BMC Pulmonary Medicine

RESEARCH

Open Access

Relation between vitamin D deficiency and *Pseudomonas aeruginosa* colonization in patients with bronchiectasis

Yu-Hua Wen[1†], Ruo-Xuan Dai[1†], Heng Yang[1†], Jie-Lu Lin[2], Ru-Jia Tao[1], Ling Yang[1], Jin-Fu Xu[1] and Hai-Wen Lu[1*]

Abstract

Background The relationship between vitamin D deficiency and *Pseudomonas aeruginosa (P. aeruginosa)* colonization in bronchiectasis patients is not well understood.

Methods This study was conducted at Shanghai Pulmonary Hospital from June 2014 to May 2018. Serum 25-hydroxyvitamin D levels were measured in patients with bronchiectasis, and clinical data including sputum culture results were collected. To investigate the relationship between vitamin D levels and *P. aeruginosa* colonization, we conducted correlation analysis and logistic regression. Additionally, in vitro experiments with bone marrow-derived macrophages (BMDMs) infected with *P. aeruginosa* strain PAO1 were performed to further explore the influence of vitamin D on the bacterial infection and inflammatory response.

Results Among the 195 patients with bronchiectasis, 83.1% (162/195) were vitamin D deficient. A significant negative correlation was observed between serum vitamin D levels and the BSI (Bronchiectasis Severity Index) score. Patients with vitamin D deficiency showed higher rates of *P. aeruginosa* colonization compared to those with adequate vitamin D levels. Female gender and vitamin D deficiency were identified as risk factors for *P. aeruginosa* colonization in patients with bronchiectasis. Additionally, serum interleukin (IL)-1β levels were significantly elevated in the vitamin D-deficiency group. In vitro experiments, 1,25-dihydroxyvitamin D [1,25D] was shown to inhibit PAO1 phagocytosis in BMDMs and to suppress IL-1β secretion.

Conclusions Vitamin D deficiency was strongly associated with an increased risk of *P. aeruginosa* colonization in patients with bronchiectasis. Furthermore, vitamin D demonstrated protective effects by reducing *P. aeruginosa* survival in cells and modulating the inflammatory dysregulation induced by the bacterium.

Keywords Vitamin D, *Pseudomonas aeruginosa*, Bronchiectasis, IL-1β

†Yu-hua Wen, Ruo-xuan Dai and Heng Yang contributed equally to this work.

*Correspondence:
Hai-Wen Lu
haiwen_lu@163.com
[1]Department of Respiratory and Critical Care Medicine, Shanghai Pulmonary Hospital, School of Medicine, Tongji University, Shanghai 200433, China
[2]Department of Pathology, Shanghai Pulmonary Hospital, School of Medicine, Tongji University, Shanghai, China

Background

Bronchiectasis is a chronic, irreversible inflammatory respiratory disease characterized by permanent bronchial dilation, often accompanied by symptoms such as chronic cough, sputum production, and recurrent respiratory infections [1, 2]. The disease follows a prolonged course with frequent acute exacerbations, leading to progressive decline in lung function and significantly impairing patients' quality of life. This imposes a substantial physical and psychological burden on patients [3, 4]. According to Cole' s vicious cycle model, infection, inflammation, and airway destruction are key factors in the pathogenesis of bronchiectasis [5]. Recent models of the "vicious vortex" also describe the pathogenesis of bronchiectasis [6]. Impaired mucociliary clearance and airway mucus retention disrupt normal host defense mechanisms, making the airways more susceptible to infection, which may persist. This, in turn, triggers inflammatory responses, causing airway damage and abnormal remodeling, ultimately leading to bronchiectasis. Studies have shown that *Pseudomonas aeruginosa (P. aeruginosa)* is a dominant bacterial pathogen of bronchiectasis and has a high isolation rate in clinical practice [7, 8]. Our preliminary research has demonstrated that *P. aeruginosa* is closely linked to the severity and prognosis of bronchiectasis. Once established, colonization with *P. aeruginosa* is difficult to eradicate, contributing to repeated exacerbations [9–11]. Furthermore, multiple studies in Europe have confirmed that *P. aeruginosa* colonization significantly heightens the risk of acute exacerbations, hospitalizations, and mortality in these patients [8, 12].

