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Short-Term Biochemical and Inflammatory Responses to 10,000 versus 50,000 IU/day Cholecalciferol in Critically Ill Adults with Low Vitamin D Status: A Prospective Quasi-Experimental Study

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ABSTRACT: Low vitamin D status is common in critically ill patients, but short-term biochemical and inflammatory responses to different supplementation regimens remain uncertain. This prospective, non-randomized, quasi-experimental pretest-posttest study included 43 adult ICU patients with serum 25-hydroxyvitamin D [25(OH)D] concentrations <30 ng/mL. Participants were stratified by baseline vitamin D status and received cholecalciferol 10,000 or 50,000 IU/day for three consecutive days. Serum 25(OH)D, total lymphocyte count (TLC), neutrophil-to-lymphocyte ratio (NLR), C-reactive protein (CRP), white blood cell count (WBC), albumin, prognostic nutritional index (PNI), and total calcium were assessed at baseline and on day 4. Serum 25(OH)D increased significantly in all four groups, and the magnitude of change differed across groups ($p=0.002$). The largest median increase occurred in vitamin D-deficient patients receiving 50,000 IU/day; post-hoc differences were significant compared with deficient patients receiving 10,000 IU/day ($p=0.002$) and insufficient patients receiving 10,000 IU/day ($p=0.003$). CRP decreased significantly in all groups, while WBC decreased significantly in three groups, although between-group changes were not significant. TLC, NLR, albumin, PNI, and total calcium showed no significant between-group differences in change. Three-day cholecalciferol supplementation was associated with rapid increases in 25(OH)D, particularly with 50,000 IU/day in deficient patients, whereas inflammatory and nutritional-immunological responses were less consistent.

INTRODUCTION

Critical illness is accompanied by systemic inflammation, immune dysregulation, and metabolic disturbances that may alter vitamin D metabolism. Low circulating 25-hydroxyvitamin D [25(OH)D] is common in ICU populations; a multicenter study reported deficiency in 59% of critically ill patients and associations with higher SOFA scores, longer mechanical ventilation, and longer ICU stay [1]. However, the prognostic significance of low 25(OH)D remains uncertain, as a larger ICU cohort found no independent association between vitamin D deficiency and hospital mortality [2].

Beyond calcium–phosphate homeostasis, vitamin D has immunomodulatory activity through vitamin D receptors expressed on multiple immune-cell populations, influencing antimicrobial peptide production, dendritic-cell function, macrophage activity, and adaptive immune responses [3,4]. Laboratory indices may capture complementary aspects of these responses. In a cohort

of 1,106 ICU patients, higher NLR and lower PNI were independently associated with mortality, particularly among patients with low nutritional risk [5].

Interventional evidence remains heterogeneous. High-dose cholecalciferol increased 25(OH)D and was accompanied by reductions in inflammatory markers in mechanically ventilated ICU patients [6]. In an Indonesian trial of moderate COVID-19, 10,000 IU/day increased 25(OH)D, although changes in TLC and NLR were not significant [7]. Meta-analyses have reported differing effects on mortality, mechanical ventilation, and ICU stay [8–10], while recent reviews continue to identify uncertainty regarding optimal dose, timing, route, and patient selection [11,12]. However, it remains unclear whether the magnitude of the early biochemical response differs according to baseline vitamin D status and supplementation dose, and whether rapid increases in 25(OH)D are accompanied by parallel changes in inflammatory and nutritional-immunological markers.

Accordingly, this study examined short-term changes in 25(OH)D, TLC, NLR, CRP, WBC, albumin, PNI, and serum calcium after three days of vitamin D3 supplementation across groups defined by baseline vitamin D status and doses of 10,000 or 50,000 IU/day.

METHODS

Study Design and Setting

This prospective, non-randomized, quasi-experimental pretest-posttest study was conducted in the intensive care unit (ICU) of Dr. Wahidin Sudirohusodo General Hospital, Makassar, Indonesia, from April to July 2026. The study evaluated short-term changes in serum 25-hydroxyvitamin D [25(OH)D], inflammatory markers, nutritional-immunological parameters, and serum calcium following three consecutive days of vitamin D3 supplementation. No untreated or placebo control group was included.

Participants

All eligible adult patients admitted to the ICU during the study period were considered for inclusion using a total sampling approach. Patients were eligible if they were aged ≥ 18 years, were hemodynamically stable with a mean arterial pressure > 65 mmHg and peripheral oxygen saturation $\geq 95\%$, had an ICU stay of at least 5 days, and had baseline serum 25(OH)D concentrations classified according to the study protocol as vitamin D deficiency (< 20 ng/mL) or insufficiency (20–29 ng/mL).

