

# Reduced Vitamin D Levels are Associated with Stroke-Associated Pneumonia in Patients with Acute Ischemic Stroke

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**Background and aim:** Stroke-associated pneumonia (SAP) is a common complication in patients with acute ischemic stroke (AIS). This study explored the potential relationship between serum vitamin D levels and SAP.

**Methods:** This study recruited 863 consecutive AIS patients. In-hospital SAP was defined as a complication that occurred after stroke, during hospitalization, that was confirmed radiographically. Serum vitamin D levels were measured within 24 hrs of admission and the patients were divided into vitamin D sufficient (>50 nmol/L), insufficient (25–50 nmol/L), and deficient (<25 nmol/L) groups.

**Results:** In this study, 102 (11.8%) patients were diagnosed with SAP. Compared to the patients without SAP, patients with SAP had significantly lower vitamin D levels ( $P = 0.023$ ). The incidence of SAP was significantly higher in patients with vitamin D deficiency than in those with vitamin D insufficiency or sufficiency (21.2% vs 16.2% & 9.5%,  $P = 0.006$ ). After adjusting for confounders, vitamin D deficiency and insufficiency were independently associated with SAP (OR = 3.034, 95% CI = 1.207–7.625,  $P = 0.018$ ; OR = 1.921, 95% CI = 1.204–3.066,  $P = 0.006$ , respectively). In multiple-adjusted spline regression, vitamin D levels showed a linear association with the risk of SAP ( $P < 0.001$  for linearity).

**Conclusion:** Reduced vitamin D is a potential risk factor of in-hospital SAP, which can help clinicians identify high-risk SAP patients.

**Keywords:** acute ischemic stroke, stroke-associated pneumonia, vitamin D

## Introduction

Stroke patients are more likely to have pneumonia during hospitalization, and the prevalence of stroke-associated pneumonia (SAP) ranges from 7.1 to 31.3%.<sup>1–5</sup> Studies have confirmed that SAP is independently associated with early mortality, prolonged hospitalization, and poor outcomes among stroke patients.<sup>1,3,6,7</sup> To improve the prognosis of stroke patients, it is necessary to identify risk factors for SAP early, to enable preventive interventions and treatment.

Vitamin D is a neurosteroid hormone that affects various diseases, including stroke, cardiovascular disease, and multiple sclerosis.<sup>8–11</sup> Clinical studies show that low vitamin D levels are common in stroke patients due to reduced vitamin D intake, a lack of outdoor exercise, and decreased physiological synthesis.<sup>8,12–14</sup> Furthermore, vitamin D has anti-inflammatory properties and low vitamin D levels may lead to increased inflammatory activity.<sup>15,16</sup> Therefore, we hypothesized that a reduction in

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vitamin D levels might be associated with the occurrence of pneumonia after acute stroke.

Given that low vitamin D levels are common in stroke patients, we aimed to explore the association between vitamin D levels and SAP in patients with acute ischemic stroke (AIS).

## Methods

### Study Design

We enrolled patients from a retrospective clinical database that included consecutive patients who were admitted to the Department of Neurology, First Affiliated Hospital of Wenzhou Medical University, within 24 hrs after the onset of ischemic stroke between October 2017 and October 2018.

This retrospective study was approved by the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University. Because this study was retrospective and all included data were anonymous, the requirement that patients give informed consent was waived.

### Inclusion and Exclusion Criteria

All patients with suspected AIS were confirmed by cranial computed tomography (CT) or magnetic resonance imaging (MRI) within 24 hrs of admission. The exclusion

criteria were as follows: (i) diagnosed with transient ischemic attack; (ii) active infection or pyrexia within 2 weeks before admission; (iii) preventive antibiotic therapy; (iv) history of any other central nervous system disease, such as brain trauma, cerebral hemorrhage, hydrocephalus, and Parkinson's disease; (v) severe hepatic disease (serum transaminase concentration exceeding twice the upper limit of the reference range within 6 months or persistent hyperbilirubinemia); (vi) severe renal disease [glomerular filtration rate (GFR)  $< 60 \text{ mL min}^{-1} 1.73 \text{ m}^{-2}$ ]; (vii) osteoporosis or taking vitamin D supplementation before stroke onset; and (viii) lack of complete medical and laboratory records. Ultimately, 863 patients were enrolled in this study and their data were analyzed (Figure 1).