Vitamin D, a fat-soluble vitamin, plays a broad biological role beyond its established functions in mineral metabolism and bone health. It also regulates immune and inflammatory responses, particularly in the lungs, where it modulates alveolar macrophage activity, potentially leading to tissue damage [13–15]. According to a study by Songlin Yu and colleagues, 55.9% of the Chinese population is vitamin D deficient [16]. Studies have demonstrated that vitamin D deficiency is closely related to chronic respiratory diseases such as chronic obstructive pulmonary disease (COPD), asthma, and cystic fibrosis, with potential consequences including worsened lung function, heightened infection risk, and increased rates of acute exacerbation [17–23]. In addition, vitamin D has a protective role against respiratory infections such as tuberculosis, enhancing antimicrobial peptide expression and inhibiting pathogen invasion [24]. Recent research has also suggested that vitamin D may provide some protection against COVID-19 infection [25, 26].

Although the vitamin D status of patients with bronchiectasis has been explored, with deficiency being linked to disease severity [27, 28], there remains a lack of research on the relationship between vitamin D levels and *P. aeruginosa* colonization in bronchiectasis patients, as well as the underlying mechanisms of this association. Therefore, this study aims to investigate the correlation between vitamin D levels and bacterial colonization, along with other clinical characteristics in patients with bronchiectasis, and to further explore the underlying mechanisms involved.

Methods

Study design

Adults (aged ≥ 18) patients had clinically significant bronchiectasis confirmed by high-resolution computed tomography from June 2014 to May 2018 in Shanghai Pulmonary Hospital were recruited in this study [1]. The study protocol was approved by the Ethics Committee of Shanghai Pulmonary Hospital, with the approval number K15-169. Patients were excluded (1) diseases which influence the synthesis of vitamin D (i.e. hepatic/renal dysfunction); (2) allergic bronchopulmonary aspergillosis/ active tuberculosis/non-tuberculous mycobacteria, poorly controlled asthma, cystic fibrosis, interstitial lung disease; (3) malignant tumor or immunodeficiency disease; (4) refused to sign informed consent.

Collect baseline information, comorbidities, course of disease, admission time, laboratory results (Hemoglobin, white blood cell (WBC) count, Albumin, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), TNF-α and IL-1β), image examination, lung function, No. of annual exacerbations, No. of annual hospitalizations. Vitamin D (25-hydroxyvitamin-D) concentration was measured by automatic electrochemical luminescence immunoanalyzer (cobas e411, Roche) according to vitamin D total kit (Roche Diagnostics, Cat. No. 05894913).

Definitions

Vitamin-D deficiency was defined as serum 25-hydroxyvitamin-D < 20ng/mL and non-deficiency as 25-hydroxyvitamin-D ≥ 20ng/mL [29]. Microbiology results were obtained from the three most recent cultures; *P. aeruginosa* colonization was defined as having two out of the three *P. aeruginosa* cultures positive [30]. Exacerbations were incidental events that had an acute deterioration in one or more symptoms (increasing sputum volume or purulence, worsening dyspnea, increased cough, declining lung function or increased fatigue/malaise) or the appearance of new symptoms (fever, pleurisy or hemoptysis requiring antibiotic treatment) [1].

Severity scoring

Bronchiectasis was assessed using a modified Reiff score, the number of lobes involved (of a total of six; the lingula was considered separate) and the degree of dilatation (tubular = 1, varicose = 2, and cystic = 3) were calculated

(range,1–18) [31]. The Bronchiectasis Severity Index (BSI) score consists of nine variables: age, body mass index, spirometry, previous hospitalization for severe exacerbations in the preceding 2 years, exacerbation in the previous year, mMRC dyspnea score, *P. aeruginosa* colonization, colonization with other microorganisms and radiological severity [32]. The possible range of points is 0 to 26, with a higher score indicating greater severity of the disease.

