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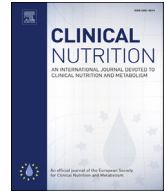
Covid-19

Vitamin D deficiency in critically ill COVID-19 ARDS patients

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SUMMARY

Background & aims: Vitamin D's pleiotropic effects include immune modulation, and its supplementation has been shown to prevent respiratory tract infections. The effectivity of vitamin D as a therapeutic intervention in critical illness remains less defined. The current study analyzed clinical and immunologic effects of vitamin D levels in patients suffering from coronavirus disease 2019 (COVID-19) induced acute respiratory distress syndrome (ARDS).

Methods: This was a single-center retrospective study in patients receiving intensive care with a confirmed SARS-CoV-2 infection and COVID-19 ARDS. 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D serum levels, pro- and anti-inflammatory cytokines and immune cell subsets were measured on admission as well as after 10−15 days. Clinical parameters were extracted from the patient data management system. Standard operating procedures included the daily administration of vitamin D_3 via enteral feeding.

Results: A total of 39 patients with COVID-19 ARDS were eligible, of which 26 were included in this study as data on vitamin D status was available. 96% suffered from severe COVID-19 ARDS. All patients without prior vitamin D supplementation (n = 22) had deficient serum levels of 25-hydroxyvitamin D. Vitamin D supplementation resulted in higher serum levels of 25-hydroxyvitamin D but not did not increase 1,25-dihydroxyvitamin D levels after 10−15 days. Clinical parameters did not differ between patients with sufficient or deficient levels of 25-hydroxyvitamin D. Only circulating plasmablasts were higher in patients with 25-hydroxyvitamin D levels ≥30 ng/ml (p = 0.029). Patients with 1,25-dihydroxyvitamin D levels below 20 pg/ml required longer mechanical ventilation (p = 0.045) and had a worse acute physiology and chronic health evaluation (APACHE) II score (p = 0.048).

Conclusion: The vast majority of COVID-19 ARDS patients had vitamin D deficiency. 25-hydroxyvitamin D status was not related to changes in clinical course, whereas low levels of 1,25-dihydroxyvitamin D were associated with prolonged mechanical ventilation and a worse APACHE II score.

1. Introduction

Vitamin D exerts pleiotropic effects with actions far beyond its classic role in mineral homeostasis. Tissue actions require two enzymatic conversions to 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D after which vitamin D has been shown to modulate the immune response amongst others. Vitamin D deficiency is highly prevalent across all age groups and countries [1], a fact of particular interest regarding respiratory infections. Vitamin D supplementation reduces the risk of acute respiratory tract infections [2], even though effects onto innate immunity after exposure to respiratory syncytial virus (RSV) are limited and its usage at various concentrations inhibits neither pro- nor anti-inflammatory responses [3,4]. A cross-sectional study in the United Kingdom (UK) showed a linear relationship between adequate vitamin D levels and reduced risk of respiratory infections, including improved pulmonary function [5]. Although vitamin D deficiency was further determined to be associated with greater illness severity, a causal relationship between vitamin D

deficiency and multiple organ dysfunction has not been established [6]. Thus, the efficacy of vitamin D as a therapeutic in critically ill patients remains controversial [7].

The current study analyzed vitamin D deficiency, clinical and immunologic effects of vitamin D supplementation in patients infected with severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), subsequently suffering from a critical coronavirus disease 2019 (COVID-19) induced acute respiratory distress syndrome (ARDS).

2. Patients, materials & methods

This is a single-center, retrospective study adhering to the STROBE-Guidelines [8]. The institutional board of the University of Wuerzburg waived the need for ethic approval (63/20-kr, 25.03.2020 and 20200528 01, 05.06.2020) due to sole chart review. Informed consent was not necessary according to local legislation (Bayerisches Krankenhausgesetz, Art. 24, Abs 4).

