Original article

# Association between 25-hydroxyvitamin D levels and COVID-19 severity

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## ARTICLE INFO

## SUMMARY

Background and aims: Despite reports on the impact of vitamin D status on coronavirus disease 2019 (COVID-19) severity, the association between low vitamin D status and severe COVID-19 remains unclear. Moreover, researchers have not determined the aforementioned association in Japanese patients. This study aimed to investigate the association between 25-hydroxyvitamin D [25(OH)D] levels and COVID-19 severity in Japanese patients.

Methods: This retrospective observational study included 117 consecutive patients with COVID-19 admitted to the Kobe City Medical Center General Hospital between October 01, 2020, and January 31, 2021. We measured the serum 25(OH)D levels using blood specimens collected within 5 days of hospital admission using liquid chromatography-tandem mass spectrometry.

Results: There were 21 (17.9%), 73 (62.4%), 19 (16.2%) and 4 (3.4%) patients with severe deficiency (<10 ng/mL), deficiency (10−<20 ng/mL), insufficiency (20−<30 ng/mL), and sufficiency (≥30 ng/mL) of vitamin D, respectively. In univariate logistic regression analyses, lower serum 25(OH)D levels [odds ratio (OR) 1.18 per 1 ng/mL decrease, 95% confidence interval (CI) 1.04−1.33, p = 0.007] were significantly associated with invasive mechanical ventilation (IMV) or death. In a multivariate logistic regression analysis, low serum 25(OH)D levels [OR 1.22 per 1 ng/mL decrease, 95% CI 1.06−1.40, p = 0.005] were significantly associated with IMV or death. The cut-off value of serum 25(OH)D levels was 10.4 ng/mL, calculated by the receiver operating characteristic curve to detect the requirement for IMV or death.

Conclusions: To the best of our knowledge, this is the first study to assess the association between vitamin D status and COVID-19 severity in Japanese patients. Low serum 25(OH)D level was detected as an independent risk factor for severe COVID-19 among Japanese patients.

## 1. Introduction

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). As of October 31, 2021, the COVID-19 pandemic has rapidly spread globally, causing more than 246 million confirmed infections and approximately 5 million deaths worldwide [1]. Severe COVID-19 causes severe pneumonia mediated by immune dysregulation [2].

Vitamin D is a fat-soluble vitamin implicated in the modulation of both innate and adaptive immune responses as well as bone

metabolism [3]. Therefore, the potential effects of vitamin D have been focused on preventing severe COVID-19. Serum 25-hydroxyvitamin D [25(OH)D] levels are used as the most reliable marker of vitamin D status [4]. It has been reported that an estimated 1 billion people have vitamin D insufficiency [serum 25(OH) D level 20—<30 ng/mL] or deficiency [serum 25(OH)D level <20 ng/mL] [5].

Prior to the COVID-19 pandemic, a meta-analysis reported an association between low serum 25(OH)D levels and the risk and severity of acute respiratory tract infections [6]. Low serum 25(OH) D level has been reported as an independent risk factor for severe COVID-19 worldwide [7—18]. In contrast, some studies have also reported no association between low serum 25(OH)D levels and severe COVID-19 [19—25]. Thus, the role of low vitamin D status as a risk factor for severe COVID-19 remains controversial. The pathology of COVID-19 is complex and remains unclear, thus warranting research to elucidate the exact association between serum 25(OH)D levels and COVID-19 severity. Despite reports on the effects of genetic variants associated with severe COVID-19 [26], there are no data on the association between serum 25(OH)D levels and COVID-19 severity in Japanese patients.

Herein, we aimed to investigate the association between 25(OH) D levels and COVID-19 severity in Japanese patients.

## 2. Materials and methods

### 2.1. Study design

This single-center, retrospective observational study was conducted in accordance with the tenets of the Declaration of Helsinki and the Ethical Guidelines for Epidemiological Research by the Ministry of Education, Culture, Sports, Science, and Technology and the Ministry of Health, Labor and Welfare of Japan. The protocol was approved by the institutional review board of the Kobe City Medical Center General Hospital, Japan (Approval No. zn210703).

### 2.2. Patients

We evaluated the records of consecutive patients with COVID-19 admitted to the Kobe City Medical Center General Hospital, Japan, from October 01, 2020, to January 31, 2021. The inclusion criteria were as follows: 1) Japanese; 2) age ≥18 years; 3) positive results for COVID-19 on reverse transcription polymerase chain reaction assays for SARS-CoV-2; and 4) informed written consent for testing blood specimens. Patients receiving vitamin D supplementation prior to the diagnosis of COVID-19 were excluded [19—21]. A total of 117 patients were included in the present study, all of whom were unvaccinated against COVID-19. Pregnant women were not included in this study.