Materials and reagents

P. aeruginosa strain PAO1 (ATCC-BAA-47; strain HER-1018) was purchased from the American Type Culture Collection. The PAO1 strain, which had been stored in glycerol at -80 °C, was taken out and resuspended in 3–4 mL of sterile lysogeny broth (LB) (Sodium chloride, 5 g/L, Sinopharm chemical reagent Co. 10019318; TRYPTONE, 10 g/L, OXOID LP0042; YEAST EXTRACT, 5 g/L, OXOID LP0021). The mixture was incubated overnight (12–16 h) at 37 °C with shaking at 220 rpm. Using a sterile inoculating loop, the PAO1-containing LB liquid was streaked onto LB agar (Agar, 15 g/L, Yeasen Biotechnology (Shanghai) Co. 70101ES76; Sodium chloride, 5 g/L, Sinopharm chemical reagent Co. 10019318; TRYPTONE, 10 g/L, OXOID LP0042; YEAST EXTRACT, 5 g/L, OXOID LP0021) plates. The plates were incubated for 24 h and then sealed with parafilm and stored at 4 °C. *P. aeruginosa* Culture and Counting: A single colony of PAO1 was picked from the streaked LB agar plates and transferred to 4 mL of LB liquid medium. The culture was incubated overnight (12–16 h) at 37 °C with shaking at 220 rpm. The next day, 200 µL of the logarithmic-phase culture was added to 4 mL of fresh LB liquid medium and incubated for an additional 2 h with shaking. A 1 mL sample of the culture was then transferred to a 1.5 mL EP tube and centrifuged at 8000 rpm for 5 minutes at 4 °C. The supernatant was discarded, and the pellet was resuspended in 1 mL of Phosphate buffer saline (PBS), followed by three washes. The culture was then serially diluted (10^1, 10^3, 10^5, 10^6, 10^7, and 10^8) using PBS, and 100 µL of each dilution was plated onto LB agar plates. The plates were inverted and incubated at 37 °C for 24 h. Colonies were counted on plates containing 30–300 colonies, and the colony count was calculated as follows: colony number*10*dilution factor (colony-forming unit (CFU) /mL)0.1,25-dihydroxyvitamin D [1,25D] (H-107, Sigma) were from Sigma-Aldrich. Absolute ethanol was used as the solvent for 1,25D.

Cell culture

Bone marrow-derived macrophages (BMDMs) from mice were prepared as previously described [33]. Bone marrow was extracted from the femur and tibia of 6–8-week-old female C57BL/6 mice. Mice were purchased from SHANGHAI SLAC LABORATORY ANIMAL CO. LTD and housed in a specific pathogen-free (SPF) facility. For bone marrow isolation, the animals were euthanized using cervical dislocation, ensuring a painless procedure that rendered the animals unconscious and led to death as quickly as possible. All experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee of Tongji University (approval number TJBE00120101). Cells were centrifuged following the removal of erythrocytes and then were differentiated into BMDMs in Dulbecco's Modified Eagle medium (DMEM, Cytiva HyClone) supplemented with 10% fetal bovine serum (FBS) (Atlanta Biologicals, S11150), 30% L929 supernatant, 1% antibiotic-antimycotic (Gibco 15240062), and 0.1% β-Mercaptoethanol (Sigma-Aldrich, M3148). The cell incubator was set to 37 °C with 5% CO2.

Antimicrobial assay

PAO1 were inoculated in 96-well plates, 1×10^6 CFU/200µL sterile LB per well. Then incubated with or without 100nM 1,25D/ethanol for 24 h, and measured the OD value at wavelength 620 nm in Multiscan Spectrum (MD SpectraMax M5). Additionally, CFU assays were performed to assess PAO1 growth.

CFU assay

BMDMs were inoculated in 12-well plates, 2×10^6 cell/500µL culture medium per well, then incubated with or without 100nM 1,25D/ethanol for 24 h, infected with PAO1 MOI = 10 for 3 h. Prior to cell lysis, the supernatant was removed. Cultured with antibiotics to remove extracellular bacteria. Gentamicin was used for this purpose. Then adding 1% Triton-X-100 100 µL/per well to lyse cell for half an hour, and mixing well to gradient dilution. For plating, the suspension was spread onto LB agar plates, which were then inverted and incubated at 37 °C for 24 h.