### Data Collection

The patients' demographic data, including age and gender, were collected from their medical records. Baseline clinical parameters were obtained, including stroke subtype (according the TOAST criteria),<sup>17</sup> previous stroke, dysphagia, current cigarette smoking, current alcohol consumption, and arterial blood pressure. Pre-existing comorbidities included hypertension, diabetes, coronary heart disease, and chronic obstructive pulmonary disease

1665 AIS patients referred to the First Affiliated Hospital of Wenzhou Medical University from 10/2017 to 10/2018.

802 patients were excluded

- 1) Transient ischemic attack (n = 65)
- 2) Active infection or pyrexia within 2 weeks before admission (n = 61)
- 3) Preventive antibiotic therapy (n = 153)
- 4) History of any other central nervous system disease (n = 80)
- 5) Severe hepatic or renal diseases (n = 103)
- 6) Osteoporosis or taking vitamin D supplementation before stroke onset (n = 79)
- 7) Missing complete medical and laboratory records (n = 261)

Total 863 patients were included

Figure 1 Study flow diagram. AIS, acute ischemic stroke.

(COPD). The baseline laboratory examinations, including vitamin D, fasting blood glucose, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), serum creatinine (SCr), blood urea nitrogen (BUN), GFR, platelets, leukocytes, and total cholesterol, were obtained within 24 hrs of admission. The stroke severity was assessed by well-trained neurologists using the National Institutes of Health Stroke Scale (NIHSS) score within 24 hrs of hospital admission.

## Vitamin D Tests

Blood samples were obtained within 24 hrs of admission. Vitamin D status was evaluated by measuring 25-hydroxyvitamin D [25(OH)D], which is the main active form of vitamin D and is a good indicator of the total vitamin D level. The serum 25(OH)D levels were measured using a competitive electrochemiluminescence protein binding assay in our hospital laboratory. According to the previous studies,<sup>18,19</sup> the patients were divided into the following three clinically relevant groups based on the 25(OH)D concentrations: vitamin D sufficient (>50 nmol/L), insufficient (25–50 nmol/L), and deficient (<25 nmol/L) groups.

## Outcome Measures

The diagnosis of in-hospital SAP was based on clinical and laboratory examinations according to the modified Centers for Disease Control and Prevention criteria for hospital-acquired pneumonia.<sup>20</sup> Patients suspected of having pneumonia based on chest X-rays and clinical symptoms were diagnosed and confirmed by chest CT and sputum cultures.<sup>21</sup> SAP was diagnosed by two experienced neurologists blinded to the results of the clinical and laboratory examinations. Only hospital-acquired pneumonia was recorded; pneumonia before stroke and community-acquired pneumonia were not considered in this study.

## Statistical Analysis

Continuous variables are presented as the mean  $\pm$  standard deviation or median and interquartile range (IQR) according to the normal or non-normal distribution of data determined by the Kolmogorov–Smirnov test. The significance of differences in continuous variables in the patient subgroups with and without SAP was analyzed with Student's *t*-test or the Mann–Whitney *U*-test, as appropriate. Categorical variables were described as relative frequencies and percentages and two groups were compared with the chi-square test. Statistical comparisons among different

vitamin D groups were assessed with the Kruskal–Wallis test or one-way analysis of variance (ANOVA) for continuous variables and Pearson's chi-square test or Fisher's exact test for categorical variables. Binary univariate logistic regression analysis was used to find confounding variables that were associated with SAP. Then, all confounding variables (defined as  $P < 0.1$ ) were included in multivariate logistic regression models to adjust for potential confounding and assess any independent predictors of SAP. An adjusted spline regression model was computed to provide more precise estimates and determine the shape of the associations between serum vitamin D as a continuous variable and the risk of SAP,<sup>22,23</sup> fitting a restricted cubic spline function with four knots (5th, 35th, 65th, and 95th percentiles). In this study, two-tailed *P*-values  $< 0.05$  were considered statistically significant. All statistical analyses were performed using R for Windows (version 3.4.1; <http://www.r-project.org/>) and SAS statistical software, version 9.2 (SAS Institute, Cary, NC, USA).