Patients receiving intensive care between March 14th and May 28th, 2020 at the University hospital Wuerzburg with a confirmed SARS-CoV-2 infection [9] and COVID-19 induced ARDS were screened for study eligibility. At the University hospital Wuerzburg, in-house procedures recommend, but do not oblige, screening of vitamin D status in all intensive care patients independent of clinical risk factors or radiographic findings of vitamin D deficiency. In order to be included in the analysis, 25-hydroxyvitamin D status on admission had to be available. Patients were excluded from the study in case vitamin D status was not available on admission as the single exclusion criterion. ARDS was classified according to the Berlin definition [10]. Prior medical history was evaluated based on written records and clinical data were collected via retrospective chart review using a patient data management system (COPRA6 RM1.0, COPRA System GmbH, Berlin, Germany). COVID-19 intensive care unit (ICU) standard operating procedures at the University hospital Wuerzburg included the administration of 200.000 IU vitamin D_3 (Vigantol®, Merck Selbstmedikation GmbH, Darmstadt, Germany) as a loading dose and 10.000 IU daily via enteral feeding. Both 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D serum levels were measured on admission as well as after 10−15 days of ICU care. Serum probes for 25-hydroxyvitamin D were analyzed at the laboratory of the University hospital Wuerzburg using the automated chemiluminescence IDS-iSYS 25 VitD assay (Immunodiagnostic Systems, Tyne & Wear, UK) according to manufacturer's instructions, whereas samples for 1,25-dihydroxyvitamin D (calcitriol) and cytokine levels were deep frozen (−80 °C) and analyzed by an external diagnostics provider (Ganzimmun Diagnostics AG, Mainz, Germany). An automated Liaison XL 1,25-dihydroxyvitamin D immunoassay (DiaSorin, Saluggia, Italy) and the BD CBA Human Inflammatory Cytokines Kit (BD Biosciences, San Jose, USA) were used. 25-hydroxyvitamin D levels were classified according to Holick [11]: Levels below 30 ng/ml were considered insufficient, levels below 20 ng/ml deficient. Severe vitamin D deficiency was defined as 25-hydroxyvitamin D below 12 ng/ml. For 1,25-dihydroxyvitamin D, levels below <20 pg/ml were considered deficient, in line with the reference range of the local laboratory. Lymphocyte subsets were analyzed with a Navios cytometer (Beckman Coulter, Krefeld, Germany) with a minimum of 3.000 events within each lymphocyte gate. The following anti-human antibodies were used: anti-CD45-Krome-Orange, anti-CD14-APCA700, anti-CD3-FITC, anti-CD4-APC, anti-CD8-ECD, anti-CD56/CD16-APC A750, anti-CD19-PC7, anti-CD38-PC5.5, anti-CD27-ECD, anti-CD20-APC750 (each Beckman Coulter, Krefeld, Germany) and anti-IgD-FITC (BD Biosciences, San Jose, USA). Reference values are based on previous publications [12,13].

2.1. Statistical analysis

Median and interquartile range (IQR, 25−75%) were calculated for all variables, as normality of the data could not be assumed. Longitudinal changes were evaluated with Wilcoxon's paired test. Mann−Whitney's rank-sum test was used for numeric variables and Fisher's exact test was applied for categorial data. Associations between different variables were correlated according to Spearman. Differences were considered significant with p < 0.05. Data analysis was conducted with Microsoft Office® 365 ProPlus (Microsoft™, Redmond, USA) and GraphPad Prism® Version 8.4.2 (GraphPad Software™, San Diego, USA).

3. Results

A total of 39 patients were eligible for retrospective analysis, of which 26 were included in this study (Fig. 1). Median age was 59.5 (51−69) years, 65% were male, 35% female. A total of 96% had severe COVID-19 induced ARDS, 62% required treatment with venovenous extracorporeal membrane oxygenation (vvECMO) (Table 1).

Four patients (15%) were taking vitamin D as a home medication and demonstrated sufficient levels of 25-hydroxyvitamin D on ICU admission (Table 2). All patients without prior vitamin D supplementation had levels below 30 ng/ml, whereas eight patients were severely deficient with levels below 12 ng/ml (31%) (Fig. 2). In comparison, 1,25-dihydroxyvitamin D had a median value of 27.5 pg/ml (19−35) on admission and was not below reference range.

After vitamin D supplementation 25-hydroxyvitamin D levels significantly increased (p = 0.002). However, median levels were still insufficient (Fig. 3A). Vitamin D supplementation did not lead to an overall increase in 1,25-dihydroxyvitamin D after 10−15 days (Fig. 3B).

After 10−15 days, clinical parameters did not differ between patients with sufficient and insufficient or deficient levels of 25-hydroxyvitamin D levels, respectively (Table 3).

Patients with 1,25-dihydroxyvitamin D below 20 pg/ml tended towards worse PaO_2/FiO_2 ratios, requiring significantly longer mechanical ventilation with higher acute physiology and chronic health evaluation (APACHE) II scores (Table 3). There was a correlation between 1,25-dihydroxyvitamin D levels after 10−15 days and the minimal recorded PaO_2/FiO_2 ratios ($r_s = 0.599$, p = 0.007) of the patients. Also 1,25-dihydroxyvitamin D levels and the duration of mechanical ventilation ($r_s = -0.641$, p = 0.003) as well as duration of ICU treatment ($r_s = -0.509$, p = 0.026) correlated inversely. There was no association between both forms of vitamin D, age, body mass index and parameters of inflammation.