### 2.3. Definitions and data collection

The severity of COVID-19 was defined according to the World Health Organization (WHO) COVID-19 ordinal scale of clinical improvement [27]. We evaluated the maximum scores of the aforementioned scale during the hospitalization of the selected patients. Laboratory data of patients, except serum 25(OH)D levels, were assessed upon hospital admission. We collected blood specimens for measuring serum 25(OH)D levels within 5 days of hospitalization [28].

### 2.4. Measurement of serum 25(OH)D levels

Serum was stored at −80 °C until assessment. We measured serum vitamin D metabolites by the modified liquid chromatography

tandem mass spectrometry method [29]. The modification point was derived from extracted vitamin D metabolites by 4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalyl) ethyl]-1,2,4-triazoline-3,5-dione to obtain high sensitivity by increasing ionization efficiency [30]. The intra- and inter-assay coefficients of variation were 3.4—9.2% and 11.9% for 25(OH)D and 13.1—19.3% and 14.7% for 24,25-dihydroxy vitamin D, respectively. The accuracy of the assay was validated using SRM 972a and SRM2973 provided by the National Institute of Standards [31]. We calculated the total serum 25(OH)D level by their summation. These levels were categorized as follows: severe deficiency (<10 ng/mL), deficiency (10—<20 ng/mL), insufficiency (20—<30 ng/mL) and sufficiency (≥30 ng/mL) [32].

### 2.5. Outcomes

The need for invasive mechanical ventilation (IMV) or death [14] was the primary outcome, and the need for oxygen therapy was the secondary outcome.

### 2.6. Statistical analyses

Statistical analyses were performed using JMP 13.2.1 (SAS Institute Inc., Cary, NC, USA). For continuous data, values are presented as median (interquartile range [IQR]). For categorical data, values are presented as number (percentage). We performed the Fisher's exact test and Wilcoxon rank-sum test for two-group comparisons. To identify risk factors for the severity of COVID-19, we conducted univariate logistic regression analyses using serum 25(OH)D levels and clinical variables associated with the severity of COVID-19, such as diabetes, chronic obstructive pulmonary disease (COPD), age, smoking, hypertension, body mass index (BMI), cancer, chronic kidney disease (CKD), and male sex [33—39]. Significant factors in univariate analyses were evaluated as potential covariates in the multivariate logistic regression analyses. Moreover, we performed the Cochran-Armitage trend test to evaluate a trend in the reduction of outcome rates with an increase in serum 25(OH)D levels. For serum 25(OH)D levels, we performed receiver operating characteristic (ROC) analyses to determine the optimal cut-off point for predicting the severity of COVID-19. All p-values <0.05 were considered statistically significant.

## 3. Results

### 3.1. Severity of COVID-19 during hospitalization

Table 1 summarizes the maximum scores of the WHO COVID-19 ordinal scale of clinical improvement during hospitalization. During hospitalization, 69 patients (59.0%) required oxygen therapy, and 14 (12.0%) required IMV or died.

### 3.2. Patient characteristics

The median concentration of serum 25(OH)D was 14.8 ng/mL (IQR 11.0—18.8) in all patients. Twenty-one (17.9%), 73 (62.4%), 19 (16.2%) and 4 (3.4%) patients had a severe deficiency, deficiency, insufficiency and sufficiency of vitamin D, respectively.

Table 2 summarizes patient characteristics stratified by the need for oxygen therapy during hospitalization. The age and BMI of patients who received oxygen therapy were significantly higher than that of those without oxygen therapy. In contrast, serum 25(OH)D levels, albumin levels, and creatinine clearance were significantly lower in patients who received oxygen therapy than that in those who did not. Moreover, patients who received oxygen therapy demonstrated a higher prevalence of smoking, hypertension, diabetes, and dyslipidemia than that in those without oxygen therapy.

**Table 1**
Maximum score of the WHO COVID-19 ordinal scale of clinical improvement during hospitalization.

| | Maximum score during hospitalization (n = 117) |
|---|---|
| Non-hospitalization, n (%) | |
| 1. No limitation of activities | 0 (0%) |
| 2. Limitation of activities | 0 (0%) |
| Hospitalization, n (%) | |
| 3. Not-required oxygen | 48 (41.0%) |
| 4. Required oxygen by mask or nasal prongs | 50 (42.7%) |
| 5. Required noninvasive ventilation or high-flow oxygen | 5 (4.3%) |
| 6. Required intubation and mechanical ventilation | 9 (7.7%) |
| 7. Required ventilation and additional organ support (pressor, RRT, ECMO) | 0 (0%) |
| 8. Death | 5 (4.3%) |

COVID-19, Coronavirus disease 2019; ECMO, extracorporeal membrane oxygenation; RRT, renal replacement therapy; and WHO, World Health Organization.