Patients were excluded if they had a contraindication to oral or enteral administration, had received vitamin D supplementation during the preceding month, were receiving long-term corticosteroid therapy or chemotherapy, had a hematologic malignancy, were pregnant or breastfeeding, had cirrhosis or chronic hepatitis, had an estimated glomerular filtration rate ≤ 60 mL/min, had biliary disease, or had undergone gastrointestinal surgery.

Sample Size

For sample-size planning, C-reactive protein (CRP) was selected as the reference continuous inflammatory outcome because a previous study in mechanically ventilated ICU patients receiving oral cholecalciferol reported the paired change data required for calculation. In that study, oral cholecalciferol 50,000 IU/day was associated with a mean CRP change of -37.44 mg/L with a standard deviation of the change of 32.11 mg/L [6].

Using a two-sided α of 0.05, 80% statistical power, and the standardized paired effect derived from these values, the minimum requirement was 8 evaluable participants per group. Allowing for approximately 20% attrition, the recruitment target was increased to 10 participants per group, corresponding to a minimum target of 40 participants across the four study groups. All four final groups exceeded the minimum evaluable requirement of eight participants. The sample-size calculation was based on the expected within-group change in CRP and was not specifically powered for all between-group comparisons of biochemical and inflammatory outcomes.

Group Allocation and Vitamin D Supplementation

Participants were first stratified according to baseline vitamin D status as deficient or insufficient. Within each stratum, vitamin D3 dose assignment was performed by alternation between 10,000 IU/day and 50,000 IU/day according to the sequence in which baseline laboratory results became available. Thus, treatment allocation was not randomized, and no random sequence generation or allocation concealment was performed.

This procedure resulted in four study groups: vitamin D deficiency receiving 10,000 IU/day, vitamin D deficiency receiving 50,000 IU/day, vitamin D insufficiency receiving 10,000 IU/day, and vitamin D insufficiency receiving 50,000 IU/day.

Vitamin D3 was administered as cholecalciferol using 5,000-IU tablets. Participants assigned to 10,000 IU/day received two tablets, whereas those assigned to 50,000 IU/day received ten tablets. Tablets were crushed and administered orally or through an enteral route once daily for three consecutive days.

Laboratory Assessment

Blood samples were obtained before the first dose of vitamin D3 and again on day 4, approximately 24 hours after completion of the three-day supplementation regimen. Laboratory measurements included serum 25(OH)D, total serum calcium, complete blood count with differential leukocyte counts, serum albumin, and CRP.

Serum 25(OH)D was measured using a chemiluminescent microparticle immunoassay (CMIA). Baseline vitamin D deficiency was defined as serum 25(OH)D <20 ng/mL and insufficiency as 20–29 ng/mL, according to the prespecified study definitions.

Total lymphocyte count (TLC) was calculated from the total white blood cell count and lymphocyte percentage as:

$$TLC = WBC \times \frac{\text{lymphocyte percentage}}{100}$$

The neutrophil-to-lymphocyte ratio (NLR) was calculated by dividing the neutrophil count by the lymphocyte count.

The prognostic nutritional index (PNI) was calculated using the formula [13] :

$$PNI = 10 \times \text{albumin (g/dL)} + 0.005 \times \text{TLC (cells}/\mu\text{L)}$$

Serum total calcium was measured before and after supplementation as a biochemical monitoring parameter related to high-dose vitamin D administration.

Study Outcomes

The principal biochemical outcome analyzed in this study was the change in serum 25(OH)D from baseline to the day-4 post-supplementation assessment. Secondary outcomes included changes in total lymphocyte count (TLC), neutrophil-to-lymphocyte ratio (NLR), C-reactive protein (CRP), white blood cell count (WBC), serum albumin, prognostic nutritional index (PNI), and total serum calcium. For each outcome, the change score (Δ) was calculated as the post-supplementation value minus the baseline value.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Macintosh, Version 30.0 (IBM Corp., Armonk, NY, USA). Continuous variables were assessed for normality using the Shapiro-Wilk test within each of the four study groups. Normally distributed baseline variables were summarized as mean $\pm$ standard deviation (SD), whereas non-normally distributed variables were summarized as median with the first and third quartiles [median (Q1–Q3)]. Age was compared across the four study groups using one-way ANOVA, whereas sex and comorbidity were compared using the Fisher–Freeman–Halton exact test.

Baseline laboratory characteristics were compared across the four groups using one-way analysis of variance (ANOVA) for normally distributed variables and the Kruskal-Wallis test for non-normally distributed variables.

For longitudinal outcomes, the distribution of the change score (Δ) was considered when selecting the inferential test. Because evidence of non-normality was present in at least one study group for each longitudinal outcome, a consistent nonparametric approach was used. Baseline-to-post-supplementation changes within each group were evaluated using the Wilcoxon signed-rank test, and the corresponding values were reported as median (Q1–Q3).

