

1 **Effect of Vitamin D₃ Supplementation vs Placebo on Hospital Length of Stay in**
2 **Patients with Severe COVID-19: A Multicenter, Double-blind, Randomized**
3 **Controlled Trial**

4
5 **Short title:** Vitamin D₃ Supplementation in COVID-19
6

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1 **Key points:**

2

3 **Question:** Can vitamin D₃ supplementation reduce hospital length of stay in

4 hospitalized patients with severe COVID-19?

5

6 **Findings:** In this double-blind, randomized, placebo-controlled trial involving 240

7 hospitalized patients with severe COVID-19, a single dose of 200,000 IU of vitamin D₃

8 supplementation was safe and effective in increasing 25-hydroxyvitamin D levels, but

9 did not significantly reduce hospital length of stay (hazard ratio, 1.12) or any other

10 clinically-relevant outcomes compared with placebo.

11

12 **Meaning:** Vitamin D₃ supplementation does not confer therapeutic benefits among

13 hospitalized patients with severe COVID-19.

1 **Importance:** Patients with COVID-19 may exhibit 25-hydroxyvitamin D deficiency,
2 but the beneficial effects of vitamin D₃ supplementation in this disease remain to be
3 proven by randomized controlled trials.

4 **Objective:** To investigate the efficacy and safety of vitamin D₃ supplementation in
5 patients with severe COVID-19.

6 **Design, Setting, and Participants:** This is a multicenter, double-blind, randomized,
7 placebo-controlled trial conducted in two centers (a quaternary hospital and a field
8 hospital) in Sao Paulo, Brazil. The trial included 240 hospitalized patients with severe
9 COVID-19. The study was conducted from June 2, 2020 to October 7, 2020.

10 **Interventions:** Patients were randomly allocated (1:1 ratio) to receive either a single
11 oral dose of 200,000 IU of vitamin D₃ or placebo.

12 **Main Outcomes and Measures:** The primary outcome was hospital length of stay,
13 defined as hospital discharge from the date of randomization or death. Secondary
14 outcomes were mortality, admission to ICU, mechanical ventilation requirement, and
15 serum levels of 25-hydroxyvitamin D, creatinine, calcium, C-reactive protein, and D-
16 dimer.

17 **Results:** Of 240 randomized patients (mean age, 56 years; 56% men), 232 (96.7%)
18 were included in the primary analysis. Log-rank test showed that hospital length of stay
19 was comparable between the vitamin D₃ supplementation and placebo groups (7.0 days
20 [95% CI, 6.1 to 7.9] and 7.0 days [95% CI, 6.2 to 7.8 days]; hazard ratio, 1.12 [95% CI,
21 0.9 to 1.5]; $P = .379$; respectively). The rate of mortality (7.0% vs 5.1%; $P = .590$),
22 admission to ICU (15.8% vs 21.2%; $P = .314$), and mechanical ventilation requirement
23 (7.0% vs 14.4%; $P = .090$) did not significantly differ between groups. Vitamin D₃
24 supplementation significantly increased serum 25-hydroxyvitamin D levels compared to

1 placebo (difference, 24.0 ng/mL [95% CI, 21.0% to 26.9%]; $P = .001$). No adverse
2 events were observed.

3 **Conclusions and Relevance:** Among hospitalized patients with severe COVID-19,
4 vitamin D₃ supplementation was safe and increased 25-hydroxyvitamin D levels, but
5 did not reduce hospital length of stay or any other relevant outcomes vs placebo. This
6 trial does not support the use of vitamin D₃ supplementation as an adjuvant treatment of
7 patients with COVID-19.

8 **Trial Registration:** ClinicalTrials.gov Identifier: NCT04449718

9

1 **Introduction**

2 A growing body of evidence has indicated that vitamin D may enhance the innate¹⁻³ and
3 adaptive immunity.^{4,5} Since antigen-presenting cells have the ability to synthesize 1,25-
4 dihydroxyvitamin D (the active form of vitamin D) from 25-hydroxyvitamin D, it has
5 been postulated that vitamin D supplementation could improve the function of
6 macrophages and dendritic cells, thereby ameliorating overall immune response.⁶ In
7 fact, insufficient vitamin D status has been suggested as a potential risk factor for non-
8 communicable⁷ and acute respiratory tract diseases,^{8,9} including viral infections.¹⁰
9 In this context, it has been recently conjectured that optimal levels of vitamin D could
10 play important immunomodulatory and anti-inflammatory roles, thereby benefiting
11 patients with COVID-19.^{11, 12} However, the putative benefits of supplementary vitamin
12 D₃ to patients with COVID-19 remain speculative and partially supported by limited
13 data from observational studies and one small-scale, non-randomized clinical trial.¹³⁻¹⁵
14 To our knowledge, this is the first randomized, double-blind, placebo-controlled trial to
15 investigate the safety and efficacy of vitamin D₃ supplementation on hospital length of
16 stay and other relevant clinical outcomes in hospitalized patients with severe COVID-
17 19. Our main *a priori* hypothesis was that a single dose of 200,000 IU of vitamin D₃
18 supplementation would increase 25-hydroxyvitamin D levels and shorten hospital
19 length of stay among these patients.

1 **Methods**

2 The study was approved by the Ethics Committee of Clinical Hospital of the School of
3 Medicine of the University of Sao Paulo and by the Ethics Committee of Ibirapuera
4 Field Hospital. All the procedures were conducted in accordance with the Declaration of
5 Helsinki. The participants provided written informed consent before being enrolled in
6 the study (Ethics Committee Approval Number 30959620.4.0000.0068). The trial
7 protocol and statistical plan are included in Supplement 1. This manuscript was written
8 according to the recommendations by the Consolidated Standards of Reporting Trials
9 (CONSORT) guidelines (see Supplement 2).

10

11 **Participants**

12 Hospitalized patients were recruited from Clinical Hospital of the School of Medicine
13 of the University of Sao Paulo (a quaternary referral teaching hospital), and from
14 Ibirapuera Field Hospital, both located in Sao Paulo, Brazil. Enrollment started on June
15 2, 2020, to August 27, 2020, with the final follow-up on October 7, 2020.

