Applied nutritional investigation

Prevalence of vitamin D deficiency and its prognostic impact on patients hospitalized with COVID-19

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ABSTRACT

Objectives: Although hypovitaminosis D appears to be highly prevalent in patients with coronavirus disease 2019 (COVID-19), its impact on their prognosis remains unclear.
Methods: In this study, serum 25-hydroxyvitamin D (Vit-D) level was measured in 200 patients hospitalized with COVID-19. The association between Vit-D and the composite endpoint of intensive care unit (ICU) admission/in-hospital death was explored using univariable and multivariable analyses. Also, serum Vit-D level in patients with COVID-19 was compared with that in age- and sex-balanced COVID-19-negative controls (i.e., 50 inpatients with sepsis).
Results: Serum Vit-D level was comparable between patients with COVID-19 and COVID-19-negative inpatients with sepsis ($P = 0.397$). No significant differences were found in serum Vit-D level according to COVID-19 severity at the time of hospital admission ($P = 0.299$). Incidence rates of the composite endpoint of ICU admission/in-hospital death did not differ significantly between patients with either Vit-D deficiency (i.e., Vit-D <20 ng/mL) or severe Vit-D deficiency (i.e., Vit-D <12 ng/mL) and those without (31% vs 35% with $P = 0.649$, and 34% vs 30% with $P = 0.593$, respectively). Vit-D level and status (i.e., Vit-D deficiency and severe Vit-D deficiency) were not prospectively associated with the risk of the composite endpoint of ICU admission/in-hospital death ($P > 0.05$ for all Cox regression models).
Conclusions: Regardless of the potential usefulness of Vit-D measurement to guide appropriate supplementation, Vit-D does not appear to provide helpful information for the stratification of in-hospital prognosis in patients with COVID-19.

Introduction

The coronavirus disease 2019 (COVID-19) continues to be a terrifying challenge for health systems worldwide, as reported daily by World Health Organization official updates [1]. In the hospital setting, therapeutic efforts to manage patients with the most common complications of this disease are not always effective [2]. Thus, approximately 20% of patients hospitalized with COVID-19 are transferred to intensive care units (ICUs) and 20% die from pulmonary and extrapulmonary complications of the disease [3]. The most unfavorable prognosis, often dictated by a lack of effective specific therapies, is recorded in older patients with greater frailty [4,5]. However, identifying circulating biomarkers that possibly suggest pathophysiological mechanisms and clinical evolution of the disease regardless of age and comorbidities, as well as direct the diagnostic and therapeutic choices, could be very helpful.

The major circulating form of vitamin D, 25-hydroxyvitamin D (Vit-D), is produced by the hepatic hydroxylation of previtamin D, which in turn is obtained in the skin from 7-dehydrocholesterol by the action of ultraviolet B rays. Subsequently, Vit-D is converted into 1,25-dihydroxyvitamin D (calcitriol), the biologically active form of

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vitamin D, through another hydroxylation reaction in the kidney and other tissues/cells (e.g., immune cells) [6,7]. Beyond having a key role in calcium homeostasis [8], Vit-D can exert direct antiviral effects and cooperate with type I interferon to enhance antiviral responses [9,10]. In addition, Vit-D can exert an immune-modulating action by regulating the production of inflammatory cytokines and inhibiting the proliferation of inflammatory cells [10].

By virtue of its antiviral and immunomodulating action, Vit-D could play a favorable role in the natural history of COVID-19 [11]. On the contrary, Vit-D deficiency status could be a prognostically unfavorable element in this infectious disease [11]. To date, various observational studies have assessed Vit-D levels in patients with COVID-19 and showed a unique result, namely the high prevalence of Vit-D deficiency [12,13]. Also, a recent meta-analysis of observational studies has shown that patients with Vit-D deficiency are more likely to get a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection [14]. However, a similar concordance of results was not observed with respect to the association between Vit-D and COVID-19-related outcomes. In fact, the inverse associations between Vit-D level and both COVID-19 severity and prognosis that emerged in some studies [15−17] were not always confirmed in others [18−22].

To make the link between serum Vit-D level and prognosis even more intricate, some intervention studies have found that Vit-D supplementation could reduce the transfer of patients with COVID-19 to ICUs or their risk of death [23]. However, in other studies, Vit-D supplementation had no favorable effects on the clinical course of COVID-19 [24,25].

In this study we investigated the prevalence of Vit-D deficiency and severe Vit-D deficiency in patients with COVID-19 who were hospitalized in an internal medicine ward compared with that in COVID-19-negative patients admitted with sepsis. In addition, considering the diverging literature results, we explored the prospective association between Vit-D and risk of ICU admission/in-hospital death as a composite endpoint in patients hospitalized with COVID-19.