Differences in change scores among the four study groups were evaluated using the Kruskal-Wallis test. When the omnibus comparison for $\Delta 25(\text{OH})\text{D}$ was statistically significant, pairwise post-hoc comparisons were performed using the Mann-Whitney U test. Six pairwise comparisons were possible; therefore, a Bonferroni-adjusted significance threshold of $p < 0.0083$ was applied to the post-hoc analyses. As an exploratory analysis, a two-way factorial analysis of variance was performed for $\Delta 25(\text{OH})\text{D}$ to examine supplementation dose, baseline vitamin D status, and their interaction. Because the distributional and

homogeneity assumptions were not fully satisfied, this analysis was interpreted as supportive rather than confirmatory, while the nonparametric four-group comparison remained the primary analysis.

One extreme post-supplementation NLR observation was retained in the primary analysis to avoid data-driven exclusion. A sensitivity analysis was subsequently performed after excluding this observation to determine whether it materially influenced the between-group comparison of Δ NLR.

All statistical tests were two-sided. A p value <0.05 was considered statistically significant, except for the Bonferroni-adjusted post-hoc comparisons. Graphical displays of baseline-to-post-supplementation changes were based on group medians.

Ethical Considerations

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Hasanuddin (Protocol No. UH26010078; Approval No. 240/UN4.6.4.5.31/PP36/2026). Written informed consent was obtained from all participants or their legally authorized representatives prior to enrollment.

RESULTS

Baseline demographic and selected clinical characteristics are presented in Table 1. No statistically significant differences were observed across the four study groups in age, sex distribution, or the presence of comorbidity (all $p>0.05$).

Table 1. Baseline demographic and selected clinical characteristics according to study group

Variable	Deficiency + 10,000 IU (n=11)	Deficiency + 50,000 IU (n=12)	Insufficiency + 10,000 IU (n=11)	Insufficiency + 50,000 IU (n=9)	p value
Age, years	50.55 $\pm$ 13.43	45.33 $\pm$ 20.16	55.91 $\pm$ 11.91	57.00 $\pm$ 13.86	0.277 ^a
Female sex, n (%)	9 (81.8)	8 (66.7)	6 (54.5)	6 (66.7)	0.608 ^b
Comorbidity, n (%)	3 (27.3)	2 (16.7)	4 (36.4)	3 (33.3)	0.744 ^b

Notes: Values are presented as mean $\pm$ SD or n (%), according to variable type. ^a One-way ANOVA. ^b Fisher–Freeman–Halton exact test. SD, standard deviation.

Baseline laboratory characteristics across the four study groups are presented in Table 2. No statistically significant between-group differences were observed for TLC, NLR, albumin, PNI, CRP, WBC, or calcium (all $p>0.05$). Baseline 25(OH)D differed significantly across groups ($p<0.001$), as expected because the study groups were defined in part according to baseline vitamin D status.

Table 2. Baseline laboratory characteristics according to study group

Variable	Deficiency + 10,000 IU (n=11)	Deficiency + 50,000 IU (n=12)	Insufficiency + 10,000 IU (n=11)	Insufficiency + 50,000 IU (n=9)	p value
25(OH)D, ng/mL	17.10 (13.50–18.50)	17.10 (10.10–19.00)	23.00 (22.70–25.50)	23.70 (20.65–25.60)	$<0.001^{b*}$
TLC, cells/ μ L	668.20 (555.50– 960.30)	615.60 (423.82– 983.17)	628.14 (525.00– 856.30)	1142.20 (629.50– 1627.30)	0.171 ^b
NLR	23.10 (9.70–38.50)	19.45 (12.43–30.33)	18.60 (12.20–25.40)	10.40 (5.31–25.50)	0.418 ^b
Albumin, g/dL	3.00 (2.80–3.70)	2.65 (2.43–3.28)	3.10 (2.80–4.00)	3.00 (2.80–3.20)	0.348 ^b

PNI	35.65 ± 6.83	32.30 ± 7.17	36.22 ± 8.79	37.00 ± 7.22	0.480 ^a
CRP, mg/L	218.39 ± 161.21	175.78 ± 92.82	145.01 ± 132.75	168.27 ± 137.96	0.628 ^a
WBC, cells/ μ L	16,260.91 ± 4,516.74	15,370.00 ± 5,199.72	14,357.27 ± 5,541.61	14,850.00 ± 5,601.71	0.850 ^a
Calcium, mg/dL	7.50 (7.00–8.40)	7.10 (7.00–7.45)	7.70 (7.40–8.00)	7.50 (7.30–8.30)	0.152 ^b

Notes: Values are presented as mean ± SD or median (Q1–Q3), according to distribution. ^a One-way ANOVA. ^b Kruskal–Wallis test. * $p < 0.05$. Abbreviations: 25(OH)D, 25-hydroxyvitamin D; TLC, total lymphocyte count; NLR, neutrophil-to-lymphocyte ratio; PNI, prognostic nutritional index; CRP, C-reactive protein; WBC, white blood cell count.

