



## Vitamin D Status and COVID-19 Clinical Outcomes in Hospitalized Patients

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### ABSTRACT

**Context:** Populations severely affected by COVID-19 are also at risk for vitamin D deficiency. Common risk factors include older age, chronic illness, obesity, and non-Caucasian race. Vitamin D deficiency has been associated with risk for respiratory infections and failure, susceptibility and response to therapy for enveloped virus infection, and immune-mediated inflammatory reaction.

**Objective:** To test the hypothesis that 25-hydroxyvitamin D[25(OH)D] deficiency is a risk factor for severity of COVID-19 respiratory and inflammatory complications.

**Design:** We examined the relationship between prehospitalization 25(OH)D levels (obtained 1–365 days prior to admission) and COVID-19 clinical outcomes in 700 COVID-19 positive hospitalized patients.

**Primary Outcomes:** Discharge status, mortality, length of stay, intubation status, renal replacement.

**Secondary Outcomes:** Inflammatory markers.

**Results:** 25(OH)D levels were available in 93 patients [25(OH)D:25(IQR:17–33)ng/mL]. Compared to those without 25(OH)D levels, those with measurements did not differ in age, BMI or distribution of sex and race, but were more likely to have comorbidities. Those with 25(OH)D < 20 ng/mL (n = 35) did not differ from those with 25(OH)D ≥ 20 ng/mL in terms of age, sex, race, BMI, or comorbidities. Low 25(OH)D tended to be associated with younger age and lower frequency of preexisting pulmonary disease. There were no significant between-group differences in any outcome. Results were similar in those ≥50 years, in male/female-only cohorts, and when differing 25(OH)D thresholds were used (<15 ng/mL and <30 ng/mL). There was no relationship between 25(OH)D as a continuous variable and any outcome, even after controlling for age and pulmonary disease.

**Conclusions:** These preliminary data do not support a relationship between prehospitalization vitamin D status and COVID-19 clinical outcomes.

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COVID-19; vitamin D; mortality; intubation; hospital discharge; inflammatory markers

## Introduction

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), can produce severe respiratory symptoms and failure and has led to hundreds of thousands of deaths worldwide. Risk factors for severe COVID-19 illness include older age, non-Caucasian race, obesity, chronic illness, and nursing home/chronic care facility residence.<sup>1–4</sup> The populations at the highest risk for COVID-related severe illness also have a high prevalence of vitamin deficiency.<sup>5,6</sup> Shared risk factors for COVID-19 and vitamin D deficiency, and findings that countries in which vitamin D deficiency is endemic have the highest COVID-related mortality,<sup>7</sup> have led to the hypothesis that vitamin D deficiency may lead to increased risk of developing COVID-19, increased severity of symptoms, and worsened outcomes.<sup>8,9</sup>

Vitamin D deficiency has been linked to susceptibility to other non-COVID respiratory tract infections and/or symptoms.<sup>10–13</sup> Interventional studies also support a link between vitamin D and risk of respiratory infection. In a meta-analysis utilizing individual patient data from over 10,000 participants in 25 randomized controlled trials, vitamin D supplementation reduced the risk of respiratory tract infection.<sup>14</sup> Vitamin D deficiency has also been linked to worse outcomes in patients with respiratory or critical illness. In over 6,000 participants in the British birth cohort study, pre-admission 25(OH)D levels were associated with lung function indices and predicted both short- and long-term outcomes.<sup>15,16</sup> In a retrospective cohort study relating prehospital 25(OH) vitamin D levels to incident acute respiratory failure in almost 2,000 critically ill adults, patients with 25(OH)D < 30 ng/mL had

significantly higher adjusted odds of acute respiratory failure/ARDS.<sup>17</sup>