Methods

COVID-19 population

Patients hospitalized with COVID-19 who were admitted to the Unit of Internal Medicine of the Santa Maria della Misericordia Hospital of Perugia in Italy between December 2020 and March 2021 were consecutively enrolled. The study protocol was developed in accordance with the principles of the Helsinki Declaration, and approved by the local ethics committee. The inclusion criteria were as follows: 1) age ≥18 y; 2) positive result on real-time reverse-transcriptase-polymerase chain reaction (RT-PCR) assays testing for SARS-CoV-2 on nasal or pharyngeal swab specimens at the time of hospital admission; and 3) informed written consent. Ongoing or previous (within the last 6 mo) Vit-D supplementation was an exclusion criterion.

Control group

For the control group, 50 age- and sex-balanced COVID-19-negative patients who were admitted with sepsis to the Unit of Internal Medicine of the Santa Maria della Misericordia Hospital of Perugia in Italy between December 2020 and March 2021 were enrolled. The inclusion criteria were as follows: 1) Age ≥18 y, 2) negative result on RT-PCR assays test for SARS-CoV-2 on nasal or pharyngeal swab specimens at the time of hospital admission, 3) informed written consent, and 4) diagnosis of sepsis according to the Sepsis-3 criteria [26] at the time of hospital admission. Ongoing or previous (within the last 6 mo) Vit-D supplementation was an exclusion criterion.

Data collection

Data on demographic characteristics, coexisting medical conditions, current treatments, laboratory tests, as well as physical and instrumental examinations performed at the time of hospital admission were collected and registered in medical records. Tests for SARS-CoV-2 on nasal or pharyngeal swab specimens were performed with RT-PCR assays (Allplex 2019-nCoV Assay, Seegene, Seoul, South Korea, or Xpert Xpress SARS-CoV-2, Cepheid, Sunnyvale, CA). Arterial and venous blood samples obtained within 48 h since hospital admission were processed according to standard laboratory techniques to determine the following laboratory variables: blood gas parameters (ABL90 FLEX blood gas analyzer, Radiometer, Brønshøj, Denmark), white cell and platelet counts (Sysmex XT-2000i, Dasit, Milano, Italy), D-dimer (BCS XP Coagulation Analyzer, Siemens, Munich, Germany), C-reactive protein (CRP), blood urea nitrogen, creatinine, bilirubin, lactate dehydrogenase, fasting glucose, and albumin (AU5800 Clinical Chemistry System, Beckman Coulter, Brea, CA). Serum Vit-D was assessed on venous blood samples obtained within 48 h since hospital admission through a radioimmunoassay (Diasorin Inc. Stillwater, Minnesota). Estimated glomerular filtration rate was calculated through the Chronic Kidney Disease Epidemiology Collaboration equation. Vit-D deficiency was defined as Vit-D level <20 ng/mL and severe Vit-D deficiency as Vit-D level <12 ng/mL [27]. A radiologic diagnosis of pneumonia was based on the presence of at least one of the following radiographic signs in either chest x-ray or high-resolution computed tomography: mono- or bilateral consolidations, ground glass opacities, and crazy paving pattern. Respiratory insufficiency was defined as the presence of peripheral oxygen saturation (SpO2) ≤90% and/or arterial partial pressure of oxygen (PaO2) ≤60 mmHg and/or the need for oxygen support at the time of admission. Calculated PaO2 per fraction of inspiration oxygen ratio (PaO2/FiO2) <300 was used to define the presence of respiratory distress. The CURB-65 score was estimated for each patient by integrating five clinical/laboratory data at the time of admission (i.e., 1 = confusion [1 point]; 2 = blood urea nitrogen >19 mg/dL [1 point]; 3 = respiratory rate ≥30/min [1 point]; 4 = systolic blood pressure <90 mmHg or diastolic blood pressure ≤60 mmHg, as assessed with a sphygmomanometer [1 point]; 5 = age ≥65 y [1 point]) [28,29]. The Charlson comorbidity index was calculated for each patient by integrating information on coexisting medical conditions [30]. Data on clinical course (i.e., in-hospital medical treatments and need of noninvasive ventilation [NIV]) and in-hospital outcomes (i.e., composite endpoint of ICU admission/in-hospital death or hospital discharge) were collected and registered in medical records.