Cytotoxicity (cell death) assay

BMDMs were inoculated in 96-well plates, 1×10^5 cell/100µL per well, with 500ng/mL Lipopolysaccharides (LPS) pre-treated for 3 h. BMDMs were incubated with or without 100nM 1,25D/ethanol for 2 h, then infected with 10 MOI PAO1 for 3 h. Cell supernatants were collected and analyzed for lactic dehydrogenase (LDH) using CytoTox 96® Non-Radioactive Cytotoxicity Assay kit from Promega and used according to the manufacturer's instructions.

Enzyme-linked immunosorbent assay (ELISA)– cytokines quantifications

BMDMs were inoculated in 96-well plates, 1×10^5 cell/100µL per well, with 500ng/mL LPS pre-treated for 3 h. BMDMs were incubated with or without 100nM 1,25D/ethanol for 2 h, then infected with 10 MOI PAO1

for 3 h. Cell supernatants were collected and analyzed for secreted interleukin (IL)-1β, TNF-α, IL-6 (eBioscience, USA) and IL-18 (ABclonal, USA) was detected using ELISA kits. ELISA kits were used according to the manufacturer's instructions.

Statistical analysis

For clinical experiments, quantitative data with a normal distributed, were analyzed with the Student's t-test, while the Mann–Whitney U-test was applied for data that were not normally distributed. For qualitative data, the χ^2 test was used to assess differences. Spearman correlation analysis was used to calculate the correlation between vitamin D levels and other clinical indicators. We first performed univariate analysis. Covariates that were significant ($P<0.05$) in univariate analysis were then included in multivariate analysis. A backward stepwise approach in the multivariate analysis was considered to keep significant covariates with a cut-point of 0.05. In vitro data was presented as mean ± standard deviation based on at least triplicate samples. The Student's t-test was used in comparison between two groups. For comparisons across more than two groups, ANOVA was applied to normally distributed variables. Statistical significance was set at $P<0.05$. All the data were analyzed with SPSS Version 20.0 software, Medcalc Version 15.2.2 software and GraphPad Prism Version 8.0 program.

Results

Characteristics of the patients with bronchiectasis

A total of 223 patients with bronchiectasis were recruited from June 2014 to May 2018 at Shanghai Pulmonary Hospital. After applying exclusion criteria, 195 patients were included in the analysis. Of these, 83.1% (162/195) were found to have vitamin D deficiency (mean concentration 13.46 ± 3.36 ng/mL), while 16.9% (33/195) were non-deficient (26.00 ± 5.98ng/mL). The vitamin D deficiency group had a significantly higher rate of *P. aeruginosa* colonization ($P=0.038$) (Fig. 1) and a greater number of affected lung segments ($P=0.042$) compared