Levels of immune cells and pro- and anti-inflammatory cytokines did not differ as a function of vitamin D levels with the only exception being circulating plasmablasts (Fig. 4). Circulating plasmablasts were significantly higher in patients with 25-hydroxyvitamin D levels ≥30 ng/ml (p = 0.029).

4. Discussion

Vitamin D deficiency has been linked to a high prevalence of viral infections [14−17], whereas specific data on COVID-19 are scarce. A large prospective UK Biobank population-based cohort study did not report alterations in vitamin D status as a modifiable risk factor of COVID-19 [18]. Furthermore, a novel study of 1.326 COVID-19 cases found no significant association between season-adjusted 25-hydroxyvitamin D status and COVID-19 positivity in multivariate logistic regression models incorporating sex, age and ethnicity [19]. Nevertheless, vitamin D deficiency is known to be present in 40−70% of critically ill patients. In our study 85% of life-
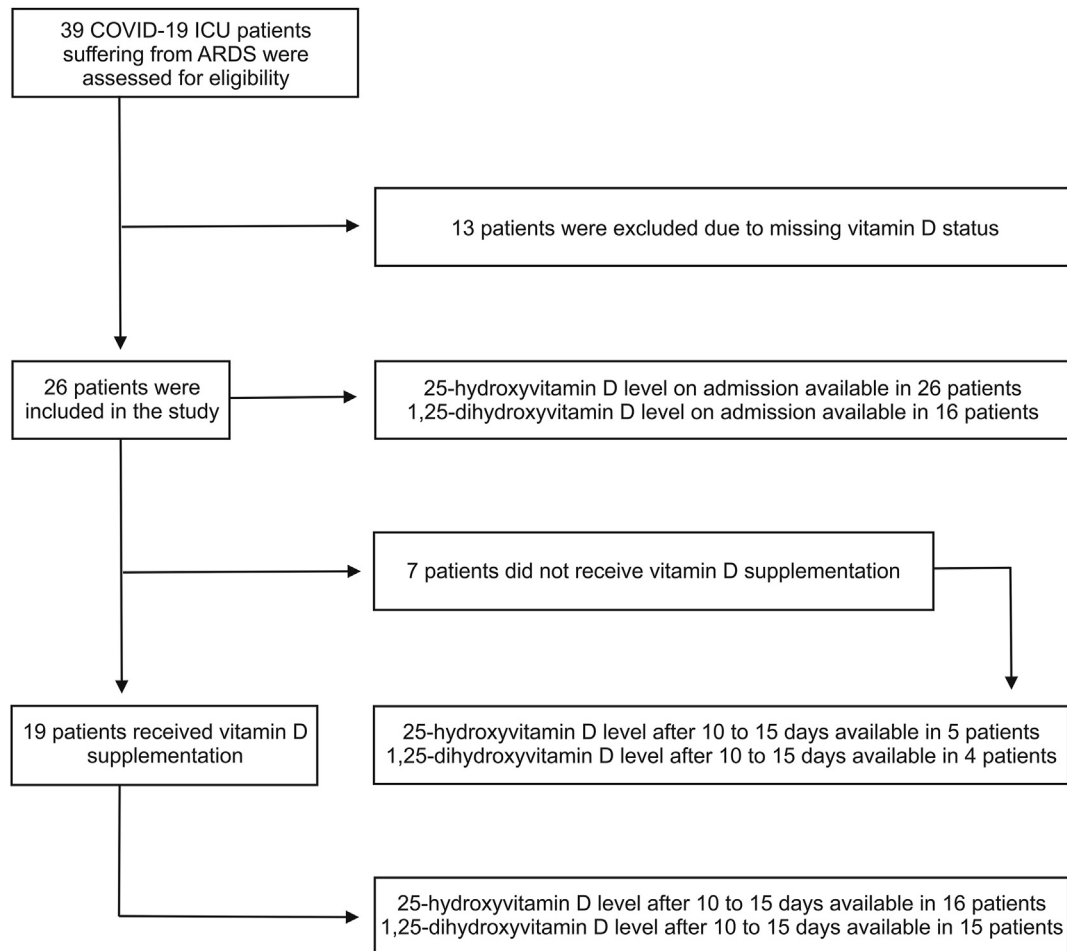
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│ 39 COVID-19 ICU patients│
│ suffering from ARDS were│
│ assessed for eligibility│
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            │
            │──────────────────►┌──────────────────────────────────────────────────┐
            │                   │ 13 patients were excluded due to missing vitamin D │
            │                   │ status                                             │
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┌─────────────────────────┐    ┌──────────────────────────────────────────────────────────────┐
│ 26 patients were        │───►│ 25-hydroxyvitamin D level on admission available in 26 patients│
│ included in the study   │    │ 1,25-dihydroxyvitamin D level on admission available in 16 patients│
└─────────────────────────┘    └──────────────────────────────────────────────────────────────┘
            │
            │──────────────────►┌──────────────────────────────────────────────────┐
            │                   │ 7 patients did not receive vitamin D supplementation│
            │                   └──────────────────────────────────────────────────┘
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┌─────────────────────────┐    ┌──────────────────────────────────────────────────────────────────┐
│ 19 patients received    │───►│ 25-hydroxyvitamin D level after 10 to 15 days available in 5 patients│
│ vitamin D supplementation│   │ 1,25-dihydroxyvitamin D level after 10 to 15 days available in 4 patients│
└─────────────────────────┘    └──────────────────────────────────────────────────────────────────┘
            │
            │──────────────────►┌──────────────────────────────────────────────────────────────────┐
                                │ 25-hydroxyvitamin D level after 10 to 15 days available in 16 patients│
                                │ 1,25-dihydroxyvitamin D level after 10 to 15 days available in 15 patients│
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Fig. 1. Flow diagram of retrospective study inclusion, availability of vitamin D levels and status regarding vitamin D supplementation.