Statistical analysis

The study sample size was calculated based on the results of a previous study [31] by assuming a type I error of 0.05, type II error of 0.2, ratio of unexposed (Vit-D ≥20 ng/mL) to exposed (Vit-D <20 ng/mL) group of 0.56, probability of event (i.e., ICU admission/in-hospital death) in the unexposed group of 0.02, and probability of event in the exposed group of 0.13. The estimated sample size was 206 patients, but we performed our analysis in 200 patients as an interim analysis. The SPSS statistical package, version 24.0 (SPSS Inc, Chicago, IL) was used for all statistical analyses. The Shapiro test was used to verify the normality of the study variables. Categorical variables were expressed as percentages and continuous variables as mean ± standard deviation or median and interquartile ranges. The independent samples *t* test, Mann−Whitney U-test, and χ^2 test were used for two-group comparisons. The Kruskal−Wallis test was used for multiple-group comparisons of nonparametric variables. The χ^2 test was used to compare multiple independent categorical variables. Correlation analyses between the study variables were performed using the Pearson's and Spearman's coefficients of correlation.

Time-to-event analyses were performed to assess the association between Vit-D and the composite endpoint of ICU admission/in-hospital death (primary endpoint), as well as the association between Vit-D and in-hospital death as a single endpoint (secondary endpoint).

For five patients, who did not meet the aforementioned endpoints and were still hospitalized at the time of the analysis, the event date was censored on April 3, 2021. The association between Vit-D, either as a continuous or categorical variable (i.e., serum Vit-D level, Vit-D deficiency, and severe Vit-D deficiency), and either the composite endpoint of ICU admission/in-hospital death or in-hospital death as a single endpoint was evaluated through Cox proportional hazard models by adjusting for potential confounders. Statistical significance was assumed if a null hypothesis could be rejected at $P < 0.05$.

Results

Characteristics of patients with COVID-19

The main characteristics of 200 patients with COVID-19 categorized according to the presence or absence of Vit-D deficiency (i.e., Vit-D <20 ng/mL vs Vit-D ≥20 ng/mL) are shown in Table 1. The prevalent symptoms reported at the time of hospital admission were fever, dyspnea, and cough (65%, 64%, and 41% of patients, respectively). According to the National Institutes of Health classification of COVID-19 severity [32], 22 (11%), 26 (13%), and 152 (76%) patients had mild (i.e., signs and symptoms of COVID-19 without shortness of breath, dyspnea, or abnormal chest imaging), moderate (i.e., lower respiratory disease during clinical assessment or imaging and SpO2 ≥94% on room air at sea level) and severe

Table 1
Characteristics of patients with COVID-19 categorized according to the presence or absence of Vit-D deficiency (Vit-D <20 ng/mL vs Vit-D ≥20 ng/mL).

	Total N = 200	Vit-D <20 ng/mL n = 160	Vit-D ≥20 ng/mL n = 40	P-value*
Age, y	74 (15)	72 (16)	78 (13)	0.031
Male sex, %	55	56	47	0.320
Body mass index, kg/m^2	26.2 (4.1)	26.4 (4.1)	25.0 (4.1)	0.063
Current smoking status, %	17	18	13	0.482
Hypertension, %	61	61	62	0.884
Type 2 diabetes, %	20	21	20	0.930
Previous cardiovascular event, %	18	19	17	0.856
Active cancer, %	8	9	5	0.375
Previous venous thromboembolism, %	5	5	5	1.000
Atrial fibrillation, %	19	19	17	0.787
Chronic obstructive pulmonary disease, %	14	14	15	0.839
Obesity, %	22	31	22	0.301
Charlson comorbidity index	4 (3−6)	4 (3−6)	4 (3−7)	0.524
Aangiotensin converting enzyme inhibitors, %	26	24	32	0.295
Angiotensin receptor blockers, %	14	14	17	0.547
Beta-blockers, %	31	29	40	0.196
Calcium channel blockers, %	22	22	22	0.932
Diuretics, %	35	34	40	0.506
Statins, %	16	16	17	0.848
Direct oral anticoagulants, %	15	14	17	0.621
Vitamin-K antagonists, %	3	4	0	0.214
Low molecular weight heparin, %	21	19	27	0.259
Antiplatelets, %	23	24	20	0.614
Insulin, %	13	14	10	0.520
Oral hypoglycemic agents, %	11	11	12	0.825
Systolic blood pressure, mmHg	131 (20)	132 (20)	130 (20)	0.573
Diastolic blood pressure, mmHg	77 (12)	78 (12)	75 (10)	0.155
Leukocytes, X 10^3/μL	8.0 (5.7−11.0)	7.9 (5.7−10.8)	8.9 (5.9−11.3)	0.480
Platelets, X 10^3/μL	220 (160−276)	220 (161−279)	224 (152−271)	0.546
D-dimer, ng/mL	895 (530−1762)	903 (493−1943)	875 (604−1455)	0.913
C-reactive protein, mg/dL	6.9 (3.6−12.1)	6.4 (3.6−11.2)	10.0 (3.3−14.6)	0.148
Fasting glucose, mg/dL	121 (102−155)	120 (101−156)	133 (110−153)	0.306
Estimated glomerular filtration rate, mL/min	69 (27)	71 (27)	62 (27)	0.079
Lactate dehydrogenase, UI/L	307 (236−432)	300 (235−406)	337 (253−469)	0.213
Albumin, g/dL	3.4 (3−3.7)	3.4 (3.0−3.7)	3.5 (3.0−3.7)	0.625
Arterial partial pressure of oxygen/ fraction of inspiration oxygen	248 (147−294)	247 (161−290)	254 (130−304)	0.715
CURB-65 score	2 (1−3)	2 (1−3)	2 (1−3)	0.296
Vit-D, ng/mL	11 (7−18)	10 (6−14)	28 (23−34)	< 0.001