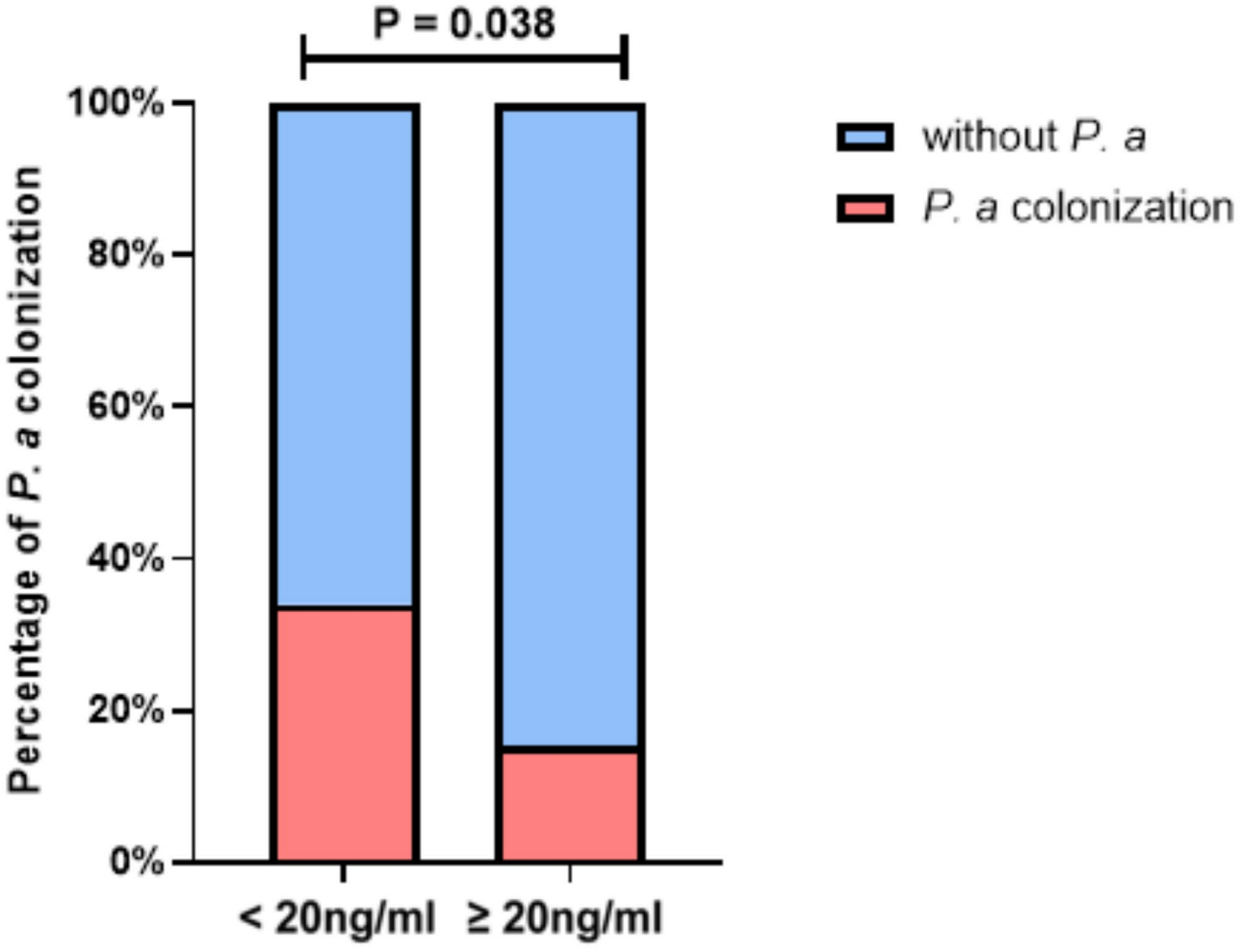


Fig. 1 The percentage of *P. aeruginosa* colonization in the vitamin D deficiency group (without *P. a*: $N=107$; *P. a* colonization: $N=55$) and the non-deficiency group (without *P. a*: $N=28$; *P. a* colonization: $N=5$). The χ^2 test was used to assess differences. *P.a*, *P. aeruginosa*

to the non-deficient group. No significant differences were observed between the groups in terms of smoking status, comorbidities, course of disease, annual exacerbations in the previous year, hospitalizations in the previous year, or lung function parameters (Table 1).

Given that vitamin D synthesis is influenced by ultraviolet radiation, and the duration and intensity of sunlight vary across seasons [34], we categorized the included patients based on the season of their visit. Upon analyzing the seasonal distribution of vitamin D concentrations, the results indicated no significant variation in vitamin D levels across different seasons ($P = 0.17$) (Fig. 1S).