Serum 25(OH)D increased significantly from baseline to post-supplementation in all four study groups (all $p < 0.05$). The magnitude of change differed significantly across the four groups ($p = 0.002$). In Bonferroni-adjusted post-hoc comparisons, the increase in 25(OH)D was greater in the deficiency + 50,000 IU group than in the deficiency + 10,000 IU group ($p = 0.002$) and the insufficiency + 10,000 IU group ($p = 0.003$). No other pairwise differences remained statistically significant. In the exploratory factorial analysis, the dose $\times$ baseline vitamin D status interaction was not statistically significant ($F(1,39) = 1.701$, $p = 0.200$, partial $\eta^2 = 0.042$), indicating no statistical evidence that the dose-associated difference in $\Delta 25(\text{OH})\text{D}$ varied according to baseline vitamin D status. The dose term was significant ($F(1,39) = 27.325$, $p < 0.001$, partial $\eta^2 = 0.412$), whereas the baseline vitamin D status term was not ($F(1,39) = 0.624$, $p = 0.434$). Serum calcium did not change significantly within any group, and the magnitude of calcium change did not differ across groups ($p = 0.682$).

Table 3. Changes in serum 25(OH)D and calcium according to study group

Parameter	Group	Baseline	Post	Δ Post–baseline	Within-group p value	Between-group p value for Δ
25(OH)D, ng/mL	Deficiency + 10,000 IU (n=11)	17.10 (13.50–18.50)	21.00 (18.10–25.00)	5.20 (2.20–9.00)	0.011 ^{a*}	
	Deficiency + 50,000 IU (n=12)	17.10 (10.10–19.00)	47.65 (37.50–51.33)	31.50 (19.70–38.10)	0.003 ^{a*}	0.002^{b*}
	Insufficiency + 10,000 IU (n=11)	23.00 (22.70–25.50)	30.40 (26.00–33.00)	4.90 (0.50–9.00)	0.008 ^{a*}	
	Insufficiency + 50,000 IU (n=9)	23.70 (20.65–25.60)	46.00 (31.45–54.20)	25.70 (5.20–29.10)	0.008 ^{a*}	
Calcium, mg/dL	Deficiency + 10,000 IU (n=11)	7.50 (7.00–8.40)	7.20 (6.90–8.40)	0.00 (–0.40–0.20)	0.440 ^a	
	Deficiency + 50,000 IU (n=12)	7.10 (7.00–7.45)	7.40 (6.50–7.98)	0.30 (–0.45–0.58)	0.582 ^a	0.682^b
	Insufficiency + 10,000 IU (n=11)	7.70 (7.40–8.00)	7.70 (7.30–8.20)	0.30 (–0.70–0.90)	0.609 ^a	
	Insufficiency + 50,000 IU (n=9)	7.50 (7.30–8.30)	7.80 (7.25–8.10)	0.30 (–0.60–0.80)	0.593 ^a	

Notes: Values are presented as median (Q1–Q3). Δ was calculated as post-supplementation minus baseline. ^a Wilcoxon signed-rank test. ^b Kruskal–Wallis test. Post-hoc pairwise comparisons for $\Delta 25(\text{OH})\text{D}$ were performed using Mann–Whitney U tests with Bonferroni correction (significance threshold $p < 0.0083$); significant differences were observed for deficiency + 10,000 versus deficiency + 50,000 IU ($p = 0.002$) and deficiency + 50,000 versus insufficiency + 10,000 IU ($p = 0.003$). * $p < 0.05$.

Figure 1 illustrates the change in median serum 25(OH)D levels from baseline to post-supplementation across the four study groups. Serum 25(OH)D increased in all groups, with the largest observed median increase in the deficiency + 50,000 IU group.

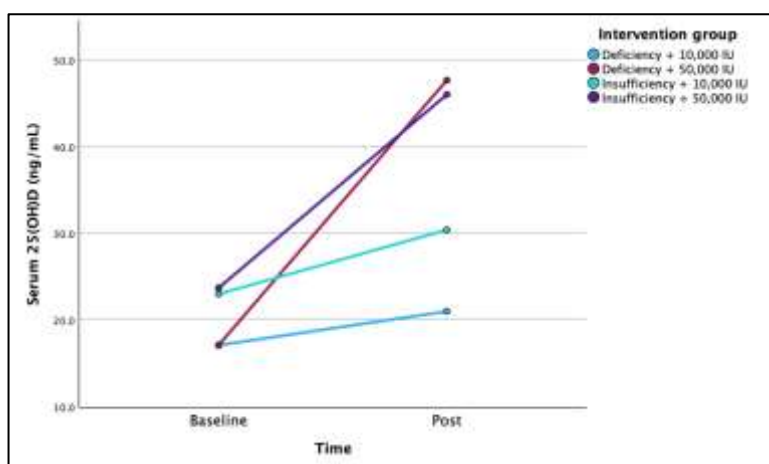


Figure 1. Changes in serum 25(OH)D levels from baseline to post-supplementation across the four study groups. Values are presented as group medians.