Table 3 outlines patient characteristics stratified by IMV requirement or death. The age and albumin-corrected calcium levels of patients who received IMV or died were significantly higher than that of those who did not. Conversely, serum 25(OH)D levels and albumin levels were significantly lower in patients who received IMV or died than that in those who did not. The prevalence

of diabetes and the concomitant use of angiotensin converting enzyme inhibitors (ACE-I) or angiotensin II receptor blockers (ARB) was higher in patients who received IMV or died than that in those who did not.

### 3.3. Logistic regression analyses for the primary and secondary outcomes

Tables 4 and 5 summarize the findings of the univariate and multivariate logistic regression analyses to identify risk factors for the need of oxygen therapy and IMV or death during hospitalization, respectively.

#### 3.3.1. Univariate analysis

In univariate logistic regression analyses, lower serum 25(OH)D levels [odds ratio (OR) 1.10 per 1 ng/mL decrease, 95% confidence interval (CI) 1.03–1.17, $p = 0.005$], diabetes (OR 9.07, 95% CI 2.56–32.17, $p = 0.001$), age $\geq$65 years (OR 5.69, 95% CI 2.24–14.42, $p < 0.001$), smoking (OR 3.42, 95% CI 1.57–7.46, $p = 0.002$), and hypertension (OR 3.97, 95% CI 1.67–9.44, $p = 0.002$) were significantly associated with oxygen therapy (Table 4). Lower serum 25(OH)D levels (OR 1.18 per 1 ng/mL decrease, 95% CI 1.04–1.33, $p = 0.007$), diabetes (OR 5.21, 95% CI 1.63–16.64, $p = 0.005$), and COPD (OR 8.42, 95% CI 1.08–65.33, $p = 0.042$) were significantly associated with IMV or death (Table 5).

**Table 2**
Patient characteristics on receiving oxygen therapy during hospitalization.

| | Overall (n = 117) | Receiving oxygen therapy | | P-value |
|---|---|---|---|---|
| | | Yes (n = 69) | No (n = 48) | |
| Age, median (IQR), years | 56 (45–70) | 64 (54–75) | 44 (28–55) | <0.001[a] |
| Male/Female, n | 83/34 | 52/17 | 31/17 | 0.221[b] |
| BMI, median (IQR), kg/m² | 23 (21–27) | 24 (23–27) | 22 (20–25) | 0.012[a] |
| Smoking, n (%) | 57 (48.7%) | 42 (60.9%) | 15 (31.3%) | 0.002[b] |
| Comorbidity, n (%) | | | | |
| Hypertension | 42 (35.9%) | 33 (47.8%) | 9 (18.8%) | 0.002[b] |
| Diabetes | 29 (24.8%) | 26 (37.7%) | 3 (6.3%) | <0.001[b] |
| Dyslipidemia | 27 (23.1%) | 22 (31.9%) | 5 (10.4%) | 0.007[b] |
| CKD | 9 (7.7%) | 8 (11.6%) | 1 (2.1%) | 0.080[b] |
| Asthma | 4 (3.4%) | 3 (4.4%) | 1 (2.1%) | 0.643[b] |
| COPD | 4 (3.4%) | 4 (5.8%) | 0 (0%) | 0.143[b] |
| Coronary artery disease | 4 (3.4%) | 4 (5.8%) | 0 (0%) | 0.143[b] |
| Liver cirrhosis | 4 (3.4%) | 2 (2.9%) | 2 (4.2%) | 1.000[b] |
| Cancer | 3 (2.6%) | 1 (1.5%) | 2 (4.2%) | 0.567[b] |
| ESRD | 2 (1.7%) | 2 (2.9%) | 0 (0%) | 0.512[b] |
| Heart failure | 1 (0.9%) | 1 (1.5%) | 0 (0%) | 1.000[b] |
| 25(OH)D, median (IQR), ng/mL | 14.8 (11.0–18.8) | 14 (10.1–17.1) | 16.1 (11.6–21.6) | 0.011[a] |
| Albumin, median (IQR), g/dL | 3.5 (3.1–4.1) | 3.2 (2.9–3.5) | 4.1 (3.7–4.4) | <0.001[a] |
| Albumin-corrected calcium, median (IQR), mg/dL | 9.2 (8.9–9.4) | 9.2 (8.9–9.6) | 9.1 (8.9–9.3) | 0.136[a] |
| CrCl, median (IQR), mL/min | 94.4 (67.1–114.8) | 83.1 (60.6–114.8) | 105.9 (86.0–117.3) | 0.008[a] |
| Phosphorus, median (IQR), mg/dL | 3.1 (2.5–3.5) | 3.0 (2.5–3.5) | 3.1 (2.2–3.2) | 0.548[a] |
| Concomitant medication before admission, n (%) | | | | |
| Aspirin | 5 (4.3%) | 5 (7.3%) | 0 (0%) | 0.077[b] |
| ACE-I or ARB | 24 (20.5%) | 17 (24.6%) | 7 (14.6%) | 0.246[b] |
| Concomitant medication during hospitalization, n (%) | | | | |
| Dexamethasone | 61 (52.1%) | 60 (87.0%) | 1 (2.1%) | <0.001[b] |
| Remdesivir | 29 (24.8%) | 29 (42.0%) | 0 (0%) | <0.001[b] |
| Tocilizumab | 1 (0.9%) | 1 (1.5%) | 0 (0%) | 1.000[b] |
| Length of hospital stay, median (IQR), days | 7 (5–11) | 10 (7–13) | 4 (2–7) | <0.001[a] |
| Oxygen therapy, n (%) | 69 (59.0%) | 69 (100%) | 0 (0%) | <0.001[b] |
| Invasive mechanical ventilation, n (%) | 12 (10.3%) | 12 (17.4%) | 0 (0%) | 0.001[b] |
| Mortality, n (%) | 5 (4.3%) | 5 (7.3%) | 0 (0%) | 0.077[b] |