Table 1 Baseline characteristics of bronchiectasis patients

Covariates	Vitamin D deficiency (25(OH)D < 20ng/ml)	Vitamin D non-deficiency (25(OH)D ≥ 20ng/ml)	*P* Value[a]
Subjects, n	162(83.1)	33(16.9)	
Females	113(69.8)	19(57.6)	0.220
Age, years	61[52,65]	55[40.5,62.5]	**0.038**
BMI	20.80±3.97	20.61±2.65	0.793
Ex-smoker or current smoking	15(9.3)	7(21.2)	0.067
Comorbidities			
Diabetes	9(5.6)	0	0.362
Coronary heart disease	3(1.9)	0	1
Hypertension	24(14.8)	5(15.2)	1
Cerebral infarction	2(1.2)	0	1
Sinusitis	11(6.8)	1(3)	0.695
Etiology			
Post-infection	37(22.8)	6(18.2)	0.650
Immune system disease	6(3.7)	0	0.592
GERD	1(0.6)	0	1
Connective tissue disease	6(3.7)	1(3.0)	1
Course of disease, years	10[5,30]	10[2,30]	0.550
Laboratory results			
Hemoglobin, g/L	125.21±15.69	129.79±11.86	0.115
WBC count > 10×10^9 cell	14(8.6)	1(3)	0.474
Albumin, g/L	38.35±4.49	39.34±3.31	0.236
CRP, mg/L	4.2[1.6,14]	5.15[1.675,13.25]	0.770
ESR, mm/h	31.5[15,53.25]	26[17,48.5]	0.747
P. aeruginosa	55(34)	5(15.2)	**0.038**
Escherichia coli	2(1.2)	0	1
Staphylococcus aureus	2(1.2)	0	1
Lung function			
FEV_1 (%)	60.06±23.52	64.78±18.44	0.310
FVC (%)	69.14±20.38	74.35±12.74	0.129
FEV_1/FVC (%)	68.70±11.09	70.85±14.63	0.529
Image examination			
Type of bronchiectasis			
Cylindrical	88(54.3)	14(42.4)	0.212
Cystic	31(19.1)	9(27.3)	0.291
Both of above	43(26.5)	10(30.3)	0.658
No. of affected lung lobes	3[2,5]	3[1.5,4]	0.269
No. of affected lung segments	8[4,12]	6[3,8]	**0.042**
Modified Reiff score	4[2,8]	4[2,8]	0.746
No. of annual exacerbations	2[1,3]	2[1,3]	0.736
No. of annual hospitalizations	1[1,2]	1[1,1]	0.075
BSI score	5[4,9]	4[2.75,6.25]	**0.034**

N (%) or mean±SD or median [interquartile range]; BMI, body mass index; GERD, gastro esophageal reflux disease; WBC, white blood cell; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; FEV1(%), FEV1% predicted (%); FVC (%), FVC percent predicted (%); No. of annual exacerbations and No. of annual hospitalizations were from the previous year

[a]Values in bold indicate $P < 0.05$

Negative correlation between BSI score and vitamin D

To further clarify the relationship between vitamin D levels and disease severity, we analyzed the correlation between vitamin D and other clinical parameters. Vitamin D levels were negatively correlated with BSI score (Fig. 2). Patients with vitamin D deficiency had higher BSI score compared to non-deficient patients (5 [4,9] vs. 4 [2.75,6.25], $P = 0.034$). A weak negative correlation was observed between vitamin D concentration and BSI score (r = -0.207, $P = 0.015$), indicating that lower vitamin D levels were associated with greater disease severity (Fig. 3).

Vitamin D deficiency as a risk factor for *P. aeruginosa* colonization

The colonization of *P. aeruginosa* significantly impacts disease severity. To further investigate the relationship between vitamin D levels and *P. aeruginosa* colonization, we examined whether vitamin D deficiency serves as a risk factor for *P. aeruginosa* colonization. We used univariate logistics analysis to explore the risk factors for *P. aeruginosa* colonization and found that female sex (OR (95%CI): 2.791(1.331,5.850), $P = 0.007$) and vitamin D deficiency (OR (95%CI): 2.879(1.053,7.869), $P = 0.039$) had statistical significance. Furthermore, multivariate logistic regression analysis revealed that female sex (OR (95%CI):2.7(1.278,5.703), $P = 0.009$) and vitamin D