## Results

### Baseline Characteristics of the Patients with and Without SAP

A total of 863 patients were eligible in the study [551 males (63.9%), 312 females (36.1%); mean age  $66.3 \pm 11.4$  years; Figure 1]. Of these, 102 (11.8%) patients were diagnosed with in-hospital SAP. The baseline characteristics of the patients with and without SAP are presented in Table 1. Compared to patients without SAP, those with SAP were more likely to be older, cigarette smokers, and had higher baseline NIHSS scores, BUN and leukocyte levels, and lower GFR levels. The median (IQR) values of vitamin D levels in non-SAP and SAP patients were 61.0 (47.7–77.0) and 55.2 (38.9–71.2), respectively. Compared to patients without SAP, those with SAP had significantly lower vitamin D levels ( $P = 0.023$ ). Meanwhile, the incidence of dysphagia and hypertension was higher in patients with SAP.

### Baseline Characteristics of All Patients According to Vitamin D Status

Table 2 summarized the basic and laboratory characteristics of the AIS patients stratified by vitamin D status. The incidence of SAP was significantly higher in patients with vitamin D deficiency ( $< 25$  nmol/L) than in those with vitamin D insufficiency or sufficiency (21.2% vs 16.2% & 9.5%, respectively,  $P = 0.006$ , Table 2). Interestingly, the

**Table 1** Differences of the Baseline Characteristics in AIS Patients with and Without SAP

Variables	Non-SAP (n=761)	SAP (n=102)	P-value*
<b>Demographic parameters</b>			
Age (years)	65.7 ± 11.5	70.2 ± 10.1	<0.001
Male, n (%)	486 (63.9%)	65 (63.7%)	0.978
<b>Clinical parameters</b>			
Stroke subtype			0.253
Large-vessel disease	600 (78.8%)	77 (75.5%)	
Cardioembolism	96 (12.6%)	20 (19.6%)	
Lacunar	50 (6.6%)	4 (3.9%)	
Other	7 (0.9%)	0	
Unknown	8 (1.1%)	1 (1.0%)	
NHSS score, median (IQR)	3.0 (1.0–5.0)	5.0 (3.0–8.0)	<0.001
Dysphagia, n (%)	91 (12.0%)	25 (24.5%)	<0.001
Current smoking, n (%)	319 (41.9%)	55 (53.9%)	0.022
Current drinking, n (%)	282 (37.1%)	44 (43.1%)	0.234
SBP (mmHg)	152.5 ± 24.2	153.4 ± 23.1	0.712
DBP (mmHg)	83.3 ± 13.9	85.4 ± 13.5	0.156
History of stroke, n (%)	105 (13.8%)	18 (17.6%)	0.296
<b>Pre-existing comorbidities</b>			
Hypertension, n (%)	628 (82.5%)	94 (92.2%)	0.013
Diabetes, n (%)	369 (48.5%)	46 (45.1%)	0.520
Coronary heart disease, n (%)	10 (1.3%)	4 (3.9%)	0.050
COPD, n (%)	9 (1.2%)	0	0.270
<b>Laboratory parameters</b>			
Vitamin D (nmol/L)	61.0 (47.7–77.0)	55.2 (38.9–71.2)	0.023
Fast blood glucose (mmol/L)	6.5 ± 2.5	6.6 ± 2.4	0.777
HDL-C (mmol/L)	1.0 ± 0.2	1.0 ± 0.3	0.989
LDL-C (mmol/L)	2.7 ± 1.0	2.6 ± 1.0	0.598
SCr (μmol/L)	71.0 (59.0–85.0)	70.0 (58.0–83.0)	0.763
BUN (mmol/L)	4.9 (4.0–5.9)	5.2 (4.1–6.7)	0.038
GFR	91.1 (74.9–102.3)	87.0 (74.2–95.5)	0.047
Leukocyte (×10 <sup>9</sup> /L)	6.9 ± 2.2	8.8 ± 3.5	<0.001
Platelet (×10 <sup>9</sup> /L)	219.0 (183.0–257.0)	215.0 (173.0–255.0)	0.465
Total cholesterol (mmol/L)	4.7 ± 1.3	4.5 ± 1.2	0.111

**Notes:** \*Continuous variables were compared between the groups by the Student's *t*-test or the Mann–Whitney test. The chi-square test was used for categorical variables.