Vit-D, 25-hydroxyvitamin D. Values are expressed as mean (standard deviation), median (25−75th percentile) or percentage.
*P-value for comparison between patients with COVID-19 with and without Vit-D deficiency.

COVID-19 (i.e., SpO2 <94% on room air at sea level, PaO2/FiO2 <300 mmHg, respiratory frequency >30 breaths/min, or lung infiltrates >50%), respectively.

Clinical course and in-hospital outcomes of patients with COVID-19

The clinical management of patients with COVID-19 was conducted according to available scientific evidence and recommendations at the time of enrollment. Corticosteroid treatment (dexamethasone 6 mg daily) was administered to 180 patients with respiratory insufficiency (90%). Antiviral therapy with remdesivir was prescribed to 48 patients who fulfilled the prescription criteria of the Italian drug agency (24%). Anticoagulant therapy was introduced in 188 patients (94%). Upon admission, radiographic signs of pneumonia were documented in 170 patients (85%). During the hospital stay, 76 patients needed NIV (38%), 24 patients were admitted to ICU (12%), 40 patients (20%) died, and 64 patients (32%) met the composite endpoint of ICU admission/in-hospital death. The median time from hospitalization to ICU admission was 2 d (range, 1−5 d), and the median time from hospitalization to death was 10 d (range, 6−16 d).

Covariates of Vit-D

Age was significantly lower in patients with Vit-D deficiency compared with those without (Table 1). A significant crude

correlation was found between Vit-D and albumin (r = 0.149; P = 0.037). No significant correlations were found between Vit-D and any of the other continuous study variables. No significant correlation emerged between Vit-D and CRP (Supplementary Fig. 1).

Vit-D level did not differ significantly according to COVID-19 severity (P = 0.299) nor according to the presence of radiographic signs of pneumonia (P = 0.532), respiratory insufficiency (P = 0.342), or respiratory distress (P = 0.383) at the time of hospital admission. No significant difference was found between the serum Vit-D level of patients who needed NIV during their hospital stay and those who did not (P = 0.303).

The rates of radiographic signs of pneumonia, respiratory insufficiency, respiratory distress, and the need of NIV did not differ significantly according to Vit-D deficiency or severe Vit-D deficiency (P = 0.932, 0.754, 0.334, and 0.611, respectively, for Vit-D deficiency, and P = 0.857, 0.700, 0.502, and 0.162, respectively, for severe Vit-D deficiency).

Vit-D and in-hospital prognosis of patients with COVID-19

Baseline characteristics of patients with COVID-19 according to the composite endpoint of ICU admission/in-hospital death are reported in Table 2, and the baseline characteristics of patients with COVID-19 categorized according to the single endpoint of in-hospital death are reported in Supplementary Table 1. The detection of radiographic signs of pneumonia, respiratory insufficiency, and respiratory distress at the

Table 2
Characteristics of patients with COVID-19 categorized according to the composite endpoint of ICU admission/in-hospital death