Changes in inflammatory markers are summarized in Table 4. TLC and NLR did not change significantly from baseline within any study group. In contrast, CRP decreased significantly in all four groups (all $p < 0.05$). WBC decreased significantly in the deficiency + 10,000 IU, deficiency + 50,000 IU, and insufficiency + 50,000 IU groups, whereas the change in the insufficiency + 10,000 IU group did not reach the predefined significance threshold ($p = 0.050$). Despite these within-group changes, the magnitude of change in TLC, NLR, CRP, and WBC did not differ significantly across the four study groups. Sensitivity analysis excluding the single extreme NLR value yielded a similar result for ΔNLR ($p = 0.664$).

Table 4. Changes in inflammatory markers according to study group

Parameter	Group	Baseline	Post	Δ Post–baseline	Within-group p value	Between-group p value for Δ
TLC, cells/μL	Deficiency + 10,000 IU (n=11)	668.20 (555.50–960.30)	694.20 (571.30–1017.80)	86.40 (–138.20–310.45)	0.477 ^a	
	Deficiency + 50,000 IU (n=12)	615.60 (423.82–983.17)	771.00 (623.80–892.25)	29.90 (–190.98–318.98)	0.638 ^a	0.993^b
	Insufficiency + 10,000 IU (n=11)	628.14 (525.00–856.30)	598.50 (343.40–1533.30)	–29.64 (–145.80–731.70)	0.959 ^a	
	Insufficiency + 50,000 IU (n=9)	1142.20 (629.50–1627.30)	1156.70 (1010.20–1292.88)	108.10 (–289.35–457.05)	0.515 ^a	

NLR	Deficiency + 10,000 IU (n=11)	23.10 (9.70–38.50)	18.90 (7.40–20.40)	–6.30 (–16.81–2.80)	0.110 ^a	
	Deficiency + 50,000 IU (n=12)	19.45 (12.43–30.33)	14.80 (7.15–20.75)	–3.62 (–14.53–3.90)	0.347 ^a	0.569^b
	Insufficiency + 10,000 IU (n=11)	18.60 (12.20–25.40)	17.70 (7.30–40.45)	–1.40 (–6.70–9.60)	0.878 ^a	
	Insufficiency + 50,000 IU (n=9)	10.40 (5.31–25.50)	12.00 (6.67–19.00)	0.27 (–10.33–8.33)	0.953 ^a	
CRP, mg/L	Deficiency + 10,000 IU (n=11)	187.40 (53.70–308.80)	133.00 (29.40–248.30)	–54.10 (–101.30 to –15.00)	0.003 ^{a*}	
	Deficiency + 50,000 IU (n=12)	140.15 (103.28–239.65)	111.90 (67.10–182.60)	–36.00 (–52.63 to –13.55)	0.002 ^{a*}	0.512^b
	Insufficiency + 10,000 IU (n=11)	116.90 (35.80–217.80)	57.30 (22.40–133.90)	–37.30 (–76.30 to –8.61)	0.003 ^{a*}	
	Insufficiency + 50,000 IU (n=9)	143.30 (58.75–230.85)	76.80 (36.55–142.35)	–70.00 (–84.55 to –22.20)	0.008 ^{a*}	
WBC, cells/μL	Deficiency + 10,000 IU (n=11)	15,520 (12,510–20,250)	12,400 (8,100–19,657)	–1,400 (–6,550 to –790)	0.003 ^{a*}	
	Deficiency + 50,000 IU (n=12)	14,790 (10,450–18,605)	9,730 (8,170–15,955)	–1,580 (–6,902.5 to –197.5)	0.002 ^{a*}	0.979^b
	Insufficiency + 10,000 IU (n=11)	15,660 (9,110–16,700)	13,300 (9,250–15,100)	–1,800 (–3,230 to –910)	0.050 ^a	
	Insufficiency + 50,000 IU (n=9)	16,350 (9,065–19,675)	10,480 (7,350–16,350)	–1,560 (–4,240 to –485)	0.008 ^{a*}	

Notes: Values are presented as median (Q1–Q3). Δ was calculated as post-supplementation minus baseline. ^a Wilcoxon signed-rank test. ^b Kruskal–Wallis test. Sensitivity analysis excluding one extreme NLR observation yielded H=1.579 and p=0.664 for the between-group comparison of Δ NLR. *p<0.05.