ACE-I, angiotensin converting enzyme-inhibitor; ARB, angiotensin II receptor blocker BMI, body mass index; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; CrCl, creatinine clearance; ESRD, end-stage renal disease; IQR, interquartile range; and 25(OH)D, 25-hydroxyvitamin D.
<sup>a</sup> Wilcoxon rank-sum test.
<sup>b</sup> Fisher's exact test.

**Table 3**
Patient characteristics on receiving invasive mechanical ventilation or death during hospitalization.

| | Overall (n = 117) | Receiving invasive mechanical ventilation or death | | P-value |
| --- | --- | --- | --- | --- |
| | | Yes (n = 14) | No (n = 103) | |
| Age, median (IQR), years | 56 (45–70) | 65 (62–83) | 55 (44–70) | 0.007[a] |
| Male/Female, n | 83/34 | 11/3 | 72/31 | 0.755[b] |
| BMI, median (IQR), kg/m$^2$ | 23 (21–27) | 26 (23–27) | 23 (21–26) | 0.067[a] |
| Smoking, n (%) | 57 (48.7%) | 7 (50.0%) | 50 (48.5%) | 1.000[b] |
| Comorbidity, n (%) | | | | |
| Hypertension | 42 (35.9%) | 8 (57.1%) | 34 (33.0%) | 0.135[b] |
| Diabetes | 29 (24.8%) | 8 (57.1%) | 21 (20.4%) | 0.006[b] |
| Dyslipidemia | 27 (23.1%) | 6 (42.9%) | 21 (20.4%) | 0.087[b] |
| CKD | 9 (7.7%) | 2 (14.3%) | 7 (6.8%) | 0.293[b] |
| Asthma | 4 (3.4%) | 0 (0%) | 4 (3.9%) | 1.000[b] |
| COPD | 4 (3.4%) | 2 (14.3%) | 2 (1.9%) | 0.070[b] |
| Coronary artery disease | 4 (3.4%) | 1 (7.1%) | 3 (2.9%) | 0.404[b] |
| Liver cirrhosis | 4 (3.4%) | 0 (0%) | 4 (3.9%) | 1.000[b] |
| Cancer | 3 (2.6%) | 1 (7.1%) | 2 (1.9%) | 0.320[b] |
| ESRD | 2 (1.7%) | 1 (7.1%) | 1 (1.0%) | 0.226[b] |
| Heart failure | 1 (0.9%) | 0 (0%) | 1 (1.0%) | 1.000[b] |
| 25(OH)D, median (IQR), ng/mL | 14.8 (11.0–18.8) | 10.3 (5.9–14.9) | 15.1 (11.6–19.3) | 0.007[a] |
| Albumin, median (IQR), g/dL | 3.5 (3.1–4.1) | 3.2 (2.4–3.5) | 3.5 (3.1–4.2) | 0.017[a] |
| Albumin-corrected calcium, median (IQR), mg/dL | 9.2 (8.9–9.4) | 9.3 (8.9–9.8) | 9.1 (8.9–9.4) | 0.042[a] |
| CrCl, median (IQR), mL/min | 94.4 (67.1–114.8) | 72.8 (49.3–91.0) | 97.9 (69.6–117.3) | 0.051[a] |
| Phosphorus, median (IQR), mg/dL | 3.1 (2.5–3.5) | 2.9 (2.3–3.2) | 3.1 (2.5–3.6) | 0.163[a] |
| Concomitant medication before admission, n (%) | | | | |
| Aspirin | 5 (4.3%) | 2 (14.3%) | 3 (2.9%) | 0.108[b] |
| ACE-I or ARB | 24 (20.5%) | 6 (42.9%) | 18 (17.5%) | 0.038[b] |
| Concomitant medication during hospitalization, n (%) | | | | |
| Dexamethasone | 61 (52.1%) | 14 (100%) | 47 (45.6%) | <0.001[b] |
| Remdesivir | 29 (24.8%) | 10 (71.4%) | 19 (18.5%) | <0.001[b] |
| Tocilizumab | 1 (0.9%) | 1 (7.1%) | 0 (0%) | 0.120[b] |
| Length of hospital stay, median (IQR), days | 7 (5–11) | 17 (11–20) | 7 (4–10) | <0.001[a] |
| Oxygen therapy, n (%) | 69 (59.0%) | 14 (100%) | 55 (53.4%) | <0.001[b] |
| Invasive mechanical ventilation, n (%) | 12 (10.3%) | 12 (85.7%) | 0 (0%) | <0.001[b] |
| Mortality, n (%) | 5 (4.3%) | 5 (35.7%) | 0 (0%) | <0.001[b] |