	Non-ICU admitted/discharged alive n = 136	ICU admitted/nonsurvivors n = 64	P-value
Age, y	72 (16)	77 (13)	0.025
Male sex, %	52	59	0.342
Body mass index, kg/m^2	26.4 (4.2)	25.6 (4.0)	0.223
Current smoking status, %	18	17	0.908
Hypertension, %	61	62	0.842
Type 2 diabetes, %	15	31	0.010
Previous cardiovascular event, %	12	19	0.314
Active cancer, %	8	9	0.761
Previous venous thromboembolism, %	5	5	0.889
Atrial fibrillation, %	18	20	0.746
Chronic obstructive pulmonary disease, %	13	16	0.650
Obesity, %	31	25	0.428
Charlson comorbidity index	4 (2−6)	5 (4−6)	0.010
Aangiotensin converting enzyme inhibitors, %	26	26	0.901
Angiotensin receptor blockers, %	12	19	0.242
Beta-blockers, %	28	39	0.114
Calcium channel blockers, %	19	28	0.151
Diuretics, %	32	42	0.175
Statins, %	16	16	0.979
Direct oral anticoagulants, %	15	16	0.865
Vitamin-K antagonists, %	3	3	0.943
Low molecular weight heparin, %	19	25	0.341
Antiplatelets, %	18	33	0.024
Insulin, %	10	19	0.088
Oral hypoglycemic agents, %	9	17	0.084
Systolic blood pressure, mmHg	132 (20)	130 (20)	0.437
Diastolic blood pressure, mmHg	78 (11)	75 (11)	0.085
Leukocytes, X 10^3/μL	7.9 (5.4−10.7)	8.4 (5.9−11.9)	0.268
Platelets, X 10^3/μL	225 (162−283)	204 (157−269)	0.252
D-dimer, ng/mL	833 (551−1748)	944 (482−1768)	0.967
C-reactive protein, mg/dL	6.2 (3.2−11)	7.4 (4.6−14.8)	0.028
Fasting glucose, mg/dL	120 (103−152)	127 (101−160)	0.703
Estimated glomerular filtration rate, mL/min	74 (26)	59 (26)	< 0.001
Lactate dehydrogenase, UI/L	3.4 (3.1−3.7)	3.3 (3−3.6)	0.113
Albumin, g/dL	279 (230−399)	366 (296−467)	0.003
Arterial partial pressure of oxygen/fraction of inspiration oxygen	260 (198−303)	152 (133−271)	< 0.001
CURB-65 score	2 (1−2)	2 (1−3)	< 0.001
Vit-D, ng/mL	11 (8−18)	11 (6−18)	0.839

ICU, intensive care unit; Vit-D, 25-hydroxyvitamin D.
Values are expressed as mean (standard deviation), median (25−75th percentile) or percentage.

time of hospital admission, as well as the need of NIV during the hospital stay were more prevalent in patients who met the composite endpoint of ICU admission/in-hospital death compared with those who did not (P = 0.013, < 0.001, < 0.001, and < 0.001, respectively). Conversely, the detection of radiographic signs of pneumonia, respiratory insufficiency, and respiratory distress at the time of hospital admission, as well as the need of NIV during the hospital stay were comparable between survivors and nonsurvivors (P = 0.055, 0.635, 0.504, and 0.827, respectively).

No significant difference was found in serum Vit-D level between patients who were admitted to ICU or who died compared with those who were not admitted to ICU/were discharged alive (Table 2) nor between patients who died and those who did not (Supplementary Table 1). No significant differences were found in the prevalence of Vit-D deficiency or severe Vit-D deficiency between patients who met the composite outcome of ICU admission/in-hospital death and those who did not (P = 0.649 and 0.593, respectively) nor between survivors and nonsurvivors (P = 1.000 and 0.357, respectively). Incidence rates of the composite endpoint of ICU admission/in-hospital death did not differ significantly between patients with either Vit-D deficiency (Vit-D <20 ng/mL) or severe Vit-D deficiency (Vit-D <12 ng/mL) and those without (31% vs 35% and P = 0.649 for Vit-D deficiency, 34% vs 30% and P = 0.593 for severe Vit-D deficiency, respectively). Rates of in-hospital death did not differ significantly across serum Vit-D quintiles

(Vit-D <6.1 ng/mL in 1st quintile, 6.1 ng/mL ≥ Vit-D <9.7 ng/mL in 2nd quintile, 9.7 ng/mL ≥ Vit-D <13.9 ng/mL in 3rd quintile, 13.9 ng/mL ≥ Vit-D <20 ng/mL in 4th quintile, Vit-D ≥20 ng/mL in 5th quintile; P = 0.769; Supplementary Fig. 2).