Figure 2 presents the changes in median inflammatory markers from baseline to post-supplementation across the four study groups. TLC and NLR showed no consistent directional change across groups, whereas CRP decreased in all four groups. WBC also generally decreased after supplementation, although the within-group change did not reach statistical significance in the insufficiency + 10,000 IU group. Despite these within-group patterns, the magnitude of change in TLC, NLR, CRP, and WBC did not differ significantly across the four groups.

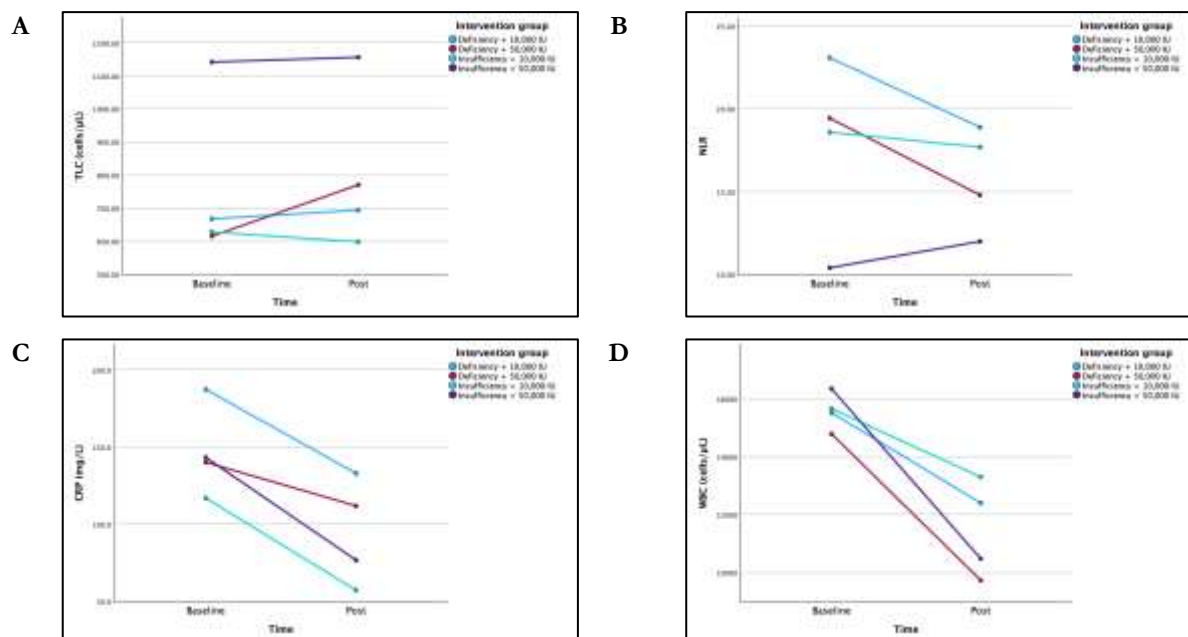


Figure 2. Changes in inflammatory markers from baseline to post-supplementation across the four study groups. (A) Total lymphocyte count (TLC), (B) neutrophil-to-lymphocyte ratio (NLR), (C) C-reactive protein (CRP), and (D) white blood cell count (WBC). Values are presented as group medians.

Neither albumin nor PNI showed a statistically significant within-group change from baseline to post-supplementation in any of the four study groups (all $p > 0.05$). Likewise, the magnitude of change did not differ significantly across groups for either albumin ($p = 0.775$) or PNI ($p = 0.941$).

Table 5. Changes in albumin and prognostic nutritional index according to study group

Parameter	Group	Baseline	Post	Δ Post–baseline	Within-group p value	Between-group p value for Δ
Albumin, g/dL	Deficiency + 10,000 IU (n=11)	3.00 (2.80–3.70)	2.80 (2.40–3.50)	–0.20 (–0.30–0.10)	0.152 ^a	0.775^b
	Deficiency + 50,000 IU (n=12)	2.65 (2.43–3.28)	2.75 (2.30–3.05)	–0.10 (–0.45–0.28)	0.449 ^a	
	Insufficiency + 10,000 IU (n=11)	3.10 (2.80–4.00)	3.10 (2.60–3.30)	0.00 (–0.70–0.30)	0.483 ^a	
	Insufficiency + 50,000 IU (n=9)	3.00 (2.80–3.20)	2.90 (2.47–3.05)	–0.37 (–0.75–0.20)	0.123 ^a	
PNI	Deficiency + 10,000 IU (n=11)	35.33 (31.06–40.80)	33.09 (28.98–38.88)	0.55 (–4.86–1.78)	0.657 ^a	0.941^b
	Deficiency + 50,000 IU (n=12)	30.59 (26.91–36.49)	30.85 (26.25–34.00)	–0.64 (–6.05–3.47)	0.638 ^a	