16

17 **Inclusion criteria**

18 Inclusion criteria were: 1) adults aged 18 years or older; 2) diagnosis of COVID-19 by
19 either polymerase chain reaction (PCR) for severe acute respiratory
20 syndrome coronavirus 2 (SARS-CoV-2) from nasopharyngeal swabs or computed
21 tomography scan findings (bilateral multifocal ground-glass opacities \geq 50%)
22 compatible with the disease; 3) diagnosis of flu syndrome with hospitalization criteria
23 on hospital admission, presenting respiratory rate \geq 24 breaths per minute, saturation $<$
24 93% on room air or risk factors for complications, such as heart disease, diabetes

1 mellitus, systemic arterial hypertension, neoplasms, immunosuppression, pulmonary
2 tuberculosis, and obesity, followed by COVID-19 confirmation before randomization.

3

4 **Exclusion criteria**

5 Exclusion criteria were: 1) patient unable to read and sign the written informed consent;
6 2) patient already admitted under invasive mechanical ventilation; 3) previous vitamin
7 D₃ supplementation (> 1000 IU/day); 4) renal failure requiring dialysis or creatinine ≥
8 2.0 mg/dL; 5) hypercalcemia defined by total calcium > 10.5 mg/dL; 6) pregnant or
9 lactating women; and 7) patients with expected hospital discharge in less than 24 hours.

10

11 **Study design and treatment**

12 This was a multicenter, double-blind, parallel-group, randomized placebo-controlled
13 trial. Eligibility screening was performed between June 2, 2020 to July 21, 2020 at
14 Clinical Hospital of the School of Medicine of the University of Sao Paulo (n = 122),
15 and from July 22, 2020 to August 27, 2020 at Ibirapuera Field Hospital (n = 118). The
16 final follow-up in both centers was on October 7, 2020. Eligible patients were assigned
17 in a 1:1 ratio into either the vitamin D₃ supplementation group or the placebo group.
18 The randomization list was created using a computer-generated code, which was
19 managed by a staff member who had no role in the study. We assessed patients' clinical
20 status, coexisting chronic diseases, demographic characteristics, self-reported body
21 weight and height, and ethnicity on hospital admission. Outcomes were assessed at
22 baseline and on hospital discharge or death records.

23 The vitamin D₃ supplementation group received an oral, single dose of 200,000 IU of
24 vitamin D₃ dissolved in a 10 mL of peanut oil solution on the same day of
25 randomization. The selected dose is within the recommended range for effectively

1 promoting vitamin D sufficiency.¹⁶ Patients in the placebo group received 10 mL of
2 peanut oil solution. The vitamin D₃ and placebo solutions were identical in color, taste,
3 smell, consistency, and container. Both were prepared by the pharmacy unit of Clinical
4 Hospital and labeled by a staff member who did not participate in the study. Allocation
5 blindness was kept until the final statistical analysis.

6

7 **Outcome measures**

8 The primary outcome was hospital length of stay, defined as the total number of days
9 that patients remained hospitalized from the date of study admission until the date of
10 hospital discharge or death. The criteria used for patient discharge were: 1) no need for
11 supplemental oxygen in the last 48 hours; 2) no fever in the last 72 hours; and 3)
12 oxygen saturation > 93% in room air without respiratory distress.

13 The secondary outcomes were: 1) mortality; 2) number of patients admitted to the
14 intensive care unit (ICU); 3) number of patients who needed mechanical ventilation and
15 duration of mechanical ventilation; and 4) serum levels of 25-hydroxyvitamin D
16 (assessed by a chemiluminescent immunoassay), calcium (assessed by a NM-BAPTA
17 method), creatinine (assessed by a colorimetric assay based on kinetic Jaffe's reaction),
18 and C-reactive protein and D-dimer (both assessed by an immunoturbidimetric assay).

19 The biochemical analyses were carried out in an accredited laboratory from Clinical
20 Hospital.

21

22 **Statistical Analysis**

23 Considering the lack of data available for sample size determination based on the
24 primary outcome (i.e., hospital length of stay after vitamin D₃ supplementation in
25 patients with severe COVID-19), the number of participants was chosen on the basis of

1 feasibility, such as resources, capacity of research staff and facility, and available
2 patients, in line with current recommendations.^{17, 18} Subsequently, we calculated sample
3 size assuming a 50% between-group difference in hospital length of stay (considering 7
4 days as a median time of stay, with an expected variability of 9 days). By considering a
5 power of 80% and a 2-sided significance level of 5% ($\alpha = .05$), the total sample was
6 estimated to be 208 patients (104 in each arm). Considering possible dropouts, and to
7 increase the power for secondary outcomes, we opted by increasing the sample size by
8 approximately 15%.

9 All analyses were carried out following the intention-to-treat principle for all
10 randomized patients, with no imputation for any missing data. Proportions were
11 compared between groups using χ^2 test and Fisher's exact test. Student's t-tests were
12 used for comparing continuous variables at baseline. The log-rank test was used to
13 compare the Kaplan-Meier estimate curves the number of days for hospital length of
14 stay, the primary outcome. Cox regression models for hospital length of stay, admission
15 to ICU and mechanical ventilation requirement were adjusted by potential confounders
16 that were not fully balanced by randomization ($P < .2$) to estimate hazard ratios (HR),
17 with corresponding 2-sided 95% CI. Generalized estimating equations (GEE) for
18 repeated measures were used for testing possible differences in laboratory parameters,
19 assuming group and time as fixed factors, with marginal distribution, and a first-order
20 autoregressive correlation matrix to test the main and interaction effects. *Post-hoc* tests
21 with Bonferroni's adjustment were performed for multiple comparisons. The
22 aforementioned statistical procedures were also carried out in *post-hoc* sensitivity
23 analyses involving patients exhibiting 25-hydroxyvitamin D deficiency (i.e., < 20
24 ng/mL).

- 1 Statistical analyses were performed with IBM-SPSS software, version 20.0.
- 2 Significance level was set at $\alpha = .05$.

1 **Results**

2 **Patients**

3 Of 1208 patients assessed for eligibility, 240 were eligible and randomly assigned to
4 either the vitamin D₃ group or the placebo group. Patients were non-eligible due to the
5 following reasons: 284 were at ICU, 263 had hospital discharge within 24 hours, 217
6 did not have COVID-19 confirmation, 95 had renal dysfunction, 37 had dementia or
7 severe mental confusion hampering their ability to provide the informed consent for
8 participation, 30 were pregnant or lactating women, 14 had hypercalcemia due to
9 metastatic neoplasm, 11 were receiving vitamin D₃ (≥ 1000 IU/day), 9 were younger
10 than 18 years, 6 were illiterate and, therefore, unable to read and sign the informed
11 consent, and 2 died before randomization.