Vitamin D's regulation of immune function may explain the link between vitamin D and COVID-19. Vitamin D levels, vitamin D metabolites, and vitamin D receptor polymorphisms play a role in immune function via actions on T lymphocyte recruitment and activation as well as modulation of innate immune responses such as induction of antimicrobial peptides and other antimicrobial effector mechanisms.<sup>18–22</sup> Vitamin D has also been shown to reduce pro-inflammatory cytokines, thereby reducing inflammation.<sup>19</sup>

Data are now becoming available regarding vitamin D and the risk of COVID-19 infection, inflammatory markers, complications, and mortality. These studies have yielded mixed results. Researchers from the University of Chicago have reported findings, not yet peer reviewed, showing a higher proportion of positive COVID-19 test results among those with vitamin D deficiency.<sup>23</sup> In an analysis of data ( $n > 500,000$ ) from the UK biobank, 25(OH)D level predicted COVID-19 infection in univariate analysis, but not after adjustment for covariates,<sup>1</sup> suggesting that the association of vitamin D with risk of COVID-19 infection may be a spurious relationship caused by the confounding effects of comorbidities. Other studies examining disease severity endpoints have utilized vitamin D levels assessed in hospitalized subjects. In a recently published study, low 25(OH)D levels, assessed during hospitalization in 37 subjects, were not associated with disease severity, imaging abnormalities, and inflammatory markers.<sup>24</sup> In contrast, in two additional small studies of hospitalized COVID-19 subjects ( $N = 73$  and  $N = 70$ ),<sup>25,26</sup> low 25(OH)D levels at admission were associated with extent of lung involvement,<sup>26</sup> need for critical care<sup>25</sup> and elevated inflammatory markers.<sup>25</sup> While one study found a relationship between low vitamin D and mortality,<sup>26</sup> the other did not.<sup>25</sup> Relationships found in these studies may be influenced by the potential effects of critical illness on measured total vitamin D levels.<sup>27–29</sup>

This emerging literature provides some support for a relationship between vitamin D deficiency and COVID-19 infection, as well as vitamin D deficiency during illness and COVID-19 severity. However, no published work has examined the relationship between prehospitalization 25(OH)D level, less likely to be impacted by critical illness, and hospital discharge, intubation, length of hospital stay, need for renal replacement, or inflammatory markers other than CRP.<sup>30</sup> If these relationships are confirmed, findings may suggest a potential role for vitamin D supplementation in

COVID-19 prevention and treatment. We hypothesized that vitamin D deficiency is associated with the severity of both respiratory and inflammatory complications of COVID-19 in hospitalized adults measured by the above outcomes.

## Methods

This study was approved by the Columbia University Institutional Review Board, which also granted waiver of the requirement for informed consent. In this retrospective medical record review, data on 700 patients admitted to Columbia University Irving Medical Center (CUIMC) in New York City from February 11, 2020, to May 10, 2020, were extracted from the electronic medical record and reviewed for an available 25(OH)D level. The cohort included non-obstetric patients 18 years of age or older who were admitted to the hospital with a positive PCR test for COVID-19<sup>4</sup> and an available serum 25(OH)D level.

## Exposure of Interest

The exposure of interest was serum 25(OH)D level obtained 1 to 365 days prior to the date of hospital admission. For patients with multiple serum 25(OH)D measurements, the measurement closest to the date of admission was used for analysis. Serum 25(OH)D level in ng/mL, assessed at any hospital or commercial laboratory, within 1 to 365 days prior to admission was available for 93 patients, defining the analytical cohort. Of the 93 vitamin D levels recorded, 76 were measured within the New York Presbyterian Hospitals laboratories via chemiluminescent immunoassay with coefficient of variations of 2.8% and 4.6% for low and high controls (Abbott Diagnostics, Lake Forest, IL), while the remainder were obtained at other hospital or commercial laboratories. Vitamin D deficiency was defined as serum 25(OH)D level less than 20 ng/mL.<sup>31</sup> This cutoff was chosen based on provocative testing in healthy adults whereby vitamin D supplementation resulted in parathyroid hormone (PTH) decrease in those with 25(OH)D less than 20 ng/mL, but not in those with 25(OH)D greater than 20 ng/mL.<sup>31</sup> Total calcium level, albumin, alkaline phosphatase, BUN, and creatinine obtained within 30 days of the serum 25(OH)D level were also manually extracted.