**Abbreviations:** BUN, blood urea nitrogen; COPD, Chronic obstructive pulmonary disease; DBP, diastolic blood pressure; GFR, glomerular filtration rate; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; NIHSS, National Institutes of Health Stroke Scale; SAP, stroke-associated pneumonia; SBP, systolic blood pressure; SCr, serum creatinine concentration.

incidence of diabetes increased as the vitamin D levels decreased ( $P < 0.001$ ). Significant differences were also observed for gender, coronary heart disease, HDL-C, and SCr among patients with different vitamin D status. Otherwise, most characteristics of the patients with low and high vitamin D levels were similar.

## Association Between the Serum Vitamin D and SAP

For the data on the stroke patients, SAP occurrence was taken as the dependent variable and vitamin D sufficiency was taken as a reference in the unadjusted and multivariate-adjusted logistic regression models (Table 3). In the unadjusted logistic regression model (univariate analysis), the odds ratio of SAP increased with decreasing serum vitamin D levels [1.850 (95% CI = 1.192–2.872) and 2.567 (95% CI = 1.066–6.182,  $P = 0.035$ )]. The test for trend was significant ( $P = 0.0015$ ). Age, baseline NIHSS score, GFR levels, hypertension, current smoking, and dysphagia were significantly associated with the risk of SAP in the univariate analyses (all  $P < 0.1$ ). After adjusting for conventional and significant confounders, including age, GFR levels, gender, diabetes mellitus, hypertension, current smoking, baseline NIHSS score and dysphagia, vitamin D deficiency ( $< 25$  nmol/L) was independently associated with the incidence of SAP (model 1: OR = 2.926, 95% CI = 1.196–7.159,  $P = 0.019$ ; model 2: OR = 3.293, 95% CI = 1.318–8.229,  $P = 0.011$ ; model 3: OR = 2.997, 95% CI = 1.185–7.584,  $P = 0.020$ ). Vitamin D insufficiency (25–50 nmol/L) was also independently associated with a lower risk of SAP (model 1: OR = 1.842, 95% CI = 1.175–2.889,  $P = 0.008$ ; model 2: OR = 1.935, 95% CI = 1.222–0.64,  $P = 0.005$ ; model 3: OR = 1.963, 95% CI = 1.228–3.136,  $P = 0.005$ ). The adjusted *P*-values for trend were 0.0011, 0.0005, and 0.0010 in models 1, 2, and 3, respectively.

Furthermore, the multiple-adjusted cubic spline regressions confirmed the association between SAP risk and serum vitamin D levels. Within the range of vitamin D less than 50 nmol/L, the spline regression showed a linear relation between an increased risk of SAP and continuously reduced serum vitamin D ( $P < 0.001$  for linearity; Figure 2).

## Discussion

To our knowledge, this is the first study to investigate the association between vitamin D levels and the development of pneumonia after AIS. We found that vitamin D deficiency and insufficiency at admission were associated with a high risk of in-hospital SAP, and the risk of SAP increased as the vitamin D levels decreased. Therefore, our results indicated that low vitamin D status could be a reliable risk factor of SAP.