ACE-I, angiotensin converting enzyme-inhibitor; ARB, angiotensin II receptor blocker; BMI, body mass index; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; CrCl, creatinine clearance; ESRD, end-stage renal disease; IQR, interquartile range; and 25(OH)D, 25-hydroxyvitamin D.
[a] Wilcoxon rank-sum test.
[b] Fisher's exact test.

**Table 4**
Univariable and multivariable logistic regression analyses for receiving oxygen therapy.

| Variable | Univariable | | | Multivariable | | |
| --- | --- | --- | --- | --- | --- | --- |
| | OR | 95% CI | P-value | OR | 95% CI | P-value |
| 25(OH)D (per 1 ng/mL decrease) | 1.10 | 1.03–1.17 | 0.005 | 1.09 | 1.00–1.18 | 0.039 |
| Diabetes | 9.07 | 2.56–32.17 | 0.001 | 7.44 | 1.78–31.20 | 0.006 |
| COPD | NA | NA | NA | | | |
| Age ≥65 years | 5.69 | 2.24–14.42 | <0.001 | 4.74 | 1.56–14.42 | 0.006 |
| Smoking | 3.42 | 1.57–7.46 | 0.002 | 4.94 | 1.86–13.10 | 0.001 |
| Hypertension | 3.97 | 1.67–9.44 | 0.002 | 2.36 | 0.83–6.72 | 0.108 |
| BMI (per 1 kg/m$^2$ increase) | 1.09 | 0.99–1.20 | 0.071 | | | |
| Cancer | 0.34 | 0.03–3.84 | 0.382 | | | |
| CKD | 6.16 | 0.74–51.01 | 0.092 | | | |
| Male sex | 1.68 | 0.75–3.76 | 0.208 | | | |

BMI, body mass index; CI, confidence interval; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; CrCl, creatinine clearance; ESRD, end-stage renal disease; OR, odds ratio; NA, not applicable; and 25(OH)D, 25-hydroxyvitamin D.

### 3.3.2. Multivariate analysis

In multivariate logistic regression analyses, lower serum 25(OH)D levels (OR 1.09 per 1 ng/mL decrease, 95% CI 1.00–1.18, p = 0.039), diabetes (OR 7.44, 95% CI 1.78–31.20, p = 0.006), age ≥65 years (OR 4.74, 95% CI 1.56–14.42, p = 0.006), and smoking (OR 4.94, 95% CI 1.86–13.10, p = 0.001) were detected as independent risk factors for oxygen therapy (Table 4). Lower serum 25(OH)D levels (OR 1.22 per 1 ng/mL decrease, 95% CI 1.06–1.40, p = 0.005) and diabetes (OR 7.03, 95% CI 1.86–26.53, p = 0.004) were detected as independent risk factors for IMV or death (Table 5).

### 3.4. Stratified analyses by 25(OH)D levels for the primary and secondary outcomes

Figure 1A depicts the associations between the rate of receiving oxygen therapy and serum 25(OH)D levels. The rate of receiving oxygen therapy by serum 25(OH)D levels were 76.2% (<10 ng/mL), 63.9% (10–<20 ng/mL), 30.0% (20–<30 ng/mL), and 25.0% (≥30 ng/mL). Figure 1B depicts the associations between the rate of receiving IMV or death and serum 25(OH)D levels. The rate of receiving IMV or death by serum 25(OH)D levels were 28.6%

**Table 5**
Univariable and multivariable logistic regression analyses for receiving invasive mechanical ventilation or death.