## Compilation of the Clinical Outcome Database

Data were extracted from the COVID-Care database.<sup>4</sup> The COVID-Care database included all patients tested for COVID-19 from March 1, 2020, moving forward. It

contains data extracted from the electronic medical record (EMR) and augmented with manually extracted data. A subset of patients testing positive was manually reviewed in a consecutive fashion starting with the first patient testing positive for SARS-CoV-2. Manually abstracted data, including the date of symptom onset and presenting symptoms, were entered into a REDCap database. Electronically extracted data included demographics, admission and discharge dates and status, vital signs, basic laboratory results, and diagnosis codes used to identify patients with preexisting medical conditions. All data were merged using RStudio.

### Outcomes of Interest

The primary outcomes of interest were discharge status (deceased/discharged to hospice, discharged (not death), still admitted), intubation status (ever, never), renal replacement during hospitalization (including hemodialysis and continuous veno-venous hemofiltration), and duration of stay. The duration of stay was calculated by subtracting the admission date from the discharge date. If the patient remained admitted at the time of this study, the duration of stay was calculated by subtracting the admission date from May 19, 2020, the date of data extraction. For patients who were intubated during the admission, the duration of intubation was also calculated.

The secondary outcomes of interest were the peaks of inflammatory markers: C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), ferritin, and interleukin 6 (IL-6). Serum measurements of these inflammatory markers were obtained routinely during admission, and the peak level during admission for each inflammatory marker was included for analysis.

### Other Variables

Other variables of interest were age, sex assigned at birth, BMI, race, and ICD-defined comorbidities, including hypertension, diabetes, chronic kidney disease, and any pulmonary disease.

### Statistical Analysis

Data are presented as frequency/percent or median (interquartile range). The characteristics of the cohort with serum 25(OH)D levels available were compared with the remainder of the 700 patients without serum 25(OH)D levels.

Among the cohort of 93 patients with total 25(OH)D levels available within 1 year prior to admission, patients with and without vitamin D deficiency were compared.

This analysis was also repeated for the following subgroups: age  $\geq 50$ , female, male, and BMI  $\geq 30$  kg/m<sup>2</sup>. Chi-square tests were used to evaluate the association between vitamin D deficiency and categorical variables; Wilcoxon rank sum tests were used to evaluate the association between vitamin D deficiency and continuous variables due to the non-normal distribution of these variables.