12 Of the 120 patients who were randomized to the vitamin D₃ group, 3 did not receive
13 intervention (1 withdrew the consent, 1 vomited immediately after ingesting the
14 supplement, and 1 was admitted to the ICU before taking vitamin D₃) and 3 were lost to
15 follow-up. Of the 120 patients who were randomized to the placebo group, 2 withdrew
16 the consent. Thus, of the 240 patients randomized, 232 (96.7%) completed the follow-
17 up (**Figure 1**).

18 Overall, patients' age was 56.3 years (SD, 14.6), BMI was 31.6 kg/m² (SD, 7.1), 56.3%
19 were men, 55% were white, 52.5% had hypertension, 35% had diabetes, 13.3% had
20 cardiovascular diseases, and 6.3% had asthma. The mean time between the onset of
21 symptoms and randomization was 10.2 days (SD, 4.3); 89.6% required supplemental
22 oxygen at baseline (183 were on oxygen therapy and 32 were on non-invasive
23 ventilation), and 59.6% had computed tomography scan findings suggestive of COVID-
24 19. Demographic and clinical characteristics did not significantly differ between groups,
25 except for sore throat, which was more prevalent in the vitamin D₃ group vs placebo

1 (38.3% vs 24.2%, $P = .026$), and PTH, which was higher in the vitamin D₃ group vs
2 placebo (50.1 vs 42.6 pg/mL, $P = .025$) (**Table 1**).

3

4 **Primary Outcome**

5 Hospital length of stay (**Figure 2**) was comparable between the vitamin D₃ group and
6 the placebo group (7.0 days [95% CI, 6.1 to 7.9] and 7.0 days [95% CI, 6.2 to 7.8 days],
7 HR, 1.12, [95% CI, 0.9 to 1.5]; $P = .379$; respectively). The Cox regression model did
8 not show any significant associations between this outcome and potential confounders.

9

10 **Secondary Outcomes**

11 There were no significant differences between the vitamin D₃ group and the placebo
12 group for mortality (7.0% vs 5.1%; $P = .590$), admission to ICU (15.8% vs 21.2%; $P =$
13 $.314$) and mechanical ventilation requirement (7.0% vs 14.4%; $P = .090$) (**Figure 3**).

14 Duration of mechanical ventilation was also comparable between the vitamin D₃ group
15 (18.1 days [95% CI, 3.5 to 32.7]) and the placebo group (11.4 days [95% CI, 7.1 to
16 15.6]; $P = .549$, respectively).

17 The Cox regression model did not show significant associations between secondary
18 outcomes and potential confounders.

19 Vitamin D₃ supplementation significantly increased 25-hydroxyvitamin D levels vs
20 placebo (difference, 24.0 ng/mL [95% CI, 21.1- 26.9]; $P < .001$) (**Figure 3**). Following
21 the intervention, 86.7% of the patients in the vitamin D₃ group showed 25-
22 hydroxyvitamin D levels above 30 ng/mL (vs 10.9% in the placebo group), and only
23 6.7% of the patients in the vitamin D₃ group exhibited 25-hydroxyvitamin D deficiency
24 (vs 51.5% in the placebo group).

25

1 ***Post-hoc Sensitivity Analyses***

2 In a sensitivity analysis involving patients with 25-hydroxyvitamin D deficiency at
3 baseline (n = 116) (**Supplementary Table 1**), vitamin D₃ supplementation significantly
4 increased 25-hydroxyvitamin D levels vs placebo (difference, 22.7 ng/mL [95% CI,
5 19.3 to 26.1]; $P < .001$) (**Figure 3**). Among the patients with 25-hydroxyvitamin D
6 deficiency, no between-group differences were observed in length of hospital stay
7 (**Figure 2**). In addition, there were no significant differences between the vitamin D₃
8 group and the placebo group for mortality (7.0% vs 1.7%; $P = .206$), admission to ICU
9 (17.5% vs 15.5%; $P = .806$), and mechanical ventilation requirement (7.0% vs 8.6%; P
10 $> .999$) (**Figure 3**). Duration of mechanical ventilation did not differ between the
11 vitamin D₃ group (15.0 days [95% CI, -12.0 to 42.0]) and the placebo group (12.6 days
12 [95% CI, -7.6 to 26.0]; $P = .730$).

13

14 **Safety and Adverse Events**

15 There were no changes in any health-related laboratory markers following the
16 intervention (**Table 2**). Vitamin D₃ supplementation was well tolerated and no severe
17 adverse events were reported throughout the trial, with the exception of one patient who
18 vomited following vitamin D₃ administration.

19

20 **Discussion**

21 This is the first randomized, double-blind, placebo-controlled trial to show that vitamin
22 D₃ supplementation is safe and increases 25-hydroxyvitamin D levels, but is ineffective
23 to improve hospital length of stay or any other clinical outcomes among hospitalized
24 patients with severe COVID-19.

1 Vitamin D has been postulated to play an important role on immune system, acting as a
2 regulator of both innate and adaptative responses.^{6, 19} Observational studies have shown
3 that 25-hydroxyvitamin D levels are associated with better clinical outcomes in
4 respiratory diseases.²⁰ Positive associations between low 25-hydroxyvitamin D levels
5 and poor prognosis among patients with COVID-19 have also been observed.²¹
6 Furthermore, a small-scale, non-randomized trial demonstrated that the administration
7 of regular boluses of vitamin D₃ before the infection was associated with better survival
8 and less severe disease among older, frail patients with COVID-19.²² In the current trial,
9 however, a single dose of 200,000 IU of vitamin D₃ supplementation failed to promote
10 any clinically relevant effects among hospitalized patients with severe COVID-19,
11 contesting the utility of supplementary vitamin D₃ as a treatment in this disease.
12 The lack of clinical benefits seen in this study was independent of the ability of vitamin
13 D₃ supplementation to increase serum 25-hydroxyvitamin D levels. In fact, following
14 the intervention, 86.7% of the patients in the supplementation arm achieved vitamin D
15 sufficiency (≥ 30 ng/mL) vs 11% only in the placebo group. In a sensitivity analysis
16 confined to the patients exhibiting 25-hydroxyvitamin D deficiency, vitamin D₃
17 supplementation remained effective in increasing 25-hydroxyvitamin D levels vs
18 placebo; yet, no clinical improvements were noted. Collectively, these analyses indicate
19 that a single oral dose of 200,000 IU of supplementation can rapidly increase 25-
20 hydroxyvitamin levels, in agreement with our hypothesis, so that the present null
21 findings cannot be attributed to the failure of increasing serum 25-hydroxyvitamin D
22 levels.
23 Despite the clinical inefficacy of vitamin D₃ supplementation, the intervention was not
24 associated with any important adverse events or meaningful changes in laboratory

1 parameters, suggesting that a relatively high-dose of vitamin D₃ can be well tolerated in
2 general and free of adverse effects in patients with COVID-19.