Table 1
Demographics and course of intensive care.

	n = 26
Female, No. patients (%)	9 (35)
Male, No. patients (%)	17 (65)
Age, years (median, IQR)	59.5 (51−69)
Transfer from regional hospital on mechanical ventilation, No. patients (%)	24 (92)
Sequential organ failure assessment score, admission (median, IQR)	15 (13−16)
Acute physiology and chronic health evaluation score, admission (median, IQR)	32.5 (25−37)
Minimal PaO_2/FiO_2, mmHg (median, IQR)	64.5 (53−72)
Severe acute respiratory distress syndrome, No. patients (%)	25 (96)
Veno-venous extracorporeal membrane oxygenation, No. patients (%)	16 (62)
Renal replacement therapy, No. patients (%)	19 (73)
Duration of intensive care, days (median, IQR)	24.5 (14−41)
Survival upon discharge from intensive care unit, No. patients (%)	18 (69)
Comorbidities	
Charlson comorbidity index (median, IQR)	2 (2−4)
Body mass index, kg/m^2 (median, IQR)	29.1 (26−32)
<30 kg/m^2, No. patients (%)	16 (61)
30 to < 35 kg/m^2, No. patients (%)	9 (35)
35 to < 40 kg/m^2, No. patients (%)	0 (0)
≥40 kg/m^2, No. patients (%)	1 (4)
Respiratory comorbidity, No. patients (%)	7 (27)
Diabetes mellitus type II, No. patients (%)	5 (19)
Coronary artery disease, No. patients (%)	3 (12)
Chronic renal insufficiency, No. patients (%)	2 (8)

IQR, Interquartile range; No., Number of.

Table 2
Vitamin D levels in n = 26 patients.

	admission	10−15 days
25-hydroxyvitamin D, ng/ml (median, IQR)	16.1 (11−25)	26.2 (19−32)
No. patients with 25-hydroxyvitamin D ≥ 30 ng/ml (%)	4* (15)	7 (27)
No. patients with 25-hydroxyvitamin D between 20 and 29.9 ng/ml (%)	5 (19)	8 (31)
No. patients with 25-hydroxyvitamin D between 12 and 19.9 ng/ml (%)	9 (35)	5 (19)
No. patients with 25-hydroxyvitamin D < 12 ng/ml (%)	8 (31)	1 (4)
25-hydroxyvitamin D levels not available, No. patients (%)	0 (0)	5 (19)
1,25-dihydroxyvitamin D, pg/ml (median, IQR)	27.5 (19−35)	21.1 (10−27)
No. patients with 1,25-dihydroxyvitamin D ≥ 20 pg/ml (%)	11 (42)	10 (39)
No. patients with 1,25-dihydroxyvitamin D < 20 pg/ml (%)	5 (19)	9 (35)
1,25-dihydroxyvitamin D levels not available, No. patients (%)	10 (39)	7 (27)