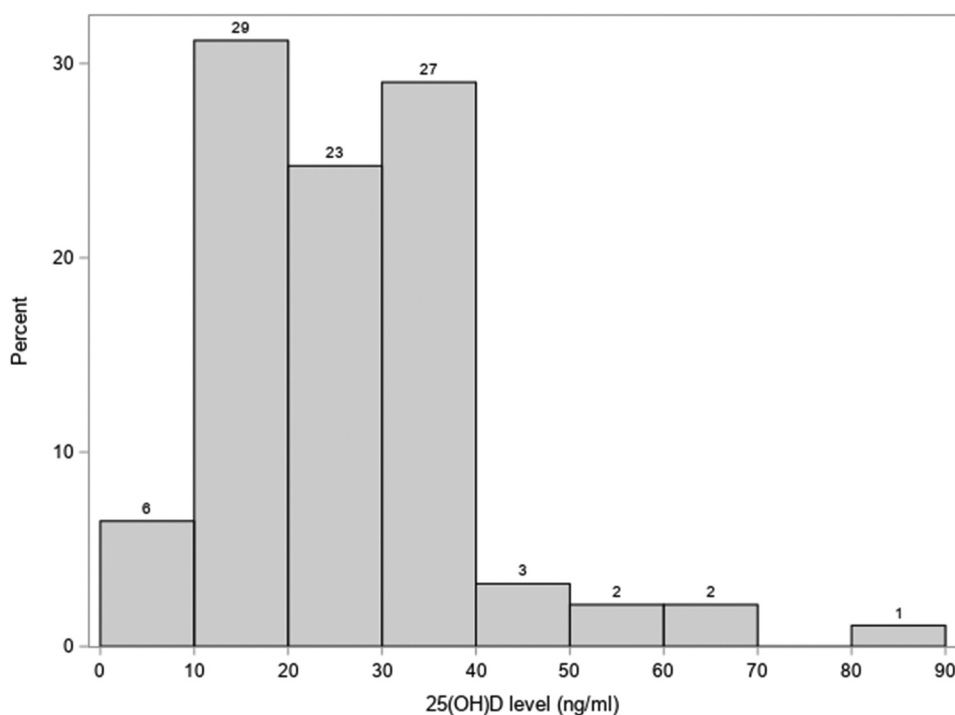
Multivariable logistic regression was used to examine the relationship between vitamin D status and categorical outcomes, adjusting for age and covariates found to be associated with vitamin D deficiency at the  $p < .10$  level. Multivariable linear regression was used to examine the relationship between vitamin D status and continuous outcomes, adjusting for age and covariates found to be associated with vitamin D deficiency at the  $p < .10$  level. For all analyses,  $p < .05$  was considered statistically significant. All analyses were conducted using SAS 9.4 (SAS Institute, Cary, NC).

### Results

Among the first 700 COVID-19 positive nonpregnant adults admitted at CUIMC, median (IQR) age was 63 (IQR: 50–74) years old, 43% were female, and median BMI was in the overweight range [28.3 (IQR: 24.6–33.3) kg/m<sup>2</sup>]. In this cohort, 22% were intubated and mortality was 21%. Characteristics of the first 1000 patients at CUIMC have been recently published.<sup>4</sup>

Of these 700, prehospitalization 25(OH)D levels were available in 93 (13.3%). The median 25(OH)D level was 25 (IQR: 17–33) ng/mL and was measured 136 (IQR: 75–248) days before admission. The distribution of 25(OH)D levels is shown in [figure 1](#). The majority of patients had a 25(OH)D level between 10 and 40 ng/mL. Compared to those without available 25(OH)D levels, those with 25(OH)D measurements did not differ in age ( $p = .61$ ), BMI ( $p = .31$ ) and race ( $p = .96$ ), tended to be female (52.7% vs. 42.0%,  $p = .053$ ), and were more likely to have ICD-defined hypertension ( $p < .01$ ), diabetes ( $p < .01$ ), chronic kidney disease ( $p < .01$ ) and pulmonary disease ( $p = .02$ ).

Vitamin D deficiency, defined as a 25(OH)D level  $< 20$  ng/mL, was present in 37.6% ( $n = 35$ ). The median 25(OH)D level of those with 25(OH)D  $< 20$  ng/mL was 16 (IQR: 14–18) ng/mL and the median 25(OH)D level of those with 25(OH)D  $\geq 20$  ng/mL was 32 (IQR: 26–36) ng/mL. As shown in [table 1](#), those with vitamin D deficiency did not differ from those who were sufficient (vitamin D  $\geq 20$  ng/mL) in terms of sex, race, BMI, or comorbidities. Vitamin D deficiency tended to be associated with younger age ( $p = .13$ ) and a lower frequency of



**Figure 1.** Histogram showing the distribution of 25-hydroxyvitamin D [25(OH)D] levels. The y-axis displays the percentage of patients with a 25(OH)D level within each category. The frequency per category is displayed on the top of each bar (total N = 93).

preexisting pulmonary disease ( $p = .07$ ). There were no between-group differences in any outcome, including intubation (29 vs 17%,  $p = .20$ ). Analyses limited to those with 25(OH)D levels measured at our institution yielded similar results (data not shown). Results were similar when analyses were limited to those over 50 years of age (Table S1),<sup>32</sup> limited to those with race categorized as black/African American (Table S2),<sup>32</sup> and when men and women were examined separately (Table S3).<sup>32</sup> Among the smaller subset of those aged 65 or over (N = 40), results were similar (data not shown). Analyses limited to those with BMI  $\geq 30$  kg/m<sup>2</sup> (n = 33) did not reveal significant relationships (data not shown). When differing 25(OH)D thresholds were used (<15 ng/mL and <30 ng/mL), the results were similar (data not shown). Due to the small number of subjects with 25(OH)D < 10 ng/ml (N = 6), we were unable to complete analyses using this threshold.