3 The strengths of this study include the randomized, double-blind, placebo-controlled
4 experimental design, the adequate power, particularly for the primary analysis, the very
5 low attrition rate (3.3%), the concomitant assessment of 25-hydroxyvitamin D levels
6 along with clinical outcomes, and the assessment of hospitalized patients with severe
7 COVID-19.

8

9 **Limitations**

10 This trial has several limitations. First, the sample size could have been underpowered
11 to detect significant changes for the secondary outcomes. Second, as the patients had
12 several coexisting diseases and were subjected to a diverse medication regimen, the
13 results could have been affected by the heterogeneity of the sample and its treatment.
14 Third, the proportion of patients with 25-hydroxyvitamin D deficiency enrolled in this
15 study was considerably lower than those reported in other cohorts,²³ possibly as a
16 consequence of differences in geographic locations. Although we conducted sensitivity
17 analyses involving patients with 25-hydroxyvitamin D deficiency, one could argue that
18 they could have been underpowered, as previously pointed out. Therefore, caution
19 should be exercised in generalizing these findings to patients from other geographical
20 regions. Finally, the findings should be also confined to the dose and supplementation
21 strategy used in this trial. Further studies should determine whether preventive or early
22 vitamin D₃ supplementation could be useful in the treatment of patients with COVID-
23 19, especially those with a mild or moderate disease.

24

25

1 **Conclusions**

2 Among hospitalized patients with severe COVID-19, a single dose of 200,000 IU of
3 vitamin D₃ supplementation was safe and increased 25-hydroxyvitamin D levels, but
4 did not reduce hospital length of stay or any other clinically relevant outcomes vs
5 placebo. Thus, this trial does not support the use of vitamin D₃ supplementation as an
6 adjuvant treatment of patients with COVID-19.

1 **Article information**

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10 **Author Contributions:** Dr. Pereira had full access to all of the data in the study and
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Figure legends

Figure 1. Flow of patients.

Figure 2. Kaplan-Meier curves for hospital length of stay. Vertical bars present single censored events. The shaded areas represent the 95% confidence intervals. The adjusted hazard ratio for total number of days that patients remained hospitalized from the date of study admission until the date of hospital discharge or death was 1.12 (95% CI, 0.9 to 1.5; $P = .379$) for the vitamin D₃ group (7.0 days [95% CI, 6.1 to 7.9]) vs the placebo group (7.0 days [95% CI, 6.2 to 7.8 days]).

Figure 3. Serum 25-hydroxyvitamin D levels, mortality, admission to intensive care unit (ICU), and mechanical ventilation requirement. The panels show the comparisons between the vitamin D₃ and placebo group for all patients ($n = 240$) (Panels A and B) and for those with 25-hydroxyvitamin D deficiency (< 20 ng/mL) ($n = 116$) (Panels C and D). For all patients, no significant differences were found between the vitamin D₃ group and the placebo group for mortality (7.0% vs 5.1%; $P = .590$), admission to ICU (15.8% vs 21.2%; $P = .314$), and need of mechanical ventilation (7.0% vs 14.4%; $P = .090$). Vitamin D₃ supplementation significantly increased 25-hydroxyvitamin D levels vs placebo (difference, 24.0 ng/mL [95% CI, 21.1- 26.9]; $P < .001$). For patients with 25-hydroxyvitamin D deficiency, there were no significant differences between the vitamin D₃ group and the placebo group for mortality (7.0% vs 1.7%; $P = .206$), admission to ICU (17.5% vs 15.5%; $P = .806$), and mechanical ventilation requirement (7.0% vs 8.6%; $P > .999$). Vitamin D₃ supplementation significantly increased 25-hydroxyvitamin D levels vs placebo (difference, 22.7 ng/mL

[95% CI, 19.3- 26.1]; $P < .001$). Box plots depict median and interquartile range. Outliers (i.e., defined as a value < 5 or > 95 percentiles) were represented by filled circles. * means $P < .05$ between Baseline and Post; # means $P < .05$ between groups at Post.

Table 1. Baseline demographic and clinical characteristics.

Table 2. Laboratory variables.

Supplementary Table 1. Baseline demographic and clinical characteristics from patients with 25-hydroxyvitamin D deficiency (< 20 ng/mL).