	Insufficiency + 10,000 IU (n=11)	35.13 (30.82– 42.72)	33.67 (26.37– 41.89)	0.00 (–8.08– 7.49)	0.721 ^a	
	Insufficiency + 50,000 IU (n=9)	34.62 (32.32– 42.67)	34.51 (29.70– 37.55)	–1.55 (–11.12– 4.43)	0.314 ^a	

Notes: Values are presented as median (Q1–Q3). Δ was calculated as post-supplementation minus baseline. ^a Wilcoxon signed-rank test. ^b Kruskal–Wallis test. * $p < 0.05$.

DISCUSSION

The most prominent finding of this study was the rapid biochemical response of circulating 25(OH)D to short-term vitamin D3 supplementation. Serum 25(OH)D increased significantly in all four groups after three days, with the largest median increase observed in patients with vitamin D deficiency receiving 50,000 IU/day. After Bonferroni correction, the increase in this group remained greater than that in the deficiency + 10,000 IU/day and insufficiency + 10,000 IU/day groups. Importantly, the comparison with the insufficiency group reflects differences in both dose and baseline vitamin D status and therefore cannot be interpreted as an isolated dose effect. Within the deficiency stratum, the 50,000-IU/day regimen was associated with a greater short-term increase in 25(OH)D than the 10,000-IU/day regimen. However, the exploratory dose $\times$ baseline vitamin D status interaction was not statistically significant, providing no evidence that the dose-associated difference in Δ 25(OH)D varied according to baseline vitamin D status. Meta-analytic evidence similarly shows that vitamin D supplementation increases circulating 25(OH)D in critically ill adults, although treatment responses vary across regimens and populations [8,10]. In mechanically ventilated patients with vitamin D-deficient sepsis, Ashoor et al. (2024) further demonstrated differences between high- and low-dose regimens in day-7 changes in procalcitonin, LL-37, and SOFA score [14], suggesting that downstream biological responses may also depend on the supplementation strategy.

The inflammatory response was less uniform. Although CRP decreased significantly within all four groups, the magnitude of CRP reduction did not differ significantly between the study groups. Thus, the significant within-group reductions should not be interpreted as evidence of a differential supplementation effect. In the absence of an untreated control group, these reductions may reflect the evolving course of critical illness and concurrent ICU treatments, in addition to any potential contribution of vitamin D3 supplementation. Hasanloei et al. (2020) similarly reported reductions in CRP and other inflammatory biomarkers after high-dose vitamin D in mechanically ventilated trauma patients [6]. In a placebo-controlled ICU trial of severe COVID-19, Bychinin et al. (2022) found a significant within-group reduction in CRP together with increases in 25(OH)D and lymphocyte-related immune responses; however, the between-group CRP difference on day 7 was not significant [15]. Conversely, Ingels et al. (2020) found that intravenous 25OHD increased circulating 25OHD in prolonged critical illness without detectable effects on innate immunity [16], illustrating that biochemical correction does not necessarily translate into parallel inflammatory changes.

A biological link between vitamin D status and inflammation remains plausible. Vitamin D receptor signaling influences innate and adaptive immune pathways, including cytokine regulation and antimicrobial responses [3]. Zhou and Hyppönen (2023) further identified a nonlinear inverse association between genetically predicted 25(OH)D and CRP, concentrated within the vitamin D-deficient range [17]. Their findings are relevant mechanistically but concern chronic low-grade inflammation in a population cohort rather than acute critical illness and therefore should not be directly extrapolated to ICU inflammatory dynamics.

WBC decreased significantly within three study groups; however, the magnitude of WBC change did not differ significantly between groups. TLC and NLR showed neither significant short-term within-group changes nor significant between-group differences in change. These divergent patterns are not necessarily contradictory because leukocyte populations and acute-phase proteins may respond over different time courses. An Indonesian randomized trial of 10,000 IU/day in moderate COVID-19 likewise produced a substantial rise in 25(OH)D without significant changes in TLC or NLR [7]. Ashoor et al. (2024) detected differences in procalcitonin and LL-37 primarily at day 7 [14], suggesting that some immune responses may require a longer observation period than the day-4 assessment used in the present analysis. The NLR sensitivity analysis also yielded a similar

between-group result after removal of the extreme observation, indicating that the primary conclusion was not dependent on that single value.