IQR, Interquartile range; No., Number of. *All of these patients were taking vitamin D as a home medication prior to their COVID-19 infection.
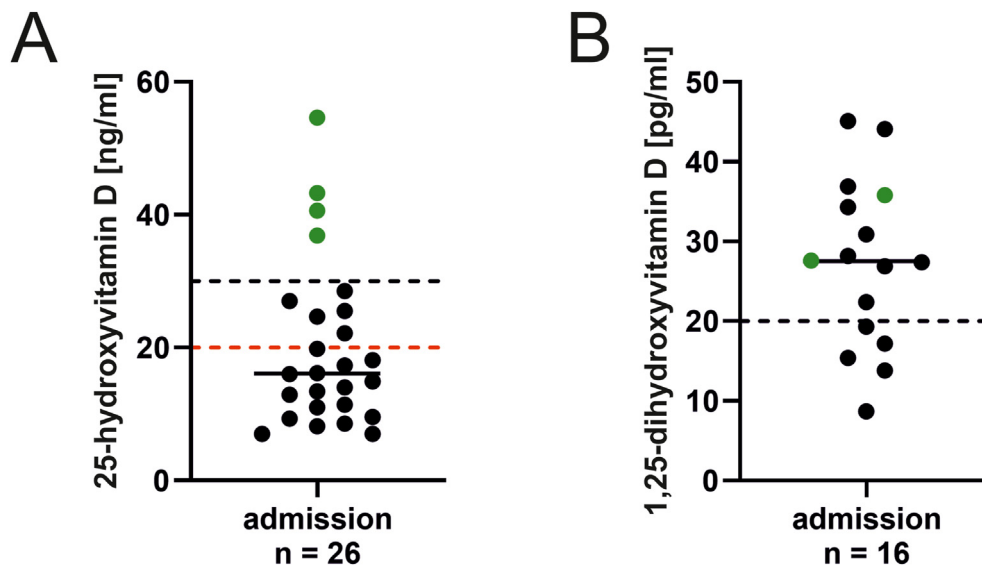

Fig. 2. A) 25-hydroxyvitamin D values below the black dashed line were insufficient, values below the red dashed line were deficient. Only four intensive care unit patients had sufficient levels of 25-hydroxyvitamin D on admission. All of these were pretreated with vitamin D (green dots). **B)** Eleven patients had sufficient levels of 1,25-dihydroxyvitamin D (above black dashed line), including two patients with vitamin D in their home medication (green dots). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
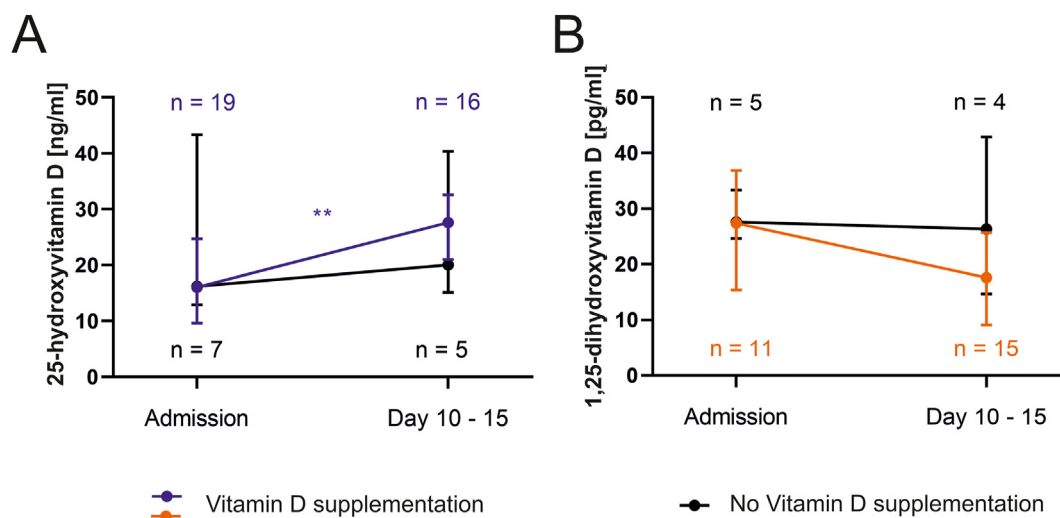

Fig. 3. 25-hydroxyvitamin D levels increased over the course of the intensive care unit stay **(A)**, whereas 1,25-dihydroxyvitamin D levels non-significantly decreased **(B)**. Vitamin D levels of patients without vitamin D supplementation were unaltered.