Regression analysis was used to examine relationships with 25(OH)D serum level as a continuous variable. The categorical outcomes were not associated with 25(OH)D in unadjusted logistic regression analyses or in analyses adjusted for age, sex and pulmonary disease (table 2). 25(OH)D level was also not significantly related to length of hospital stay in unadjusted or adjusted analyses (data not shown). Inflammatory

markers were similarly not associated with 25(OH)D level in adjusted and unadjusted models (table 3).

## Discussion

To investigate the hypothesis that vitamin D deficiency is associated with severity of both respiratory and inflammatory complications of COVID-19, we examined relationships between retrospectively available prehospitalization serum vitamin D levels and death, hospital discharge, intubation, length of hospital stay, need for renal replacement and inflammatory markers in 93 hospitalized adults. To our knowledge, this is the first study to examine relationships between prehospitalization serum vitamin D levels and a range of COVID-19 clinical outcomes, including intubation and inflammatory markers. These data do not clearly support a relationship between serum 25(OH)D either as a categorical (with various thresholds, <15 ng/mL, <20 ng/mL, and <30 ng/mL) or continuous variable and COVID-19 clinical outcomes in the whole cohort or subgroups based on age, race and sex.

Studies examining the relationship of vitamin D levels and COVID-19 clinical disease severity outcomes are limited and have yielded mixed results. In a small group of 37 patients, Pizzini et al. found that low 25(OH)D assessed at hospitalization was not associated with several measures of disease severity, including CT

**Table 1.** Characteristics of subjects by vitamin D status.

	Vitamin D Deficiency ( $< 20$ ng/mL) N = 35	Normal Vitamin D ( $\geq 20$ ng/mL) N = 58	p-value
Median Vitamin D level (ng/mL)	16 (IQR: 14–18)	32 (IQR: 26–36)	
<b>Cohort Characteristics</b>			
Age (Years)	58 (IQR: 36–74)	64 (IQR: 54–73)	0.13
Sex (% Female)	18 (51%)	31 (53%)	0.85
BMI ( $\text{kg}/\text{m}^2$ )	28.5 (IQR: 23.1–33.8)	27.0 (IQR: 22.4–32.2)	0.49
Race (% Black/African American)	32%	28%	0.69
<b>ICD-Defined Comorbidities:</b>			
Hypertension	27 (77%)	49 (84%)	0.37
Diabetes	23 (66%)	37 (64%)	0.85
Chronic Kidney Disease	18 (51%)	28 (48%)	0.77
Any Pulmonary Disease	7 (20%)	22 (38%)	0.07
<b>Predefined Outcomes</b>			
<b>Discharge status:</b>			
Deceased/Discharged to Hospice	8 (23%)	14 (24%)	0.32
Discharged (not death)	25 (71%)	37 (64%)	
Still Admitted – Intubated	2 (6%)	2 (3%)	
Still Admitted – Not Intubated	0 (0%)	5 (9%)	
Deceased	8 (23%)	14 (24%)	0.89
Ever-Intubated	10 (29%)	10 (17%)	0.20
Length of Intubation (#days)	8 (IQR: 4–34)	15 (IQR: 7–23)	0.69
Renal Replacement (HD or CVVH)	10 (29%)	14 (24%)	0.64
Length of Stay (#days)	8.8 (IQR: 4.9–16.7)	9.4 (IQR: 5.0–16.2)	0.83
<b>Peak Level Inflammatory Markers:</b>			
ESR (mm/hr)	83 (IQR: 54–114)	80 (IQR: 59–110)	0.99
CRP (mg/L)	118 (IQR: 37–274)	131 (IQR: 62–223)	0.97
Ferritin (ng/mL)	1030 (IQR: 555–1802)	933 (IQR: 311–2165)	0.65
IL-6 (pg/mL)	12 (IQR: 5–76)	26 (IQR: 8–63)	0.65