**Table 2** Baseline Characteristics of Patients with Acute Ischemic Stroke According to Different Vitamin D Status

Variables	All Patients	Vitamin D Status			P-value*
		Vitamin D Deficiency (n = 33)	Vitamin D Insufficiency (n = 240)	Vitamin D Sufficiency (n = 590)	
SAP, n (%)	102 (11.8%)	7 (21.2%)	39 (16.2%)	56 (9.5%)	0.006
<b>Demographic parameters</b>					
Age (years)	66.2 ± 11.4	63.2 ± 12.1	66.9 ± 12.7	66.1 ± 10.8	0.212
Gender					0.002
Male, n (%)	551 (63.8%)	22 (66.7%)	131 (54.6%)	398 (67.5%)	
Female, n (%)	312 (36.2%)	11 (33.3%)	109 (45.4%)	192 (32.5%)	
<b>Clinical parameters</b>					
Stroke subtype					0.520
Large-vessel disease	677 (78.4%)	29 (87.9%)	188 (78.3%)	460 (78.0%)	
Cardioembolism	116 (13.4%)	2 (6.1%)	35 (14.6%)	79 (13.4%)	
Lacunar	54 (6.3%)	2 (6.1%)	11 (4.6%)	41 (6.9%)	
Other	7 (0.8%)	0	4 (1.7%)	3 (0.5%)	
Unknown	9 (1.0%)	0	2 (0.8%)	7 (1.2%)	
NHSS score, median (IQR)	3.0 (1.0–6.0)	3.0 (1.0–7.0)	3.0 (1.0–5.75)	3.0 (1.0–6.0)	0.781
Thrombolysis, n (%)	32 (3.7%)	1 (3.0%)	8 (3.3%)	23 (3.9%)	0.906
Dysphagia, n (%)	116 (13.4%)	8 (24.2%)	36 (15.0%)	72 (12.2%)	0.101
Current smoking, n (%)	374 (43.3%)	16 (48.5%)	117 (48.8%)	241 (40.8%)	0.095
Current drinking, n (%)	326 (37.8%)	14 (42.4%)	98 (40.8%)	214 (36.3%)	0.401
SBP (mmHg)	152.6 ± 24.1	154.1 ± 27.0	154.1 ± 23.5	151.9 ± 24.2	0.446
DBP (mmHg)	83.5 ± 13.8	86.1 ± 12.7	83.7 ± 13.9	83.3 ± 13.9	0.505
History of stroke, n (%)	123 (14.3%)	5 (15.2%)	45 (18.8%)	73 (12.4%)	0.058
<b>Pre-existing comorbidities</b>					
Hypertension, n (%)	722 (83.7%)	25 (75.8%)	192 (80.0%)	505 (85.6%)	0.065
Diabetes, n (%)	415 (48.1%)	19 (57.6%)	138 (57.5%)	258 (43.7%)	<0.001
Coronary heart disease, n (%)	14 (1.6%)	2 (6.1%)	6 (2.5%)	6 (1.0%)	0.037
COPD, n (%)	9 (1.0%)	1 (3.0%)	3 (1.2%)	5 (0.8%)	0.454
<b>Laboratory parameters</b>					
Fast blood glucose (mmol/L)	6.5 ± 2.5	6.7 ± 2.6	6.8 ± 2.5	6.4 ± 2.5	0.122
HDL-C (mmol/L)	1.0 ± 0.2	0.9 ± 0.3	1.0 ± 0.3	1.0 ± 0.2	0.016
LDL-C (mmol/L)	2.7 ± 1.0	2.9 ± 1.3	2.7 ± 0.9	2.7 ± 1.0	0.252
SCr (μmol/L)	71.0 (59.0–85.0)	69.0 (57.0–101.0)	67.0 (55.0–81.0)	71.0 (61.0–85.0)	0.013
BUN (mmol/L)	4.9 (4.0–6.0)	4.7 (4.0–7.1)	4.8 (3.8–6.0)	5.0 (4.1–6.0)	0.445
GFR	90.5 (74.7–101.1)	87.2 (67.8–112.4)	90.5 (75.3–100.6)	90.7 (75.1–101.1)	0.870
Leukocyte (×10 <sup>9</sup> /L)	7.2 ± 2.4	7.6 ± 3.2	7.3 ± 2.6	7.1 ± 2.3	0.191
Platelet (×10 <sup>9</sup> /L)	218.0 (181.0–257.0)	217.0 (188.0–259.0)	231.0 (186.0–264.0)	215.0 (181.0–255.0)	0.148
Total cholesterol (mmol/L)	4.7 ± 1.3	5.0 ± 1.7	4.7 ± 1.2	4.7 ± 1.2	0.545

**Notes:** \*Continuous variables were compared between the groups by the Kruskal–Wallis test or one-way analysis of variance (ANOVA). The Pearson's chi-square test or Fisher's exact test was used for categorical variables.