| Variable | Univariable | | | Multivariable | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | P-value | OR | 95% CI | P-value |
| 25(OH)D (per 1 ng/mL decrease) | 1.18 | 1.04—1.33 | 0.007 | 1.22 | 1.06—1.40 | 0.005 |
| Diabetes | 5.21 | 1.63—16.64 | 0.005 | 7.03 | 1.86—26.53 | 0.004 |
| COPD | 8.42 | 1.08—65.33 | 0.042 | 4.75 | 0.53—42.40 | 0.163 |
| Age ≥65 years | 2.83 | 0.91—8.81 | 0.073 | | | |
| Smoking | 1.06 | 0.35—3.24 | 0.919 | | | |
| Hypertension | 2.71 | 0.87—8.42 | 0.086 | | | |
| BMI (per 1 kg/m² increase) | 1.09 | 0.95—1.25 | 0.205 | | | |
| Cancer | 3.88 | 0.33—45.88 | 0.281 | | | |
| CKD | 2.29 | 0.43—12.29 | 0.335 | | | |
| Male sex | 1.58 | 0.41—6.05 | 0.506 | | | |

BMI, body mass index; CI, confidence interval; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; CrCl, creatinine clearance; ESRD, end-stage renal disease; OR, odds ratio; and 25(OH)D, 25-hydroxyvitamin D.

(<10 ng/mL), 9.7% (10—<20 ng/mL), 5.0% (20—<30 ng/mL), and 0% (≥30 ng/mL). The Cochran-Armitage trend test displayed significant trends in reduced rates of receiving oxygen therapy and, receiving IMV or death with an increase in serum 25(OH)D levels (p = 0.001 and p = 0.017, respectively).

### 3.5. ROC analyses to identify optimal cut-off values of 25(OH)D levels for the primary and secondary outcomes

The cut-off value of serum 25(OH)D levels was 18.8 ng/mL [Area under the curve (AUC) = 0.64, p = 0.003], as calculated by the ROC curve to detect the need for oxygen (Fig. 2A). On the other hand, the value of serum 25(OH)D levels was 10.4 ng/mL (AUC = 0.72, p = 0.003), as calculated by the ROC curve to detect the need for IMV or death (Fig. 2B).

## 4. Discussion

In this study, we investigated the association between serum 25(OH)D levels and COVID-19 severity in Japanese patients. Low serum 25(OH)D level was an independent risk factor for oxygen therapy, IMV or death. In the stratified analysis by 25(OH)D levels, the rates of receiving oxygen therapy, IMV or death reduced with increasing serum 25(OH)D levels. To the best of our knowledge, this is the first study to identify low serum 25(OH)D level as a risk factor of severe COVID-19 in Japanese patients.

Vitamin D requires two hydroxylation steps for activation. First, it gets metabolized by 25-hydroxylase in the liver to 25(OH)D. Second, 25(OH)D 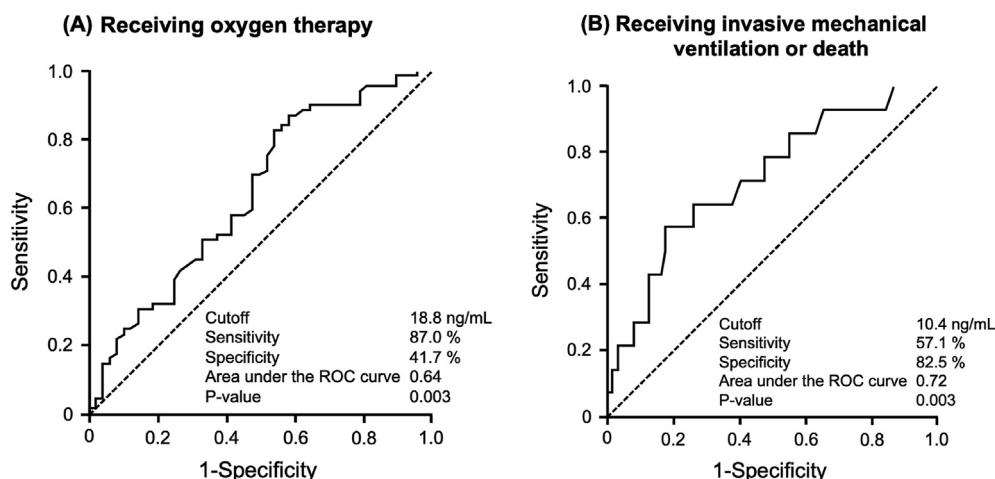gets metabolized by 1α-hydroxylase in the kidney to 1 alpha, 25-dihydroxy vitamin D [1,25(OH)₂D], which is an active form of vitamin D. 1,25(OH)₂D regulates not only calcium metabolism but also the immune system, such as innate and adaptive immune responses. 1,25(OH)₂D induces the expression of cathelicidin and defensin, which are antimicrobial peptides, and of genes typical for regulatory T cells. Furthermore, it inhibits the secretion of proinflammatory T helper (Th) 1 cell cytokines (e.g., interleukin [IL] 2, IL9, IL22, interferon-γ, and tumor necrosis factor α) and promotes the production of the more anti-inflammatory Th2 cytokines (e.g., IL3, IL4, IL5, and IL10) [40]. However, 1α-hydroxylase is tightly regulated by the parathyroid hormone, fibroblast growth factor 23, as well as 1,25(OH)₂D. Contrarily, serum 25(OH)D levels increase with vitamin D intake; therefore, these are used as markers of the vitamin D status.

