	1.0 (1.0-2.0)	1.0 (1.0-2.0)
Time for hospital length of stay, days	6.0 (4.0-8.0)	7.0 (5.0-10.0)
Body-mass index, kg/m^2 [3]	32.2 ± 6.7	32.1 ± 7.5
Body-mass index category, n (%)		
< 18.5 kg/m^2	0 (0)	1 (1.1)
18.5 - 24.9 kg/m^2	8 (8.6)	13 (14.4)
25.0 - 29.9 kg/m^2	29 (31.2)	24 (26.7)
≥ 30 kg/m^2	56 (60.2)	52 (57.8)
Acute COVID-19 symptoms, n (%)		
Cough	87 (86.1)	82 (82.8)
Fatigue	81 (80.2)	86 (86.9)
Fever	73 (72.3)	69 (69.7)
Myalgia	61 (60.4)	60 (60.6)
Joint pain	42 (41.6)	33 (33.3)
Runny nose	36 (35.6)	37 (37.4)
Diarrhea	33 (32.7)	40 (40.4)
Nasal congestion	34 (33.7)	34 (34.3)
Sore throat	36 (35.6)	23 (23.2)
Coexisting diseases, n (%)		
Hypertension	54 (53.5)	49 (49.5)
Diabetes	39 (38.6)	29 (29.3)
Cardiovascular disease	14 (13.9)	13 (13.1)
Rheumatic disease	10 (9.9)	10 (10.1)
Asthma	6 (5.9)	7 (7.1)
Chronic obstructive pulmonar disease	5 (5.0)	5 (5.1)
Chronic kidney disease	2 (2.0)	0 (0)
Concomitant medications, n (%)		
Anticoagulant	94 (93.1)	85 (85.9)

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Table 1 *(Continued.)*

Characteristic	Vitamin D_3 group (n = 101)	Placebo group (n = 99)
Antibiotic	85 (84.2)	86 (86.9)
Glucocorticoid	67 (66.3)	63 (63.6)
Antihypertensive	55 (54.5)	46 (46.5)
Proton pump inhibitor	40 (39.6)	41 (41.4)
Antiemetic	39 (38.6)	49 (49.5)
Analgesic	39 (38.6)	46 (46.9)
Hypoglycemic	23 (22.8)	20 (20.2)
Hypolipidemic	14 (13.9)	14 (14.1)
Thyroid	8 (7.9)	8 (8.1)
Antiviral [4]	4 (4.0)	3 (3.0)
Dose of glucocorticoid at randomization, mg [5]	5.1 ± 10.3	4.2 ± 4.3
Cumulative dose of glucocorticoid, mg [5]	45.7 ± 81.4	33.6 ± 33.0
Oxygen supplementation, n (%)		
Oxygen therapy	72 (71.3)	82 (82.8)
Non-invasive ventilation	13 (12.9)	12 (12.1)
No oxygen therapy	16 (15.8)	5 (5.1)
Computed tomography findings, n (%)		
Ground-glass opacities ≥ 50%	48 (53.3)	53 (62.4)
Ground-glass opacities < 50%	42 (46.7)	32 (37.6)

[1]Values are mean ± SD, median (IQR), or n (%). Continuous variables were analyzed by independent *t* test. Percentages were analyzed by chi-square or Fisher's exact test. COVID-19, coronavirus disease 2019. [2]Pardo is the exact term used in Brazilian Portuguese, meaning "mixed ethnicity", according to the Brazilian Institute of Geography and Statistics. [3]Body mass index data were missing for 8.5% patients (n=17; 8 in the vitamin D_3 group and 9 in the placebo group). [4]Included 3 patients from the vitamin D_3 group and 3 patients from the placebo group receiving 75mg of oseltamivir twice per day for 5 days, 1 patient from the vitamin D_3 group receiving 400mg of acyclovir twice per day for herpes zoster prophylaxis. [5]Glucocorticoid information was standardized in dexamethasone doses.