Table 3
Comparison of clinical parameters between patients with sufficient and deficient vitamin D levels.

	25-hydroxyvitamin D after 10–15 days			1,25-dihydroxyvitamin D after 10–15 days		
	≥30 ng/ml	<30 ng/ml	p	≥20 pg/ml	<20 pg/ml	p
	n = 7	n = 14		n = 10	n = 9	
Clinical characteristics on admission to the intensive care unit (median, IQR)						
SOFA score	16 (13–16)	14.5 (13–16)	0.490	15 (13–16)	15 (13–17)	0.728
APACHE II score	33 (24–37)	29.5 (25–37)	0.812	32.5 (30–37)	25 (23–36)	0.117
Creatinine, mg/dl	1.3 (0.7–2.2)	1.1 (0.8–1.7)	0.971	1.1 (0.7–2.3)	0.9 (0.8–1.4)	0.720
Calcium, mmol/l	1.1 (1.0–1.2)	1.2 (1.1–1.2)	0.133	1.2 (1.1–1.2)	1.2 (1.2–1.3)	0.081
Interleukin-6, pg/ml	279 (131–666)	508 (142–1465)	0.585	245 (83–1888)	666 (344–1580)	0.182
Lymphocytes, x1000/μl	0.9 (0.8–1.5)	0.8 (0.6–1.2)	0.382	0.9 (0.6–1.8)	0.8 (0.5–1.1)	0.546
Clinical characteristics on day 10–15 of intensive care (median, IQR)						
SOFA score	15 (10–18)	14 (10–18)	0.711	13 (8–16)	16 (12–18)	0.117
APACHE II score	36 (32–37)	35 (26–38)	0.407	32 (23–37)	37 (34–45)	**0.048**
Creatinine, mg/dl	1.7 (0.9–2.1)	1 (0.6–1.7)	0.361	1 (0.7–1.8)	1.1 (0.9–2.1)	0.560
Calcium, mmol/l	1.1 (1.1–1.2)	1.2 (1.1–1.2)	0.899	1.1 (1.1–1.2)	1.1 (1.1–1.3)	0.530
Interleukin-6, pg/ml	74 (56–145)	109 (36–328)	0.757	74 (52–220)	145 (52–437)	0.340
Lymphocytes, x1000/μl	1.8 (1.2–2.5)	1.4 (0.7–1.8)	0.225	1.6 (0.9–2)	1.4 (1.1–1.8)	0.556
Characteristics, therapy and outcome						
Age, years (median, IQR)	63 (51–70)	63 (49–69)	0.571	62 (50–70)	55 (49–66)	0.509
Body mass index, kg/m^2 (median, IQR)	31 (24–35)	29 (25–31)	0.597	28 (24–31)	29 (28–36)	0.203
Duration of intensive care, days (median, IQR)	41 (21–43)	21.5 (13–40)	0.231	25 (20–40)	42 (19–45)	0.234
Mechanical ventilation, days (median, IQR)	29 (18–36)	21.5 (14–33)	0.278	19 (15–28)	34 (19–39)	**0.045**
Minimal PaO$_2$/FiO$_2$ (median, IQR)	66 (58–68)	65 (51–81)	0.596	68 (59–77)	58 (51–64)	0.074
vvECMO, No. patients (%)	5 (71)	9 (64)	0.999	5 (50)	8 (89)	0.141
Renal replacement therapy, No. patient (%)	6 (86)	9 (64)	0.613	7 (70)	8 (89)	0.582
Survival, No. patients (%)	5 (71)	10 (71)	0.999	8 (80)	6 (67)	0.629

APACHE, acute physiology and chronic health evaluation; IQR, Interquartile range; No., Number of.; SOFA, sequential organ failure assessment; vvECMO, veno-venous extracorporeal membrane oxygenation.

threatening COVID-19 ARDS patients were deficient for 25-hydroxyvitamin D. All patients not receiving vitamin D in their home medication suffered from levels <30 ng/ml. These findings indicate a remarkably high prevalence of vitamin D deficiency in critically ill COVID-19 patients. Only a minority were deficient for 1,25-dihydroxyvitamin D, whereas studies suggest that vitamin D deficiency favors the production of 1,25-dihydroxyvitamin D over 24,25-dihydroxyvitamin D to maintain calcium homeostasis as long as possible [20]. 1,25-dihydroxyvitamin D does not reflect body vitamin D stores [21] but is the biologically active metabolite mediating most of vitamin D's endocrine effects. This includes cardiovascular and immune-modulatory functions [22] as well as antiviral activity modulated by cathelicidin and innate interferon pathways in bronchial epithelial cells [23]. Calcitriol has therefore become an interesting agent in supplementation trials [24].