Values represent median (interquartile range) or frequency (percentages); BMI= body mass index, ICD=International Classification of Diseases, HD=hemodialysis, CVVH=Continuous Veno-Venous Hemofiltration, ESR=erythrocyte sedimentation rate, CRP=C reactive protein, IL-6=interleukin 6

abnormalities and lung function impairment.<sup>24</sup> Among 70 hospitalized COVID-19 positive patients  $\geq$  age 65, Baktash et al.<sup>25</sup> found no difference in mortality between those with admission 25(OH)D levels  $\leq 12$  ng/mL ( $\leq 30$  nmol/L) and those with 25(OH)D levels  $> 12$  ng/mL; however, patients with 25(OH)D levels  $\leq 12$  ng/mL did have higher incidence of noninvasive ventilation and high dependency unit admission. Neither of these studies adjusted for any demographic characteristics.<sup>24,25</sup> Two additional studies (N = 73 and N = 156 hospitalized COVID-19 patients) have documented significant relationships between low admission 25(OH)D and mortality, as well as other indices of disease severity such as extent of total lung involvement<sup>26</sup> and ICU admission.<sup>33</sup>

**Table 2.** Multiple logistic regression for primary outcomes.

Outcome	Unadjusted		
	OR <sub>VitD</sub>	95% CI	p-value
Deceased	1.01	0.97 to 1.04	0.76
Ever-Intubated	0.98	0.94 to 1.02	0.31
Renal Replacement (HD or CVVH)	0.99	0.95 to 1.03	0.49
Outcome	Adjusted <sup>a</sup> OR <sub>VitD</sub>		
Deceased	1.00	0.96 to 1.04	0.90
Ever-Intubated	0.98	0.93 to 1.03	0.40
Renal Replacement (HD or CVVH)	1.00	0.95 to 1.04	0.81

HD = hemodialysis, CVVH = Continuous Veno-Venous Hemofiltration, OR<sub>VitD</sub> = odds ratio for a 1 unit increase in vitamin D level, CI = confidence interval  
<sup>a</sup>Logistic regression model containing vitamin D level as a continuous variable, adjusting for age, sex, and any pulmonary disease

Some additional studies addressing this issue have been posted, but not yet peer-reviewed. A retrospective multicenter study (not yet peer-reviewed) of 212 cases of COVID-19 linked low vitamin D levels to higher odds of severe and critical outcomes in three hospitals in Southern Asian countries.<sup>34</sup> A severe outcome was defined by hypoxia, respiratory distress, or abnormal blood gas results, and a critical outcome was defined as respiratory failure requiring intensive care. Analyses did not adjust for potentially confounding factors such as age and comorbidities. In one study unique for reported assessment of prehospitalization vitamin D levels,

**Table 3.** Multiple linear regression analyses for inflammatory markers.

CI	CI	CI	CI
ESR (mm/hr)	0.34	0.31	0.28
CRP (mg/L)	-0.51	0.95	0.59
Ferritin (ng/mL)	14.36	64.04	0.82
IL-6 (pg/mL)	-0.28	0.88	0.75
Outcome	Adjusted <sup>a</sup> B <sub>VitD</sub>	Standard error	p-value
ESR (mm/hr)	0.06	0.33	0.86
CRP (mg/L)	-0.35	1.02	0.73
Ferritin (ng/mL)	56.17	69.30	0.42
IL-6 (pg/mL)	-0.13	0.99	0.89