Three Cox proportional hazard models were plotted (Models 1, 2, and 3), including the time to ICU admission/in-hospital death as the time variable, ICU-admission/in-hospital death as the status variable, and the following independent variables: Model 1 has Vit-D (serum Vit-D level in Model 1a, Vit-D deficiency in Model 1b, and severe Vit-D deficiency in Model 1c), age, and male sex; Model 2 has Vit-D (serum Vit-D level in Model 2a, Vit-D deficiency in Model 2b, severe Vit-D deficiency in Model 2c), age, male sex, and coexisting medical conditions/current treatments varying significantly according to the in-hospital prognosis (i.e., type 2 diabetes, antiplatelet therapy, and Charlson comorbidity index); and Model 3 has Vit-D (serum Vit-D level in Model 3a, Vit-D deficiency in Model 3b, severe Vit-D deficiency in Model 3c), age, male sex, and clinical/laboratory parameters varying significantly according to the in-hospital prognosis (i.e., CURB-65 score, PaO2/FiO2, CRP, lactate dehydrogenase, and estimated glomerular filtration rate). In none of the three models, Vit-D was significantly associated with ICU admission/in-hospital death (Table 3). Independent predictors of ICU admission/in-hospital death were type 2 diabetes in Model 2 (P = 0.015, 0.016, and 0.015 in Models 2a, 2b, and 2c, respectively) and PaO2/FiO2 in Model 3 (P = 0.007, 0.006, and 0.006 in

Table 3
Risk of intensive care unit admission/in-hospital death according to Vit-D in patients with COVID-19

Model 1[*]	1a		HR	95% CI	P-value
		Vit-D	1.013	0.991−1.035	0.240
	1b		HR	95% CI	P-value
		Vit-D deficiency[‡]	0.783	0.427−1.436	0.430
	1c		HR	95% CI	P-value
		Severe Vit-D deficiency[‡]	0.912	0.551−1.510	0.720
Model 2[§]	2a		HR	95% CI	P-value
		Vit-D	1.015	0.992−1.037	0.202
	2b		HR	95% CI	P-value
		Vit-D deficiency[‡]	0.778	0.420−1.441	0.424
	2c		HR	95% CI	P-value
		Severe Vit-D deficiency[‡]	0.881	0.533−1.457	0.622
Model 3[¶]	3a		HR	95% CI	P-value
		Vit-D	1.015	0.991−1.039	0.231
	3b		HR	95% CI	P-value
		Vit-D deficiency[‡]	0.891	0.462−1.718	0.731
	3c		HR	95% CI	P-value
		Severe Vit-D deficiency[‡]	0.841	0.490−1.441	0.528

CI, confidence interval; HR, hazard ratio.
[*]Adjusted for age and male sex.
[†]Vit-D <20 ng/mL.
[‡]Vit-D <12 ng/mL.
[§]Adjusted for age, male sex, type 2 diabetes, antiplatelet therapy, and Charlson comorbidity index.
[¶]Adjusted for age, male sex, CURB-65 score, arterial partial pressure of oxygen/fraction of inspiration oxygen, C-reactive protein, lactate dehydrogenase, and estimated glomerular filtration rate.

Models 3a, 3b, and 3c, respectively). Cox regression plots of the age- and sex-adjusted cumulative hazard of the composite outcome of ICU admission/in-hospital death according to Vit-D deficiency and severe Vit-D deficiency are reported in Figure 1. No significant prospective association emerged between Vit-D (serum Vit-D level, Vit-D deficiency, severe Vit-D deficiency) and in-hospital death as single endpoint (Supplemental Table 2).

Comparison of Vit-D between patients with COVID-19 and COVID-19-negative controls

The main characteristics of age- and sex-balanced COVID-19 negative controls are reported in Supplementary Table 3. Serum Vit-D level was comparable between patients with COVID-19 and COVID-19-negative inpatients with sepsis (P = 0.397). Prevalence of Vit-D deficiency was 80% and 78% in patients with COVID-19 and COVID-19-negative inpatients with sepsis, respectively (P > 0.05 for comparison between the two groups). Prevalence of severe Vit-D deficiency was 53% and 50% in patients with COVID-19 and COVID-19-negative inpatients with sepsis, respectively (P > 0.05 for comparison between the two groups).

Discussion

In this prospective study of patients hospitalized for COVID-19, two main results emerged. First, patients with COVID-19 had comparable Vit-D levels to those of age- and sex-balanced COVID-19-negative inpatients with sepsis. Second, serum Vit-D level was not cross-sectionally associated with any of the clinical parameters of COVID-19 severity nor prospectively associated with the in-hospital prognosis of patients with COVID-19.