Prior to the COVID-19 pandemic, a meta-analysis found a significant association between low serum 25(OH)D levels and the severity of acute respiratory tract infections [5]. In addition, a meta-analysis of randomized controlled trials showed that daily or weekly vitamin D supplementation significantly reduced the risk of acute respiratory tract infections [41].

Observational studies worldwide have also found low serum 25(OH)D levels as an independent risk factor for severe COVID-19 [7—18]. Despite inconsistent definitions of severe COVID-19 as outcomes, many studies have demonstrated a significant association between low serum 25(OH)D levels [as a continuous variable [7—9], <10 ng/mL [10,11], <12 ng/mL [13—15], <12.5 ng/mL [16] or <20 ng/mL [12,14,17,18] and the severity of COVID-19 in multivariate analyses. In contrast, no association between low serum 25(OH)D levels and severe COVID-19 has also been reported in
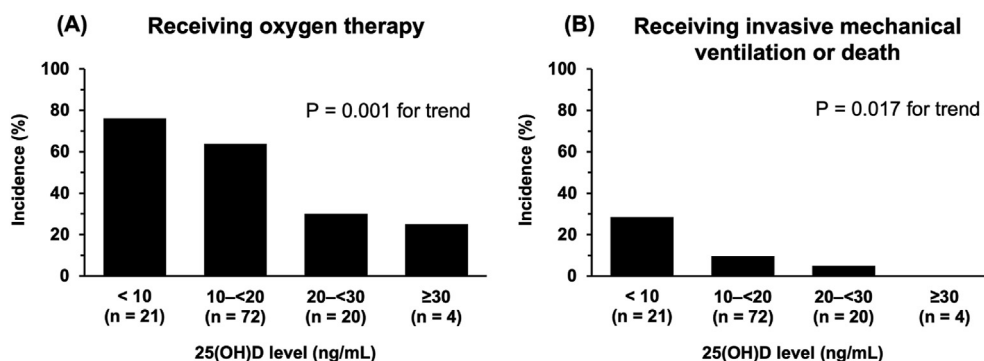


**Fig. 1.** Incidence of (A) receiving oxygen therapy and (B) receiving invasive mechanical ventilation or death based on serum 25(OH)D levels. The rate of (A) receiving oxygen therapy and, (B) receiving invasive mechanical ventilation or death are compared by serum 25(OH)D levels (severe vitamin D deficiency [<10 ng/mL], deficiency [10—<20 ng/mL], insufficiency [20—<30 ng/mL] and sufficiency [≥30 ng/mL]). The Cochran-Armitage trend test displays a trend in reduced rates of (A) receiving oxygen therapy and, (B) receiving IMV or death with increased serum 25(OH)D levels (p = 0.001 and p = 0.017, respectively). IMV, invasive mechanical ventilation; 25(OH)D, 25-hydroxyvitamin D.

**(A) Receiving oxygen therapy**

| | |
|---|---|
| Cutoff | 18.8 ng/mL |
| Sensitivity | 87.0 % |
| Specificity | 41.7 % |
| Area under the ROC curve | 0.64 |
| P-value | 0.003 |

**(B) Receiving invasive mechanical ventilation or death**

| | |
|---|---|
| Cutoff | 10.4 ng/mL |
| Sensitivity | 57.1 % |
| Specificity | 82.5 % |
| Area under the ROC curve | 0.72 |
| P-value | 0.003 |

**Fig. 2.** Area under ROC curves obtained by the univariate logistic regression analysis of serum 25(OH)D levels for (A) receiving oxygen therapy and, (B) receiving invasive mechanical ventilation or death. ROC, receiver operating characteristic; 25(OH)D, 25-hydroxyvitamin D.

other observational studies [19—25]. Therefore, the status of vitamin D as a risk factor for severe COVID-19 remains controversial. Ben-Eltriki et al. performed a meta-analysis of 24 observational studies comprising 3637 participants, based on original research studies published up to March 30, 2021. They reported that low serum 25(OH)D levels (<10 to <30 ng/mL) were significantly associated with a higher risk of death or developing severe COVID-19 pneumonia [42]. However, more studies are required to confirm the association between low serum 25(OH)D levels and severe COVID-19, considering the insufficient number of participants, ethnicity, or differences in treatment of COVID-19 in each hospital.