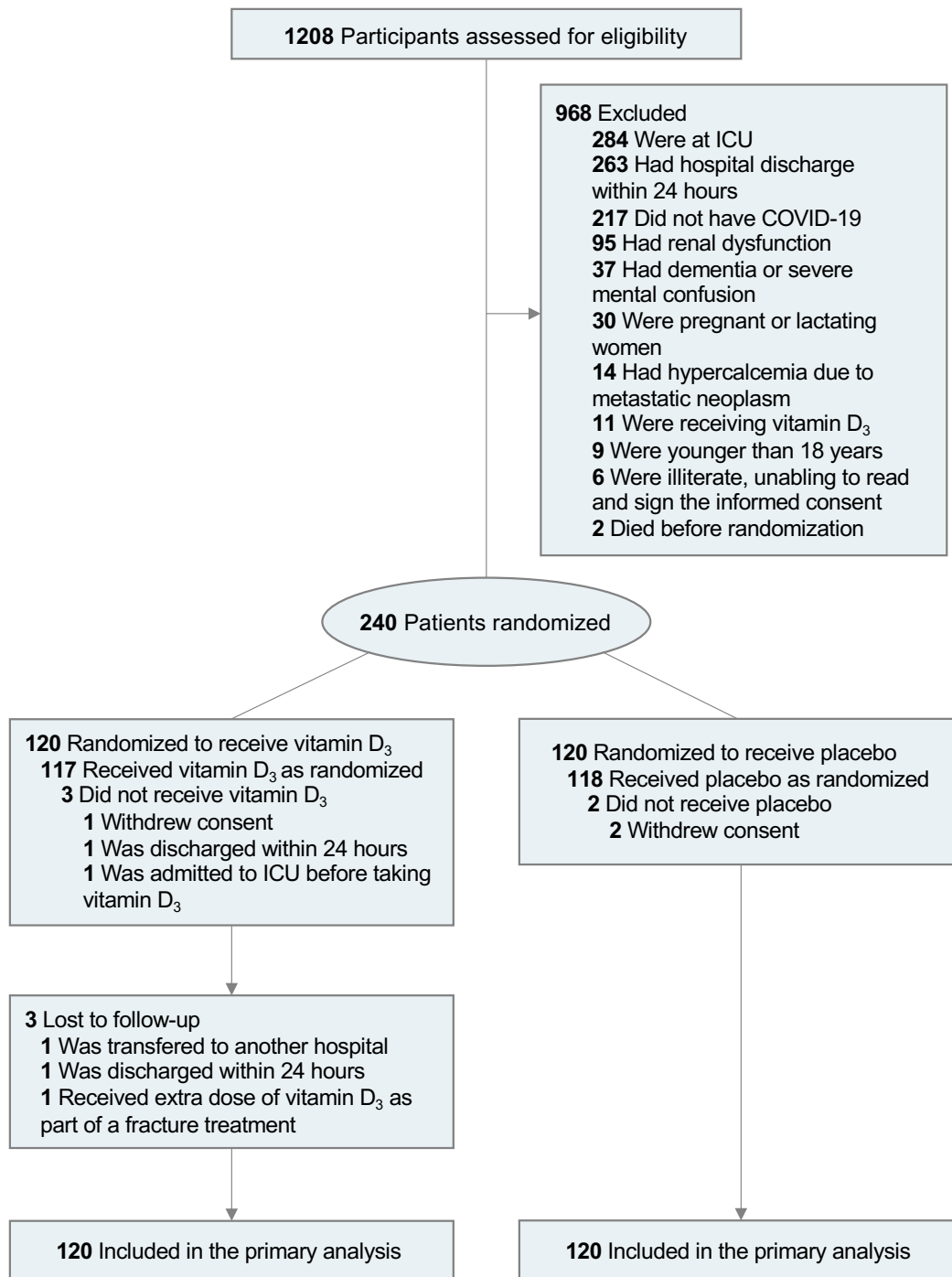


Figure 1. Flow of patients.

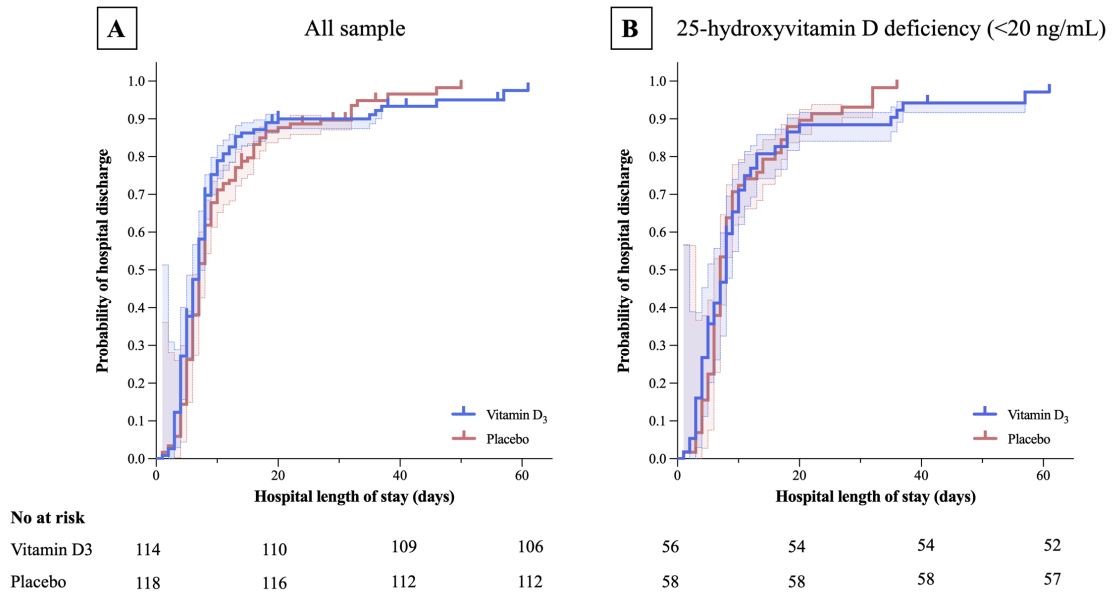


Figure 2. Kaplan-Meier curves for hospital length of stay.