ESR = erythrocyte sedimentation rate, CRP = C reactive protein, IL-6 = interleukin 6, B<sub>VitD</sub> = regression coefficient for vitamin D

<sup>a</sup>Linear regression model containing vitamin D level as a continuous variable, adjusting for age, sex, and any pulmonary disease



Raharusuna et al.<sup>35</sup> reported (not yet peer reviewed) substantially increased COVID-related mortality associated with prehospitalization 25(OH)D level <20 ng/mL in 780 confirmed COVID-19 positive hospitalized cases in Indonesia. In this study, the overall mortality rate (48.7%) was considerably higher than in our study sample (23.7%), with a larger discrepancy in those aged 50 or more (78.8% vs 28.0% in our study). In addition to the increased sample size, the higher prevalence of the outcome of interest in the study by Raharusuna et al. may have enabled the detection of the relationship between the outcome and vitamin D status. Of note, the non-peer-reviewed findings of Raharusuna et al. have since come under scrutiny.<sup>36</sup>

*In vitro* studies investigating effects of vitamin D metabolites in respiratory epithelial cells infected with respiratory viruses support an effect of 1,25 (OH)<sub>2</sub> vitamin D to reduce the production of an array of pro-inflammatory cytokines, including TNF and IL-6.<sup>19</sup> This role of vitamin D in regulating immune function and reducing inflammation may support a potential relationship between vitamin D deficiency and inflammatory markers in COVID-19. In a small group of 37 patients, Pizzini et al. found that low 25(OH)D assessed at hospitalization was not associated with serum ferritin, CRP and IL-6; D-dimer levels were unexpectedly found to be moderately *positively* associated with 25(OH)D levels at disease onset.<sup>24</sup> In contrast, among 70 hospitalized COVID-19 positive patients  $\geq$  age 65, Baktash et al.<sup>25</sup> documented higher peak D-dimer levels in those with admission 25(OH)D levels  $\leq$ 12 ng/mL ( $\leq$ 30 nmol/L) and Gennari et al. reported an inverse relationship between 25(OH)D levels and IL-6 levels at hospital admission.<sup>33</sup> One large study indirectly examined the relationship between vitamin D, CRP levels, and COVID-19 severity using a combination of cross-sectional data from the 2009–2010 cycle of the National Health and Nutritional Examination Survey (NHANES) and clinical data from patients in China.<sup>30</sup> The authors documented an inverse relationship between vitamin D levels and CRP levels in 9211 healthy patients using cross-sectional data from NHANES. They adjusted for age and income level, but not for comorbidities or sex. They then examined the association between CRP and COVID-19 clinical outcomes among 793 patients in China, conjecturing that vitamin D deficiency may be linked to worsened COVID-19 clinical outcomes through its effect on CRP. While their sample sizes were considerably larger, enabling the detection of the aforementioned associations, vitamin D status was not examined directly in COVID-19 patients. Although our sample size is smaller, our study directly examines the relationship of vitamin D status and CRP levels among COVID-19 positive patients and found no significant association.

Because vitamin D deficiency and poor outcomes in the context of COVID-19 infection share several common risk factors including older age, non-Caucasian race, obesity, and chronic illness,<sup>1–3,5,6</sup> addressing these confounding variables in analyses is particularly important. Several of the aforementioned studies did not adjust for confounding variables.<sup>24,25,34</sup> We conducted analyses in subgroups based on age and BMI in order to reduce possible confounding by these variables. Analyses limited to subjects with age 50 and over or BMI 30 kg/m<sup>2</sup> or over did not reveal significant relationships between vitamin D level and COVID-19 clinical and inflammatory outcomes assessed.

Of note, the majority of published studies investigating the relationship between 25(OH)D levels and COVID-19 outcomes measured 25(OH)D levels at the time of disease onset/presentation. Studies assessing 25(OH)D levels at the time of hospitalization may differ from those using prehospitalization 25(OH)D levels in several ways. While assessment at the time of hospitalization avoids the biased sampling inherent in the inclusion of only those with available prehospitalization levels, it may be subject to the effect of critical illness on vitamin D binding protein, thus impacting the total vitamin D level.<sup>27–29</sup> Differential effects of binding protein status may be one explanatory factor for differences in relationships seen using pre-hospitalization compared to at-hospitalization 25(OH)D levels.