Albumin and PNI remained unchanged across all groups despite the rapid increase in 25(OH)D. This is biologically reasonable in acute critical illness. Serum albumin is strongly affected by inflammation, reprioritization of hepatic protein synthesis, increased capillary permeability, redistribution, and catabolism and should not be interpreted as a direct marker of short-term nutritional response [18]. Because PNI incorporates both albumin and TLC, limited changes in these components also constrain short-term changes in PNI. Nevertheless, PNI retains prognostic value: Wullur et al. (2024) found lower PNI to be independently associated with mortality in an ICU cohort [5], while a meta-analysis of 21,239 critically ill patients found moderate predictive performance of PNI for acute kidney injury [13]. Thus, prognostic utility does not imply responsiveness to a three-day supplementation period.

Total serum calcium did not change significantly within or between groups. This finding is reassuring for the short observation interval but should not be interpreted as establishing treatment safety. Holmes et al. (2020) reported hypercalcemia, defined using ionized calcium, in 40% of vitamin D-deficient trauma ICU patients receiving 10,000 IU/day for two weeks despite the absence of hypervitaminosis D [19]. In contrast, Bychinin et al. (2022) observed no hypercalcemia during a shorter ICU vitamin D3 trial [15]. Differences in treatment duration, dose, population, and calcium measurement limit direct comparison. The present study measured total rather than ionized or albumin-corrected calcium; therefore, these data are best interpreted as short-term biochemical monitoring rather than a comprehensive safety assessment.

Taken together, the findings reflect a broader pattern in the literature: increasing circulating 25(OH)D after supplementation is relatively reproducible, whereas inflammatory and clinical responses are more heterogeneous. Menger et al. (2022) reported reductions in mortality, ICU stay, and mechanical ventilation in pooled RCTs [8], whereas Kaur et al. (2022) found no significant benefit for these outcomes [9]. The more recent meta-analysis by Zheng et al. (2025) again suggested benefits for several clinical outcomes but noted substantial heterogeneity and insufficient information for firm conclusions [10]. Recent reviews similarly emphasize the relevance of baseline vitamin D status, illness severity, dose, route, and patient selection when interpreting supplementation studies [12,20].

The present study therefore contributes short-term, dose-stratified data from an Indonesian ICU population while examining biochemical, inflammatory, and nutritional-immunological markers concurrently. Interpretation should account for the non-randomized dose allocation, absence of an untreated control group, modest group sizes, heterogeneous critical illnesses, concurrent ICU treatments, and the short follow-up interval. Although age, sex, and comorbidity did not differ significantly across the study groups, residual differences in baseline clinical characteristics and acute illness severity may still have influenced the observed biochemical and inflammatory responses. The evolving clinical course and concomitant ICU therapies could also independently affect CRP, WBC, albumin, PNI, and other laboratory markers. Consequently, significant within-group changes should not be interpreted as evidence of differential responses between supplementation groups when corresponding between-group differences in change were not observed. The data most clearly demonstrate a rapid increase in 25(OH)D, with the largest increase in deficient patients receiving 50,000 IU/day, while inflammatory responses were selective and albumin and PNI remained relatively stable. Longer randomized studies with serial biomarker and clinical assessments are needed to determine whether these early biochemical changes are followed by sustained immunological or clinical benefit.

CONCLUSION

Three days of vitamin D3 supplementation were followed by a significant increase in serum 25-hydroxyvitamin D [25(OH)D] across all study groups, with the largest median increase observed in vitamin D-deficient patients receiving 50,000 IU/day. CRP decreased significantly within all groups and WBC decreased within three groups; however, the magnitude of change in these inflammatory markers did not differ significantly between study groups. TLC, NLR, albumin, PNI, and total serum calcium likewise showed no significant between-group differences in change. These findings indicate that short-term vitamin D3 supplementation was associated with a rapid increase in circulating 25(OH)D, whereas inflammatory and nutritional-immunological responses were less consistent. Given the non-randomized allocation, absence of an untreated control group, potential influence of baseline clinical differences and acute illness severity, concurrent ICU treatments, and the short follow-up period, the observed within-group inflammatory changes cannot be attributed specifically to vitamin D3 supplementation.

Larger randomized studies with longer follow-up and serial biomarker assessments are warranted to determine whether early biochemical responses translate into sustained immunological or clinical benefits.

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Author Contributions

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Agussalim Bukhari: Conceptualization, Methodology, Supervision, Validation, Interpretation of Findings, and Writing – Review & Editing.

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All authors critically reviewed the manuscript, approved the final version, and agreed to be accountable for the accuracy and integrity of the work.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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Ethics Statement

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Hasanuddin (Protocol No. UH26010078; Approval No. 240/UN4.6.4.5.31/PP36/2026). Written informed consent was obtained from all participants or their legally authorized representatives prior to enrollment.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request, subject to applicable ethical, privacy, and institutional restrictions.

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