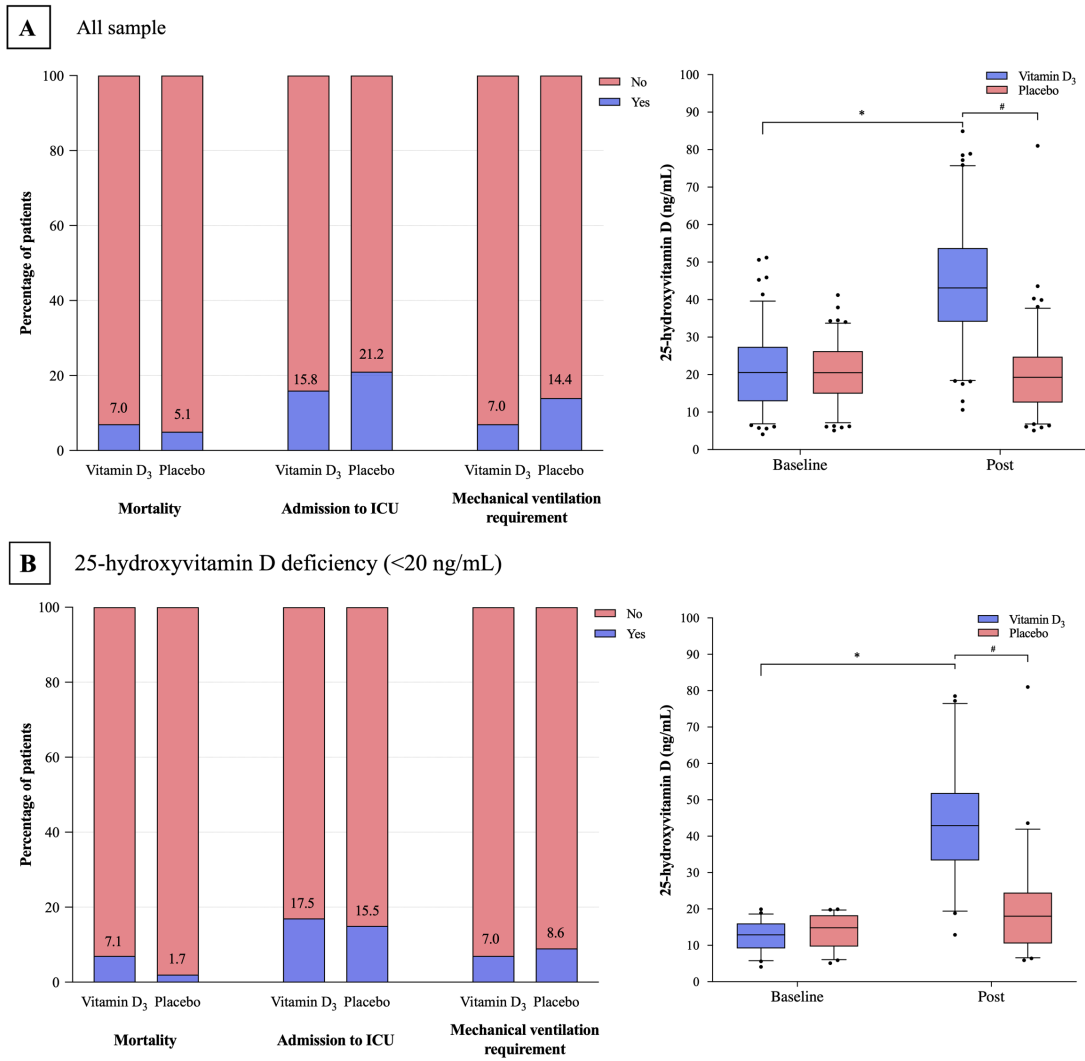


Figure 3. Serum 25-hydroxyvitamin D levels, mortality, admission to intensive care unit (ICU), and mechanical ventilation requirement. * means $P < .05$ between Baseline and Post; # means $P < .05$ between groups at Post.

Table 1. Baseline Demographic and Clinical Characteristics

	Vitamin D ₃ (n = 120)	Placebo (n = 120)	<i>P</i> value
Age, mean (SD), y	56.8 (14.2)	55.8 (15.0)	.584
Sex, No. (%)			
Male	70 (58.3)	65 (54.2)	.515
Female	50 (41.7)	55 (45.8)	
Race, No. (%)			
White	62 (51.7)	70 (58.3)	.399
Brown	37 (30.8)	36 (30.0)	
Black	20 (16.7)	14 (11.7)	
Asian	1 (0.8)	0 (0.0)	
Days since symptoms onset, mean (SD)	10.3 (4.7) [n=116]	10.2 (3.8) [n=119]	.787
Body mass index, mean (SD), kg/m ²	31.9 (6.5) [n=109]	31.3 (7.6) [n=110]	.548
Underweight, No./total (%)	0 (0)	2 (1.8)	.067
Normal, No./total (%)	9/109 (8.3)	19/110 (17.3)	
Overweight, No./total (%)	37/109 (33.9)	31/110 (28.2)	
Obesity, No./total (%)	63/109 (57.8)	58/110 (52.7)	
Acute COVID-19 symptoms, No. (%)			
Fever	86 (71.7)	81 (67.5)	.575
Cough	103 (85.8)	99 (82.5)	.596
Fatigue	98 (81.7)	100 (83.3)	.865
Joint pain	46 (38.3)	35 (29.2)	.172
Myalgia	69 (57.5)	71 (59.2)	.896
Nasal congestion	39 (32.5)	43 (35.8)	.683
Runny nose	44 (36.7)	44 (36.7)	>.999
Sore throat	46 (38.3)	29 (24.2)	.026
Diarrhea	41 (34.2)	46 (38.3)	.591
Coexisting diseases, No. (%)			
Hypertension	68 (56.7)	58 (48.3)	.196
Cardiovascular disease	16 (13.3)	16 (13.3)	>.999
Diabetes	49 (40.8)	35 (29.2)	.058
Chronic obstructive pulmonary disease	7 (5.8)	5 (4.2)	.554
Asthma	8 (6.7)	7 (5.8)	.790

Chronic kidney disease	2 (1.7)	0 (0.0)	.489
Rheumatic disease	13 (10.8)	10 (8.3)	.511
Concomitant medications, No. (%)			
Antibiotic	102 (85.0)	105 (87.5)	.708
Anticoagulant	110 (91.7)	103 (85.8)	.220
Analgesic	45 (37.5)	52 (43.7)	.430
Corticosteroids	77 (64.2)	73 (60.8)	.689
Antihypertensive	67 (55.8)	56 (46.7)	.196
Hypoglycemic	26 (21.7)	24 (20.0)	.874
Hypolipidemic	15 (12.5)	18 (15.0)	.708
Antiemetic	45 (37.5)	55 (45.8)	.239
Antiviral	4 (3.3)	4 (3.3)	>.999
Proton pump inhibitor	47 (39.2)	49 (40.8)	.895
Thyroid	10 (8.3)	10 (8.3)	>.999
Oxygen supplementation, No. (%)			
No oxygen therapy	16 (13.3)	9 (7.5)	
Oxygen therapy	86 (71.7)	97 (80.8)	.210
Non-invasive ventilation	18 (15.0)	14 (11.7)	
Computed tomography findings, No. (%)			
Ground-glass opacities < 50%	61 (50.8)	66 (55.0)	
Ground-glass opacities ≥ 50%	47 (39.2)	39 (32.5)	.543
Not available	12 (10.0)	15 (12.5)	
Laboratory variables			
Haemoglobin, mean (SD), g/L	13.1 (2.1)	12.8 (2.1)	.298
Neutrophils count, mean (SD), x10 ³ /mm ³	6.7 (4.0) [n = 119]	7.2 (3.6) [n = 120]	.281
Lymphocyte count, mean (SD), x10 ³ /mm ³	1.2 (0.5) [n = 119]	1.1 (0.8) [n = 120]	.849
Platelet count, mean (SD), x10 ³ /mm ³	305.4 (116.4)	286.7 (128.5)	.239
Erythrocyte sedimentation rate, mean (SD), mm	58.4 (40.6) [n = 117]	60.9 (36.7) [n = 119]	.627
C-reactive protein, mean (SD), mg/L	79.6 (75.2) [n = 119]	90.4 (80.4) [n = 120]	.286
D-dimer, mean (SD), ng/mL	2091 (5001.3) [n = 119]	1720.7 (3630.1) [n = 119]	.514
Albumin, mean (SD), g/L	3.1 (0.5) [n = 110]	3.0 (0.4) [n = 100]	.454
Gamma globulins, mean (SD), g/L	1.1 (0.4) [n = 110]	1.1 (0.3) [n = 100]	.337