Our study has several limitations. Most notably, the small number of subjects with available 25(OH)D levels may have limited our ability to detect differences between those with and without vitamin D deficiency. We had 80% power with a two-tailed alpha of 5% to detect a 28% point increase in mortality, assuming that the mortality in the non-vitamin D deficient group was 20%. Due to limited sample size, we included vitamin D levels obtained within 365 days prior to admission.<sup>17</sup> Vitamin D levels were only available in a small subset of patients and those with a 25(OH)D level differed from those patients without available 25(OH)D level in that they were more likely to have underlying comorbidities. Thus, our analysis may not be generalizable to those who had fewer comorbidities, were healthier and had a lower risk of dying. It is possible that those with available 25(OH)D measurements represented a cohort with more interaction with the healthcare system prior to the COVID-related admission. Such individuals may have benefitted from better prehospitalization management of underlying chronic conditions, which may have lowered risk for COVID-related complications, affecting our ability to detect between-group differences. Additionally, such individuals may have been more likely to receive vitamin D supplementation prior to admission. Unfortunately, data on vitamin D supplementation were not available. In this

cohort, data on race were obtained from the medical record and data input on admission. Sample size was limited and categorization was incomplete due to missing data. Other limitations relate to the measurement of 25(OH)D levels in this study. A chemiluminescent immunoassay was utilized to obtain the majority of 25(OH)D levels in this study. Chemiluminescent immunoassays are known to have variable fidelity in the measurement of 25(OH)D<sub>2</sub>, which can result in underestimation of 25(OH)D, especially in patients who are taking vitamin D<sub>2</sub>.<sup>37</sup> Underestimation may result in misclassification of patients into the vitamin D deficient group. While the majority of 25(OH)D levels were measured at our institution, 18% of 25(OH)D levels available for analysis were measured at other labs, which may have used different assays. Analyses limited to those with 25(OH)D levels measured at our institution yielded similar results. Finally, only one 25(OH)D measure was used, which may not be representative of patients' overall 25(OH)D status.

Our study also has several strengths. The use of the comprehensive COVID-Care database allowed us to investigate previously unreported relationships between vitamin D status and detailed information on clinical outcomes including intubation, length of hospital stay, and need for renal replacement among others. Second, we examined prehospitalization vitamin D levels, which may better assess vitamin D status than measures obtained at hospitalization when critical illness can affect vitamin D binding protein, thus impacting the total vitamin D level.<sup>27–29</sup> In order to reduce the effect of possible confounders, we adjusted for age, sex, and pulmonary disease in regression analyses, and conducted subgroup analyses based on age, sex, and BMI in order to reduce possible confounding by these variables. Finally, our analysis included a diverse group of patients including men and women with variable BMI and comorbidities that may increase risk for complications related to COVID-19.<sup>38–40</sup>

In summary, this study is the first examination of the relationship between prehospitalization vitamin D status and mortality, intubation, length of hospital stay, need for renal replacement, and various inflammatory markers amongst a cohort of COVID-19 positive hospitalized patients. Our study adds to a small body of literature investigating the association of vitamin D deficiency with COVID-19 outcomes with mixed results. Our findings do not support a relationship between prehospitalization vitamin D deficiency and mortality, respiratory, or inflammatory outcomes in COVID-19 positive hospitalized adults. More definitive investigation of our hypotheses would benefit from assessment of vitamin D levels in a larger sample of COVID-19 positive patients, with adjustment for

multiple covariates.

## Disclosure Statement

The authors have nothing to disclose.

## Data Availability

All data/results generated during this study are included in this published article or in the data repositories listed in References. All datasets analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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