In our study, 1,25-dihydroxyvitamin D levels ≥20 pg/ml after 10–15 days of intensive care were associated with lower APACHE II scores and a significantly lower number of days on mechanical ventilation. Although the study was not powered to detect survival differences, both parameters reflect lower disease severity and faster pulmonary recovery. This is in line with a trial in cardiac surgery showing risk reductions for organ dysfunction and mortality for every pg/ml increment in 1,25-dihydroxyvitamin D [25]. In addition, 1,25-dihydroxyvitamin D is known to shift proinflammatory Th1 and Th17 responses towards anti-inflammatory Th2 responses [17]. Interestingly, none of the vitamin D forms altered cytokine production in our COVID-19 patients. Only circulating plasmablasts were higher in patients with sufficient 25-hydroxyvitamin D levels. Plasmablasts have been implicated in the establishment of immune memory as well as the buildup of specific antibody titers [26] and it can be hypothesized that vitamin D levels might affect immune memory against SARS-CoV-2. Nevertheless, the relevance of this single cell type alteration is questionable as we did not observe any changes in clinical parameters and others have suggested that 25-hydroxyvitamin D levels might just mirror the severity of illness as a negative acute phase reactant [27]. Moreover, there is no

conclusive evidence that vitamin D supplementation improves the outcome of critically ill patients. In our patients, vitamin D supplementation only increased 25-hydroxyvitamin D levels, which were not related to alterations in clinical parameters. As serum levels of 1,25-hydroxyvitamin D were not affected, the current vitamin D regimen was likely inefficacious and our results independent of vitamin D supplementation. A single-center retrospective observational study showed that vitamin D deficiency was associated with increased ICU length of stay without impacting mortality [28]. In the subsequent randomized, single-center VITdAL-ICU trial high-dose vitamin D did not reduce hospital length of stay, ICU length of stay, hospital mortality or 6-month mortality [29]. These results were confirmed by the multicenter VIOLET trial in 1.078 ICU patients with 25-hydroxyvitamin D levels <20 ng/ml on admission. No significant differences with respect to 90-day mortality, clinical or safety endpoints were found [30]. However, a secondary analysis of the VITdAL-ICU trial suggested that patients staying more than seven days on the ICU had a mortality benefit from vitamin D supplementation [31]. Further trials are ongoing and results of the VITD-ALIZE study (ClinicalTrials.gov Identifier: NCT03188796) enrolling 2.400 ICU patients are expected in 2021.