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Table 2. Cytokines, Chemokines, Growth Factors, and Laboratory Outcomes

Outcomes	Vitamin D_3 group (n = 101)		Placebo group (n = 99)		*P* [1]	*P* [2]	*P* [3]	*P* [4]
	Baseline	Post	Baseline	Post				
Cytokines								
Interleukin-1β, pg/mL	1.9 ± 2.2	1.8 ± 2.4	2.2 ± 3.7	2.7 ± 5.5	0.39	0.44	0.39	0.39
Interleukin-4, pg/mL	263.4 ± 1072.8	236.6 ± 988.7	220.2 ± 783.6	213.5 ± 780.9	0.33	0.25	0.33	0.33
Interleukin-6, pg/mL [*]	26.8 ± 48.7	16.4 ± 41.2	25.3 ± 40.5	17.4 ± 38.6	0.42	0.45	0.42	0.42
Interleukin-10, pg/mL [*]	28.0 ± 21.7	14.2 ± 10.3	33.6 ± 40.1	17.5 ± 18.9	0.62	0.59	0.62	0.62
Interleukin-12p70, pg/mL	6.9 ± 25.6	4.2 ± 8.2	3.8 ± 7.2	4.7 ± 11.5	0.10	0.10	0.10	0.10
Interleukin-17A, pg/mL	12.8 ± 78.0	12.4 ± 70.8	7.6 ± 20.2	6.9 ± 18.5	0.88	0.87	0.88	0.88
IFN-γ, pg/mL [*]	11.3 ± 17.8	7.7 ± 12.7	9.1 ±13.2	7.3 ± 10.0	0.40	0.71	0.40	0.40
TNF-α, pg/mL [*]	29.5 ± 14.2	25.9 ± 12.1	28.1 ± 17.3	26.7 ± 13.9	0.11	0.14	0.11	0.11
GM-CSF, pg/mL	3.4 ± 5.2	2.9 ± 4.8	3.0 ± 4.1	4.4 ± 9.7	0.04	0.05	0.04	0.04
Chemokines								
Interleukin-8, pg/mL [*]	22.9 ± 22.9	18.3 ± 22.0	22.0 ± 21.6	19.1 ± 20.5	0.31	0.40	0.31	0.31
IP-10, pg/mL [*]	3283.0 ± 3340.3	861.9 ± 972.5	3747.2 ± 3702.0	747.8 ± 1337.4	0.24	0.27	0.24	0.24
MIP-1β, pg/mL	12.4 ± 59.3	18.0 ± 102.8	21.5 ± 73.4	50.4 ± 233.4	0.21	0.21	0.21	0.21
MCP-1, pg/mL [*]	1172.2 ± 1208.2	754.0 ± 594.0	1159.7 ± 961.0	870.2 ± 564.9	0.33	0.38	0.33	0.33
Growth factor								
VEGF, pg/mL [*]	278.8 ± 399.9	221.3 ± 297.5	391.7 ± 1242.8	271.4 ± 868.6	0.20	0.19	0.20	0.20
Laboratory								
25-hydroxyvitamin D, ng/mL [*, **]	21.1 ± 10.1 [a]	44.6 ± 14.7 [b]	20.2 ± 8.1 [a]	19.8 ± 10.5 [a]	<0.001	<0.001	<0.001	<0.001
C-reactive protein, mg/L [*, 5]	77.5 ± 70.5	21.8 ± 41.6	82.9 ± 72.6	20.5 ± 35.3	0.47	0.34	0.47	0.47
Leukocyte count, x $10^3/mm^3$ [*, 5]	8.4 ± 4.2	9.3 ± 3.9	8.9 ± 3.6	9.4 ± 3.9	0.30	0.26	0.30	0.31

Values are mean ± SD. Data were analyzed by Generalized Estimating Equations (GEE) with normal distribution and identity link function with first-order autoregressive correlation matrix. [1]*P* value represents group by time interaction adjusted by cumulative glucocorticoid doses (39.7 mg). [2]*P* value represents group by time interaction adjusted by cumulative glucocorticoid doses and time from symptom onset to enrollment. [3]*P*

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value represents group by time interaction adjusted by cumulative glucocorticoid doses and baseline 25-hydroxyvitamin D levels. [4]*P* value represents group by time interaction adjusted by cumulative glucocorticoid doses and hospitals from which patients were recruited. [5]Data were missing for 1.0% (n=2) of the patients in the vitamin D_3 group at post. $^{*}P < 0.05$ for main effect of time. $^{**}P < 0.05$ for main effect of group. Different letters indicate significant differences ($P < 0.05$).

SI conversion factors: To convert 25-hydroxyvitamin D to nmol/L, multiply values by 2.496. GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN-γ, interferon-γ; IP-10, interferon-inducible protein-10; MCP-1, monocyte chemoattractant protein-1; MIP-1β, macrophage inflammatory protein-1β; Post, post-intervention, TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

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