Prevalence of Vit-D deficiency in patients hospitalized with COVID-19

In line with the literature data [33,34], a high prevalence of Vit-D deficiency and severe Vit-D deficiency emerged in this cohort of patients hospitalized with COVID-19, with 80% and 53% of enrolled patients having shown these two conditions, respectively. However, the prevalence of Vit-D deficiency and severe Vit-D deficiency was not dissimilar to that observed in COVID-19-negative inpatients with sepsis. This finding suggests a possible pathophysiological link between Vit-D and infections. In this regard, two different albeit nonmutually exclusive speculations are plausible, with the first relating to a possible direct causality and the second to a possible reverse causation between Vit-D and infections.

With regard to the first hypothesis (i.e., direct causality), the state of Vit-D deficiency, possibly preexisting to the contact with pathogens, could affect an increased probability of getting both viral and bacterial infections. Indeed, evidence shows that Vit-D deficiency can promote different viral infections [35], including COVID-19 [12]. In addition, a significant association between hypovitaminosis D and increased susceptibility to sepsis has been reported [36].

However, although Vit-D plays an undoubted role in modulating the immune response to infections [10], the literature on this topic currently remains very controversial [37]. On the other hand,
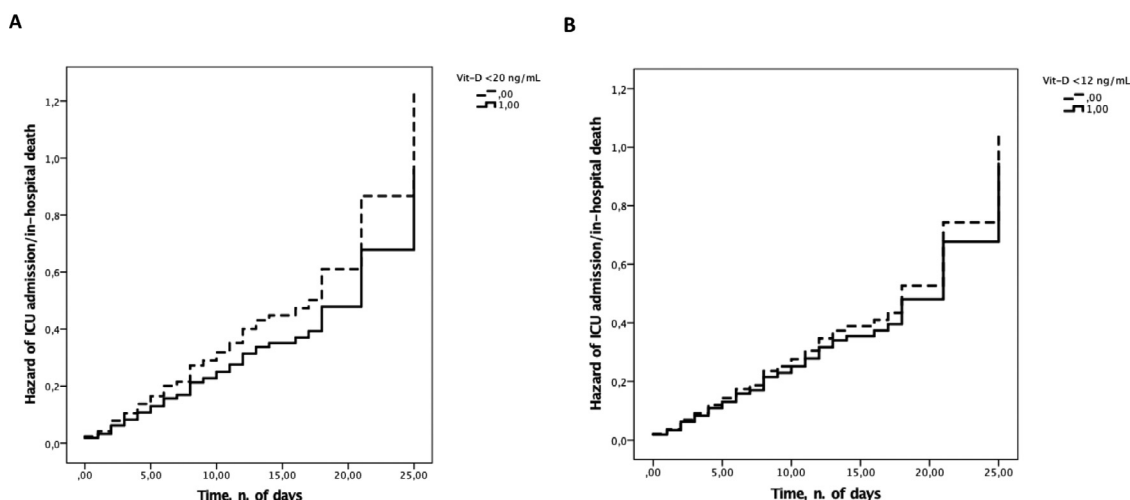

Fig. 1. Age- and sex-adjusted hazard of intensive care unit admission/in-hospital death according to (A) 25-hydroxyvitamin D deficiency and (B) severe 25-hydroxyvitamin D deficiency.

reverse causation also could explain the association between low serum Vit-D level and COVID-19. In this regard, a combination of factors characterizing the population affected by COVID-19 (e.g., preferential involvement of older age groups, state of profound debilitation and malnutrition related to the course of the disease, reduced sun exposure due to default isolation preceding and after hospitalization) [4,5,38,39] may contribute to the reduction in serum level of Vit-D. Partly supporting this interpretation, a non-specific marker of nutritional status as reduced albumin, which is particularly prevalent among patients with COVID-19 [40], was directly correlated with Vit-D level in this study.

Nonetheless, regardless of the direction of the association between COVID-19 and hypovitaminosis D, this latter condition has been proposed to alter the homeostasis of numerous biologic processes, thereby possibly promoting the progression of the disease independently from its influence on immune response.