Creatinine, mean (SD), mg/dL	0.9 (0.3)	0.8 (0.2)	.149
Urea, mean (SD), mg/dL	40.1 (20.1) [n = 120]	37.8 (14.5) [n = 119]	.309
Phosphorus, mean (SD), mg/dL	3.0 (0.6) [n = 117]	3.0 (0.8) [n = 116]	.790
25-hydroxyvitamin D, mean (SD), ng/mL	21.0 (10.2) [n = 118]	20.6 (8.1) [n = 118]	.747
Parathyroid hormone, mean (SD), pg/mL	50.1 (27.3) [n = 113]	42.6 (21.5) [n = 110]	.025
Calcium, total, mean (SD), mg/dL	8.7 (0.5) [n = 118]	8.7 (0.5) [n = 119]	.811
Total cholesterol, mean (SD), mg/dL	164.3 (43.3) [n = 116]	164.5 (47.2) [n = 115]	.975
LDL-cholesterol, mean (SD), mg/dL	101.3 (34.6) [n = 116]	99.3 (39.1) [n = 114]	.681
HDL-cholesterol, mean (SD), mg/dL	34.6 (11.4) [n = 116]	34.5 (11.0) [n = 114]	.916
Triglycerides, mean (SD), mg/dL	178.6 (75.5) [n=116]	192.5 (93.6) [n = 114]	.218

For continuous variables, groups were compared using independent t-test.

For categorical variables, groups were compared using χ^2 test or Fisher's exact test, as appropriate.

Table 2. Laboratory variables.

All patients	Vitamin D ₃ group		Difference (Baseline – Post)	Placebo group		Difference (Baseline – Post)	<i>P</i> value ^a	<i>P</i> value ^b
	Baseline	Post		Baseline	Post			
Variables								
Haemoglobin, mean (SD), g/L	13.1 (2.1)	12.7 (2.4)	0.4 (3.0) [n = 111]	12.8 (2.1)	12.7 (2.1)	0.2 (3.1) [n = 114]	.525	.595
Neutrophils count, mean (SD), x10 ³ /mm ³	6.7 (4.0)	7.0 (4.2)	-0.4 (3.6) [n = 111]	7.2 (3.6)	7.1 (4.9)	0.1 (5.0) [n = 114]	.460	.426
Lymphocyte count, mean (SD), x10 ³ /mm ³	1.2 (0.5)	1.8 (0.9)	-0.7 (0.8) [n = 111]	1.1 (0.8)	2.0 (1.1)	-0.9 (0.8) [n = 114]	.060	.075
Platelet count, mean (SD), x10 ³ /mm ³	305.4 (116.4)	367.5 (137.9)	-63.3 (100.7) [n = 111]	286.7 (128.5)	357.7 (141.1)	-70.6 (106.7) [n = 114]	.566	.597
Erythrocyte sedimentation rate, mean (SD), mm	58.4 (40.6)	48.6 (39.1)	8.9 (33.0) [n = 101]	60.9 (36.7)	44.2 (33.6)	17.1 (36.1) [n = 99]	.110	.102
C-reactive protein, mean (SD), mg/L	79.6 (75.2)	28.2 (54.0)	47.9 (70.6) [n = 109]	90.4 (80.4)	22.9 (42.1)	61.3 (79.1) [n = 107]	.156	.190
D-dimer, mean (SD), ng/mL	2091 (5001.3)	1589.4 (2484.8)	592.6 (5150.7) [n = 119]	1720.7 (3630.1)	1284.0 (1791.4)	316.3 (2049.7) [n = 119]	.843	.620
Creatinine, mean (SD), mg/dL	0.9 (0.3)	1.0 (0.7)	-0.1 (0.6) [n = 112]	0.8 (0.2)	0.9 (0.6)	-0.1 (0.7) [n = 112]	.928	.927
Urea, mean (SD), mg/dL	40.1 (20.1)	49.0 (46.8)	-9.6 (42.4) [n = 112]	37.8 (14.5)	46.6 (50.4)	-8.4 (49.4) [n = 113]	.956	.839
Phosphorus,	3.0 (0.6)	3.5 (0.7)	-0.5 (0.8)	3.0 (0.8)	3.7 (1.3)	-0.6 (1.4)	.334	.596

mean (SD), mg/dL			[n = 108]			[n = 108]		
Parathyroid hormone, mean (SD), pg/mL	50.1 (27.3)	35.0 (19.8)	13.3 (23.3) [n = 100]	42.6 (21.5)	36.7 (19.5)	7.5 (16.1) [n = 99]	.013	.049
Total calcium, mean (SD), mg/dL	8.7 (0.5)	9.1 (0.6)	-0.4 (0.5) [n = 106]	8.7 (0.5)	9.1 (0.5)	-0.4 (0.6) [n = 106]	.968	.890
Total cholesterol, mean (SD), mg/dL	164.3 (43.3)	183.2 (48.7)	-16.0 (30.4) [n = 104]	164.5 (47.2)	187.1 (50.3)	-22.5 (35.8) [n = 101]	.137	.170
LDL-cholesterol, mean (SD), mg/dL	101.3 (34.6)	111.9 (37.0)	-8.0 (24.8) [n = 104]	99.3 (39.1)	112.0 (40.2)	-12.5 (28.1) [n = 101]	.223	.243
HDL-cholesterol, mean (SD), mg/dL	34.6 (11.4)	37.5 (11.1)	-2.9 (8.0) [n = 104]	34.5 (11.0)	37.5 (10.3)	-3.7 (11.0) [n = 101]	.666	.553
Triglycerides, mean (SD), mg/dL	178.6 (75.5)	221.7 (115.3)	-41.1 (88.7) [n = 104]	192.5 (93.6)	251.9 (129.2)	-54.5 (106.6) [n = 101]	.244	.336

^a *P* value represents time by group interaction, calculated by Generalized Estimating Equations (GEE) with normal distribution and identity link function with AR (1) correlation matrix.