**Abbreviations:** BUN, blood urea nitrogen; COPD, Chronic obstructive pulmonary disease; DBP, diastolic blood pressure; GFR, glomerular filtration rate; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; NIHSS, National Institutes of Health Stroke Scale; SAP, stroke-associated pneumonia; SBP, systolic blood pressure; SCr, serum creatinine concentration.

In this study, 11.8% of the patients were diagnosed with pneumonia after acute stroke during hospitalization, which is consistent with previous findings.<sup>3,24–26</sup> We found that age and the severity of stroke (NIHSS score) affected

the risk of SAP, and these were identified as risk factors for SAP in other population-based studies.<sup>27,28</sup> Moreover, in our study, 24.5% of the patients with SAP developed dysphagia, which was significantly increased compared to

**Table 3** Multivariate Adjusted Odds Ratios for the Association Between Vitamin D Levels and SAP

	OR (95% CI) <sup>a</sup>			P-value for Trend
	Vitamin D Deficiency (< 25 nmol/L)	Vitamin D Insufficiency (25–50 nmol/L)	Vitamin D Sufficiency (> 50 nmol/L)	
Unadjusted P-value	2.567 (1.066–6.182) 0.035	1.850 (1.192–2.872) 0.006	Reference	0.0015
Model 1* P-value	2.926 (1.196–7.159) 0.019	1.842 (1.175–2.889) 0.008	Reference	0.0011
Model 2 <sup>†</sup> P-value	3.293 (1.318–8.229) 0.011	1.935 (1.222–3.064) 0.005	Reference	0.0005
Model 3 <sup>‡</sup> P-value	2.997 (1.185–7.584) 0.020	1.963 (1.228–3.136) 0.005	Reference	0.0010

**Notes:** <sup>a</sup>Reference OR (1.000) is the Vitamin D sufficiency for SAP. \*Model 1: adjusted for age, sex; <sup>†</sup>Model 2: adjusted for covariates from Model 1 and further adjusted for diabetes mellitus, hypertension and current smoking; <sup>‡</sup>Model 3: adjusted for covariates from Model 2 and further adjusted for baseline NIHSS scores, dysphagia and glomerular filtration rate.