We measured serum 25(OH)D levels using blood specimens collected within 5 days of hospital admission. The half-life of serum 25(OH)D is 2—3 weeks [43]; therefore, serum 25(OH)D levels measured within 5 days of hospital admission were equated with those measured during hospital admission. We performed logistic regression analyses to assess the risk factors of severe COVID-19 using factors such as diabetes, COPD, age, smoking, hypertension, BMI, cancer, CKD, and male sex, which were previously reported in meta-analyses [33—39]. We did not exclude confounding factors associated with COVID-19 severity and vitamin D levels from the beginning to analyze with various diseases related to severe COVID-19. Consequently, we identified low serum 25(OH)D level as an independent risk factor for oxygen therapy, IMV or death. In the stratified analyses by 25(OH)D levels, the Cochran-Armitage trend test displayed significant trends of reduced rates of receiving oxygen therapy and, receiving IMV or death with increased serum 25(OH)D levels. In other words, it demonstrated the possible association between low serum 25(OH)D levels and severe COVID-19. The ROC analysis demonstrated that the cut-off value of serum 25(OH)D levels to detect IMV requirement or death was 10.4 ng/mL, consistent with previously reported cut-off values of <10 ng/mL [10,11], <12 ng/mL [13—15] or <12.5 ng/mL [16] for severe COVID-19.

In univariate logistic regression analysis, other than low serum 25(OH)D levels, diabetes and COPD were significantly associated with IMV requirement or death (Table 5). Subsequently, in multivariate logistic regression analysis, other than low serum 25(OH)D levels, only diabetes was detected as an independent risk factor for IMV or death (Table 5). Although it is not clear why diabetes is associated with increased severity of COVID-19, the presence of an underlying chronic inflammatory state and impaired immune response in patients with diabetes may contribute to severe COVID-

19 [44]. Besides, low serum 25(OH)D level have been suggested as a risk factor of diabetes [45]. However, in this study, both of them were detected as independent risk factors for IMV or death. While COPD was significantly associated with IMV requirement or death in univariate analysis, there was no association among them in multivariate analysis. A possible reason is that the number of COPD patients with COVID-19 was small.

Albumin levels were significantly lower in patients who received IMV or died than that in those who did not (Table 3). Hypoalbuminemia may occur due to pulmonary capillary leakage as well as decreased hepatic synthesis of albumin by acute inflammation and catabolism by oxidative stress in the presence of COVID-19 [46]. Although it was not clear whether these responses occurred at hospital admission, hypoalbuminemia might occur as a result of progression of COVID-19 in this study.

The concomitant use of ACE-I or ARB was higher in patients who received IMV or died than that in those who did not (Table 3). Whether these medicines had a negative impact on COVID-19 was discussed in the early phase of the COVID-19 pandemic. However, subsequently, this hypothesis was not supported by the large randomized controlled trial [47]. In fact, careful interpretation is needed to avoid misinterpretation of the results, because these medicines are used against the diseases associated with severe COVID-19 such as diabetes, CKD and heart failure.

To summarize, we identified low serum 25(OH)D level as a risk factor of severe COVID-19 in Japanese patients. In other words, low serum 25(OH)D levels were associated with severe COVID-19.

This study had some limitations. First, few patients were retrospectively analyzed at a single center; thus, we could not generalize our results to other settings. Second, we did not consider the possible impact of seasonal variations on serum 25(OH)D levels from October 2020 to January 2021. Third, in our study, the association of serum 25(OH)D level with COVID-19 severity may be the result of reverse causality due to observational design. Whether COVID-19 leads to a reduction in serum 25(OH)D level remains controversial and unclear [48]. However, we considered that the serum 25(OH)D levels which we measured were likely to reflect the status before SARS-CoV-2 infection, since the half-life of serum 25(OH)D is 2—3 weeks. Fourth, SARS-CoV-2 variants were not identified in our patients, similar to previous reports. For reference, B.1.1.214 and B.1.1.284 were the most common COVID-19 variants in Japan during the study period [49].

## 5. Conclusions

To the best of our knowledge, this is the first study to assess the association between vitamin D status and COVID-19 severity in Japanese patients. Low serum 25(OH)D level was detected as an independent risk factor for oxygen therapy, IMV or death in Japanese patients with COVID-19.

## Ethics approval and consent on the participation

Approval No: zn210703.

## Authors' contributions

TT and NT conceived and designed this study. ME dispensed blood samples for measuring the serum 25(OH)D levels, whereas TT, NT, and TS pretreated blood samples for that. NT measured the serum 25(OH)D levels. TT and TS collected patient data from electronic medical records. TT and NT analyzed data. HI, TH, KT and NM supervised the conduct of this study. TT and NT drafted the manuscript. All authors have substantially contributed to its revision. All authors have read and approved the final manuscript.

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## Declaration of competing interest

The authors declare that they have no competing interests.

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