Vit-D and COVID-19 clinical course

In this study, we did not find any association between Vit-D and any clinical parameter of COVID-19 severity at the time of hospital admission or during hospitalization, including the presence of radiologic signs of pneumonia, respiratory insufficiency, respiratory distress, and the need for NIV. Also, serum Vit-D level, Vit-D deficiency, and severe Vit-D deficiency were not prospectively associated with the composite outcome of ICU admission/in-hospital death nor with in-hospital death as a single endpoint (Table 3; Supplementary Table 2). Conversely, as already reported in previous studies [41,42], PaO2/FiO2 and type 2 diabetes were also significant and independent predictors of a poor prognosis in this cohort of patients hospitalized with COVID-19. Our finding of the lack of an association between serum Vit-D level and severity of COVID-19 fits into the context of a series of conflicting observational studies with either a retrospective or prospective design exploring the association between Vit-D and COVID-19 severity/outcomes. Indeed, some retrospective studies in patients hospitalized for COVID-19 have found a significant association between low Vit-D level and either disease severity or in-hospital outcomes [43−45], but others have not confirmed this relationship [33,46]. Similarly, discrepant results have been highlighted in some prospective studies examining the association between low Vit-D level and either COVID-19 severity or prognosis [18,19,22,31,47]. Also, further fueling inconsistencies in the literature on Vit-D, in contrast with previous studies suggesting a possible J-shaped relationship between serum Vit-D and either cardiovascular mortality or all-cause mortality [48,49], we did not find any significant difference in rates of in-hospital death across Vit-D quintiles (Supplementary Fig. 2).

The reasons why the results of this study may diverge or agree with those from other studies may be the most varied. First, the prospective analysis of the association between Vit-D and COVID-19 prognosis, as performed in this study, provides more reliable results than those derived from retrospective studies due to the intrinsic limitations of the latter ones (e.g., impossibility of assessing the temporal relationship between the study variables, and potential confounding effect due to unmeasured variables). In addition, compared with other prospective studies that have documented a negative impact of Vit-D deficiency on the prognosis of patients with COVID-19, the population of this study was older [18,19,22,33,47], which might have masked a possible weak association between low Vit-D level and in-hospital prognosis. Furthermore, in this study, the period of observation lasted until the occurrence of ICU admission/in-hospital death or until the conclusion of hospitalization (i.e., on average 13 d for patients who were discharged alive). Therefore, a relatively short-term follow up might have

compromised the possibility of detecting a long-term impact of Vit-D deficiency on COVID-19 prognosis. Based on these observations, our findings cannot be generalized to younger populations of patients with COVID-19 nor they can be extended to the relationship between Vit-D and long-term prognosis of patients with COVID-19.

However, discrepancies between previous studies and our study may, at least in part, be attributed to different methods of measuring circulating levels of Vit-D. To this regard, measurement of total serum Vit-D may have the limitation of underestimating possible interindividual differences in the proportion of free Vit-D (bioactive form) and Vit-D bound to the Vit-D binding protein (DBP). Indeed, a well-known high variability exists in DBP affinity for Vit-D, which can be affected by several DBP genetic variants and pathophysiological conditions [50]. In addition, DBP circulating levels can be significantly influenced by the activation of systemic inflammation [50]. Overall, the disagreement of the results from observational studies relating to the relationship between Vit-D level and COVID-19 prognosis mirrors discrepancies that emerged in the few intervention studies exploring the effects of Vit-D supplementation in patients with COVID-19 [23−25].

Limitations

Some limitations of this study deserve attention. First, the population examined in this study was numerically limited, confined to a relatively advanced age group, and followed for a relatively short period of hospitalization. Therefore, generalizability of the observed results to other clinical settings must be considered with extreme caution. Second, the relatively small sample size that was included in this interim analysis may undermine the reliability of the observed results. Third, the assay we used to measure serum Vit-D has the intrinsic limitation of accounting only for total Vit-D, which is mostly bound to DBP. Accordingly, some factors influencing circulating DBP levels, including systemic inflammation [50], may have confounded Vit-D measurements. Such a limitation might be overcome by demonstrating the replicability of the study results when also incorporating free Vit-D measures or, alternatively, pre-COVID-19 Vit-D measures from the same population. However, in the setting of our study, neither the option of measuring free Vit-D nor that of recording pre-COVID-19 Vit-D measures from the same population was available. Finally, the control population (i.e., COVID-19-negative inpatients with sepsis), albeit balanced in terms of age and sex with that of patient with COVID-19, was numerically too small to allow for an adequate adjustment of between-group differences in Vit-D for a consistent number of confounding factors. However, comparable results have been previously reported for the prevalence of hypovitaminosis D in populations similar to our control group [51,52].

Conclusions

In patients with COVID-19 hospitalized in an internal medicine ward, the prevalence of Vit-D deficiency is extremely high but not dissimilar to that seen in COVID-19-negative patients hospitalized for sepsis. Low levels of Vit-D neither highlight more severe COVID-19 nor predict an unfavorable in-hospital prognosis. Although the long-term prognostic value of Vit-D and the clinical impact of Vit-D supplementation in COVID-19 remain uncertain, our data do not support the utility of Vit-D measurement for the prognostic stratification of patients hospitalized with COVID-19.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.nut.2021.111408.

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