Strengths of this report include the investigation of a clearly defined patient population suffering from life-threatening COVID-19 induced ARDS, monitoring of both 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D serum levels to distinguish the importance of both isoforms on intensive care in COVID-19 induced ARDS, as well as mechanistic insights into the impact of vitamin D levels onto pro- and anti-inflammatory immune responses. Limitations of our study include the low number of patients. A minority of patients replenished their vitamin D storages within the observation period most likely due to short follow-up time or inadequate dose regimes. Moreover, we did not have a control group of patients without vitamin D supplementation and inevitably missed sampling during the initial disease phase as the majority of patients were referred to our tertiary care center. Last, we do not have data on cytoplasmatic vitamin D receptor occupancy and function [32].
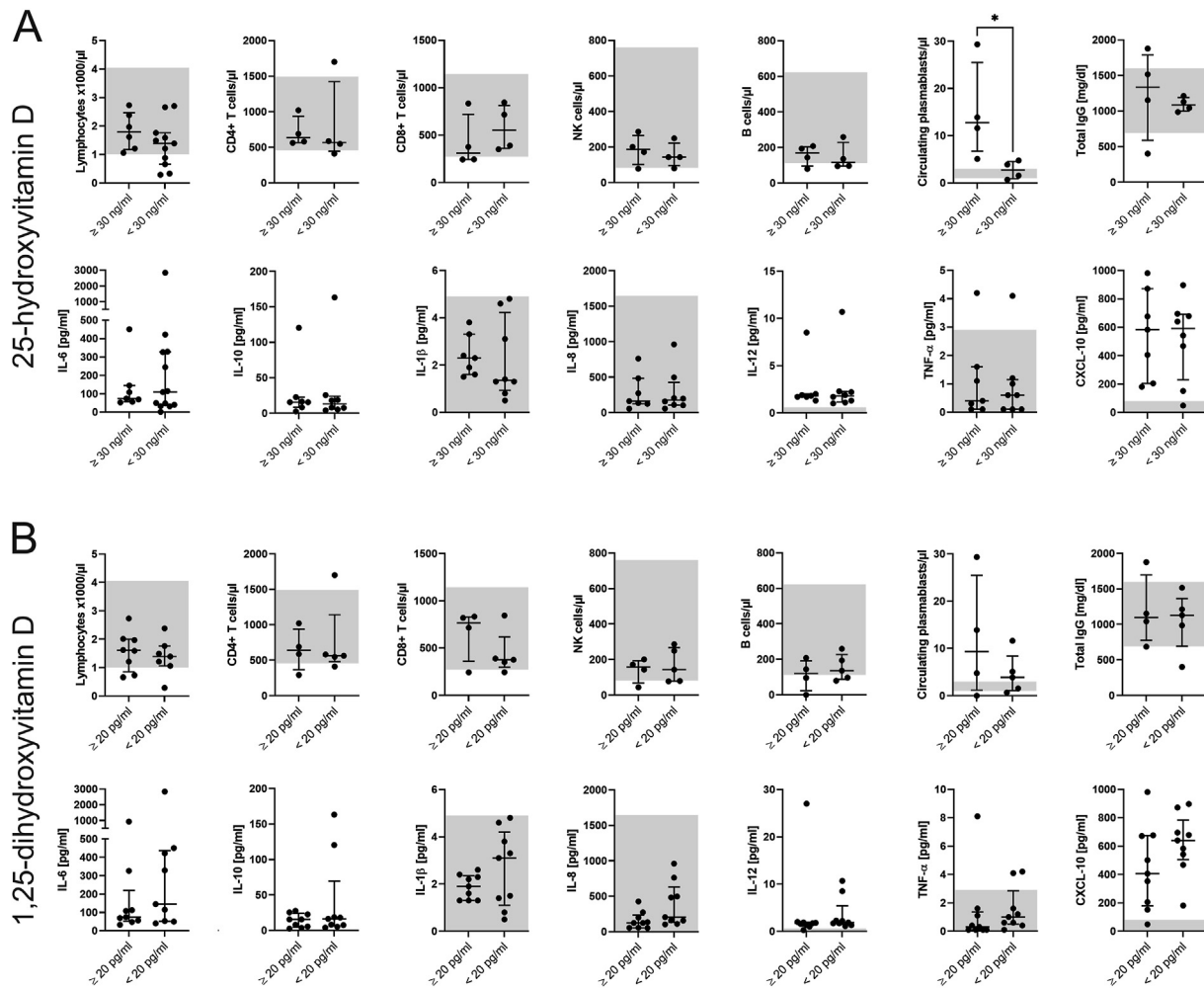
Fig. 4. Unaltered immune response against SARS-CoV-2 as a function of vitamin D levels. Patients were divided in two groups based on levels of 25-hydroxyvitamin D **(A)** as well as 1,25-dihydroxyvitamin D **(B)** after 10−15 days. Absolute numbers of the most important immune cell subsets and immunglobulin (Ig) G levels (upper row in each panel) just as main pro- and anti-inflammatory cytokines (lower row in each panel) were compared. Circulating plasmablasts were significantly higher in patients with 25-hydroxyvitamin D levels ≥30 ng/ml, whereas the other parameters did not differ between the groups, probably speaking against major effects of vitamin D on the immune response of critically ill COVID-19 patients. IL, interleukin; TNF, tumor necrosis factor.

5. Conclusion

The majority of critically ill COVID-19 ARDS patients suffered from vitamin D deficiency. Low levels of 25-hydroxyvitamin D were not related to changes in clinical course. Low levels of 1,25-dihydroxyvitamin D were associated with prolonged mechanical ventilation, whereas low-dose vitamin D supplementation did not impact the biologically active metabolite. Both forms should be included in monitoring of vitamin D status with future interventional studies targeting the usefulness of calcitriol administration in COVID-19 patients.

Author contributions

QN, PK, CL, and PM contributed substantially to the conception and design of the study, the acquisition, analysis, interpretation of the data and drafted the article.

CS contributed substantially to the conception and design of the study, interpretation of the data and revised the manuscript.

JH, TS, MS, PH contributed substantially to the acquisition of the data.

JS, DR, KA contributed substantially to the interpretation of the data and critical revision of the article.

All authors provided final approval of the version submitted for publication.

Conflict of interest

None.

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