**Abbreviations:** OR, odds ratio; CI, confidence level; SAP, stroke-associated pneumonia.

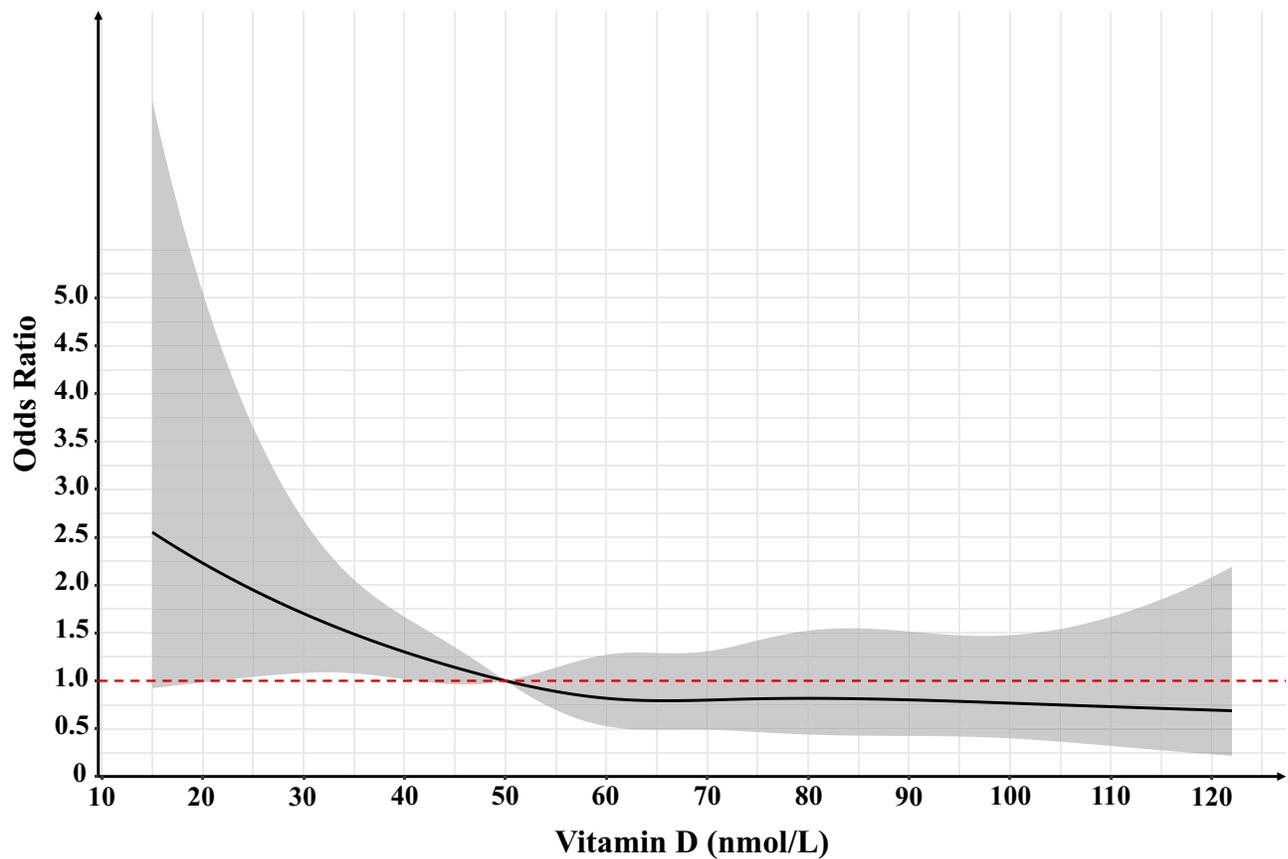
those without SAP. Adjusted logistic regression analysis showed that the presence of dysphagia was associated with the risk of SAP (OR = 1.855, 95% CI = 1.049–3.278). Currently, only a few published studies have explored the effect of dysphagia in stroke patients.<sup>29–31</sup> Our findings are in accordance with a study investigated by Martino et al,<sup>32</sup> who found that dysphagic patients have up to an 11-fold increased risk of SAP. Other recent studies have reported the same results.<sup>33,34</sup> Furthermore, consistent with many studies, we found that hypertension and current smoking were associated with pneumonia after stroke.<sup>3,5,35</sup>

In the previous studies, several biological indicators had been described as promising biomarkers related to SAP. A Korean group found that AIS patients with higher neutrophil-to-lymphocyte ratios were more likely to catch SAP.<sup>26</sup> Many observational clinical research found that procalcitonin was useful in the early diagnosis of SAP.<sup>36–38</sup> Another study found that salivary substance P (SP) levels may be a biomarker to predict SAP because AIS patients with a low frequency of spontaneous swallowing had low salivary SP levels.<sup>39</sup> Recently, Zapata-Arriaza et al suggested that pro-adrenomedullin predicted SAP and the combination of serum amyloid A protein/soluble urokinase-type plasminogen activator receptor could improve its predictive value.<sup>40</sup> Compared with the above biomarkers, vitamin D is an original biomarker that is routinely tested clinically.

In this study, the total prevalence of vitamin D deficiency and insufficiency was 31.6%. A database of 41,504 patients from the USA showed that the prevalence of

vitamin D deficiency ( $\leq 30$  ng/mL) was 63.6%.<sup>41</sup> In another study in Kampala, Uganda, where the sunshine is adequate, the prevalence was only 15%.<sup>42</sup> Thus, the various incidence of low vitamin D levels may be the result of different methods for measuring vitamin D and different durations of sunlight exposure. Furthermore, we found that the OR of the vitamin D deficiency group was higher than that of the vitamin D insufficiency group, which indicated that the vitamin D level was closely linked to the development of SAP. Therefore, we used restricted cubic spline regression to explore the relation between serum vitamin D as a continuous parameter and the risk of SAP. Consequently, the restricted cubic spline curve confirmed a linear relation between an elevated SAP risk and continuously decreasing vitamin D levels.