^b *P* value represents between-group comparisons for the difference, calculated by independent t-test.

Supplementary Table 1. Baseline Demographic and Clinical Characteristics from patients with 25-hydroxyvitamin D deficiency (< 20 ng/mL).

	Vitamin D ₃ (n = 58)	Placebo (n = 58)	<i>P</i> value
Age, mean (SD), y	58.9 (15.4)	56.1 (14.3)	.308
Sex, No. (%)			
Male	31 (53.4)	28 (48.3)	.577
Female	27 (46.6)	30 (51.7)	
Race, No. (%)			
White	26 (44.8)	34 (58.6)	.340
Brown	19 (32.8)	15 (25.9)	
Black	12 (20.7)	9 (15.5)	
Asian	1 (1.7)	0 (0)	
Days since symptoms onset, mean (SD)	10.5 (5.7)	10.4 (3.7)	.932
Body mass index, mean (SD), kg/m ²	32.0 (6.8) [n = 50]	31.7 (8.4) [n = 54]	.832
Underweight, No./total (%)	0 (0)	2/54 (3.7)	.275
Normal, No./total /(%)	5/50 (10.0)	8/54 (14.8)	
Overweight, No./total /(%)	15/50 (30.0)	18/54 (33.3)	
Obesity, No./total (%)	30/50 (60.0)	26/54 (48.1)	
Acute COVID-19 symptoms			
Fever	39 (67.2)	40 (69.0)	.842
Cough	50 (86.2)	51 (87.9)	.782
Fatigue	47 (81.0)	49 (84.5)	.623
Joint pain	20 (34.5)	15 (25.9)	.312
Myalgia	33 (56.9)	35 (60.3)	.706
Nasal congestion	16 (27.6)	26 (44.8)	.053
Runny nose	21 (36.2)	22 (37.9)	.848
Sore throat	24 (41.4)	13 (22.4)	.028
Diarrhea	16 (27.6)	22 (37.9)	.235
Coexisting diseases, No. (%)			
Hypertension	35 (60.3)	28 (48.3)	.192
Cardiovascular disease	6 (10.3)	8 (13.8)	.569
Diabetes	27 (46.6)	19 (32.8)	.129
Chronic obstructive pulmonary disease	5 (8.6)	5 (8.6)	>.999

Asthma	4 (6.9)	2 (3.4)	.679
Chronic kidney disease	1 (1.7)	0 (0.0)	>.999
Rheumatic disease	6 (10.3)	3 (5.2)	.490
Concomitant medications, No. (%)			
Antibiotic	47 (81.0)	50 (86.2)	.452
Anticoagulant	52 (89.7)	45 (77.6)	.079
Analgesic	23 (39.7)	30 (51.7)	.192
Corticosteroids	35 (60.3)	32 (55.2)	.573
Antihypertensive	34 (58.6)	29 (50.0)	.351
Hypoglycemic	12 (20.7)	13 (22.4)	.821
Hypolipidemic	9 (15.5)	9 (15.5)	>.999
Antiemetic	19 (32.8)	24 (41.4)	.336
Antiviral	2 (3.4)	2 (3.4)	>.999
Proton pump inhibitor	18 (31.0)	24 (41.4)	.246
Thyroid	6 (10.3)	5 (8.6)	.751
Oxygen supplementation, No. (%)			
No oxygen therapy	11 (19.0)	3 (5.2)	
Oxygen therapy	38 (65.5)	52 (89.7)	.008
Non-invasive ventilation	9 (15.5)	3 (5.2)	
Computed tomography findings, No. (%)			
Ground-glass opacities < 50%	27 (46.5)	18 (31.0)	
Ground-glass opacities ≥ 50%	24 (41.4)	31 (53.4)	.246
Not available	7 (12.1)	9 (15.5)	
Laboratory variables			
Haemoglobin, mean (SD), g/L	13.3 (2.2)	12.6 (2.1)	.114
Neutrophils count, mean (SD), x10 ³ /mm ³	6.02 (3.73)	7.55 (3.82)	.031
Lymphocyte count, mean (SD), x10 ³ /mm ³	1.19 (0.53)	1.10 (0.52)	.353
Platelet count, mean (SD), x10 ³ /mm ³	301.8 (130.7)	292.3 (141.9)	.707
Erythrocyte sedimentation rate, mean (SD), mm	62.3 (39.3)	66.4 (40.0)	.587
C-reactive protein, mean (SD), mg/L	78.5 (72.6)	81.4 (82.7)	.843
D-dimer, mean (SD), ng/mL	2,950 (6,957)	2,044 (4,541)	.412
Albumin, mean (SD), g/L	2.95 (0.49)	2.98 (0.45)	.801

Gamma globulins, mean (SD), g/L	1.15 (0.44)	1.12 (0.33)	.665
Creatinine, mean (SD), mg/dL	0.86 (0.32)	0.84 (0.27)	.769
Urea, mean (SD), mg/dL	38.7 (19.2)	38.8 (14.9)	.975
Phosphorus, mean (SD), mg/dL	2.97 (0.64)	3.09 (0.73)	.356
25-hydroxyvitamin D, mean (SD), ng/mL	12.6 (4.0)	13.9 (4.7)	.108
Parathyroid hormone, mean (SD), pg/mL	52.7 (30.0)	43.9 (24.4)	.098
Calcium, total, mean (SD), mg/dL	8.54 (0.52)	8.67 (0.52)	.170
Total cholesterol, mean (SD), mg/dL	161.3 (46.1)	169.6 (50.8)	.369
LDL-cholesterol, mean (SD), mg/dL	98.7 (36.8)	104.3 (41.6)	.453
HDL-cholesterol, mean (SD), mg/dL	35.2 (13.0)	32.8 (12.2)	.318
Triglycerides, mean (SD), mg/dL	170.3 (80)	201.0 (91.9)	.062

For continuous variables, groups were compared using independent t-test.

For categorical variables, groups were compared using χ^2 test or Fisher's exact test, as appropriate.