To our knowledge, no other studies have explored the association between vitamin D and SAP. However, many studies have investigated the associations between vitamin D and respiratory diseases. A recent study found that vitamin D status was inversely associated with the risk of community-acquired pneumonia in community-living adults.<sup>43</sup> However, a cross-sectional analysis in the Netherlands Epidemiology of Obesity study found that vitamin D concentrations were not associated with lung function or airway inflammation in 6671 people in the general population.<sup>44</sup> It's worth noting that our participants were the patients who suffered from acute stroke and were vulnerable to infection. In addition, in a murine model of intratracheal lipopolysaccharide challenge, Dancer et al found that vitamin D deficiency may lead to exaggerated



**Figure 2** Association between vitamin D levels and risk of PSD. The reference vitamin D was 50 nmol/L. The both edges of the gray area represent 95% confidence intervals. And the black solid curve is the ORs value. ORs and 95% confidence intervals derived from restricted cubic spline regression, adjusting for the same variables as model 3 in Table 3, with knots placed at the 5th, 35th, 65th, and 95th percentiles of the distribution of serum vitamin D.

alveolar inflammation, epithelial damage, and hypoxia.<sup>45</sup> Other studies have proved that, in disease settings, vitamin D has positive effects on maintaining lung epithelial integrity and inhibiting inflammatory responses.<sup>46,47</sup> Vitamin D supplementation ameliorated pulmonary morphological alternations in LPS-induced bronchopulmonary dysplasia, inhibited IFN- $\gamma$  overproduction and, thereby, suppressed inflammation.<sup>48</sup> Vitamin D was also confirmed to enhance the ability of neutrophils to kill *Streptococcus pneumoniae* and inhibit excessive inflammation and apoptosis.<sup>49</sup> Similar beneficial effects of vitamin D were observed in paraquat-induced lung fibrosis; vitamin D exhibited its anti-inflammatory effects by decreasing the secretion of pro-inflammatory cytokines.<sup>50</sup> A study of male C57Bl6 mice concluded that supplemental vitamin D in advance can attenuate acute anti-inflammatory actions in the ischemia-reperfusion brain.<sup>51</sup>

Our research team also enrolled AIS patients from this retrospective clinical database between January 2018 and January 2019 to develop an easy-to-use nomogram model

for predicting the risk of SAP in AIS patients.<sup>52</sup> The nomogram contained predictors including age, NIHSS score on admission, atrial fibrillation, nasogastric tube intervention, mechanical ventilation, fibrinogen, and leukocyte count. Furthermore, the SAP nomogram showed good predictive performance in training cohort and validation cohort, and it was superior to other conventional models. However, we did not collect the data of vitamin D for analysis before, so vitamin D was not included in this nomogram model as a predictor. Although these two studies had 231 overlapped patients, the purposes and methods of these two studies were completely different. Therefore, we think that the potential overlap between the patients in these two studies does not affect the result of the study and the exploration of the clinical characteristics of SAP patients from different perspectives.

This study has several limitations. First, because it was a retrospective, single-center analysis, we cannot conclude a causal relationship between vitamin D levels and SAP. Additional large-scale, prospective clinical cohorts are

needed to confirm our findings. Second, data on eating habits, nutrient intake, and parathyroid hormone levels were not available. All enrolled patients were elderly people from a single center and we assumed that there was no significant difference in their dietary habits. Third, serum vitamin D was measured only once on admission; further longitudinal studies are needed to evaluate the effects of dynamic changes in vitamin D levels after stroke on the outcome. Finally, our analysis did not consider certain drugs that can influence vitamin D levels.

## Conclusions

In conclusion, our results indicate that low serum vitamin D on admission is associated with in-hospital SAP in patients with AIS. This study may provide insights for further clinical trials to examine whether vitamin D supplementation can prevent or reduce the burden of SAP after AIS.

## Abbreviations

AIS, acute ischemic stroke; BUN, blood urea nitrogen; CI, confidence interval; COPD, Chronic obstructive pulmonary disease; DBP, diastolic blood pressure; GFR, glomerular filtration rate; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; NIHSS, National Institutes of Health Stroke Scale; OR, odds ratio; SAP, stroke-associated pneumonia; SBP, systolic blood pressure; SCr, serum creatinine concentration.

## Data Sharing Statement

The data supporting this study is available from the corresponding author for reasonable request.

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## Disclosure

The authors declare that there are no conflicts of interest in this work.

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