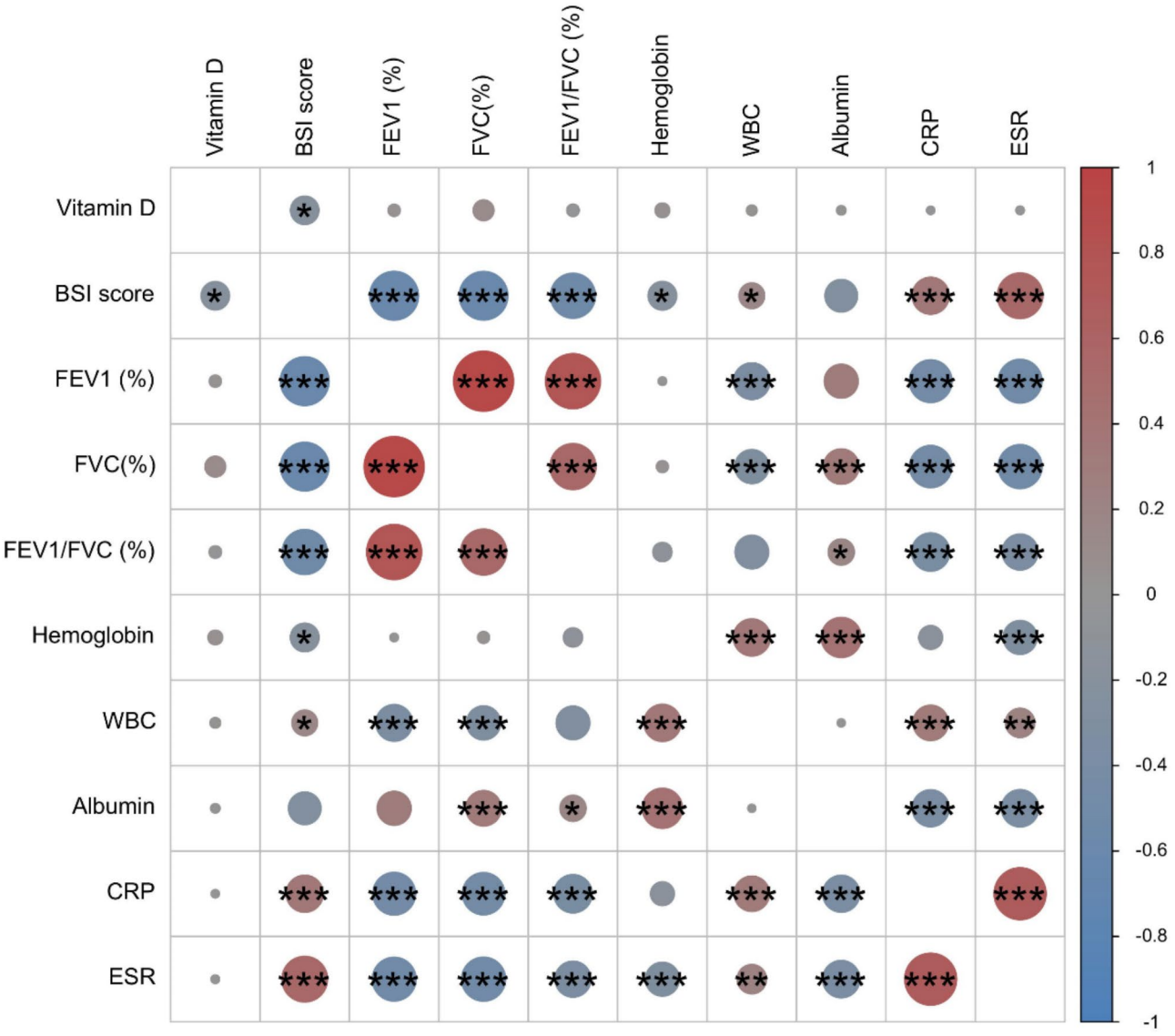


Fig. 2 Correlation between vitamin D levels and other clinical indicators. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ as determined by Spearman correlation analysis. The size of the dots represents the absolute value of the r-value; the scale on the right indicates the r-value, with small values shown in blue, mid-range values in gray, and large values in red

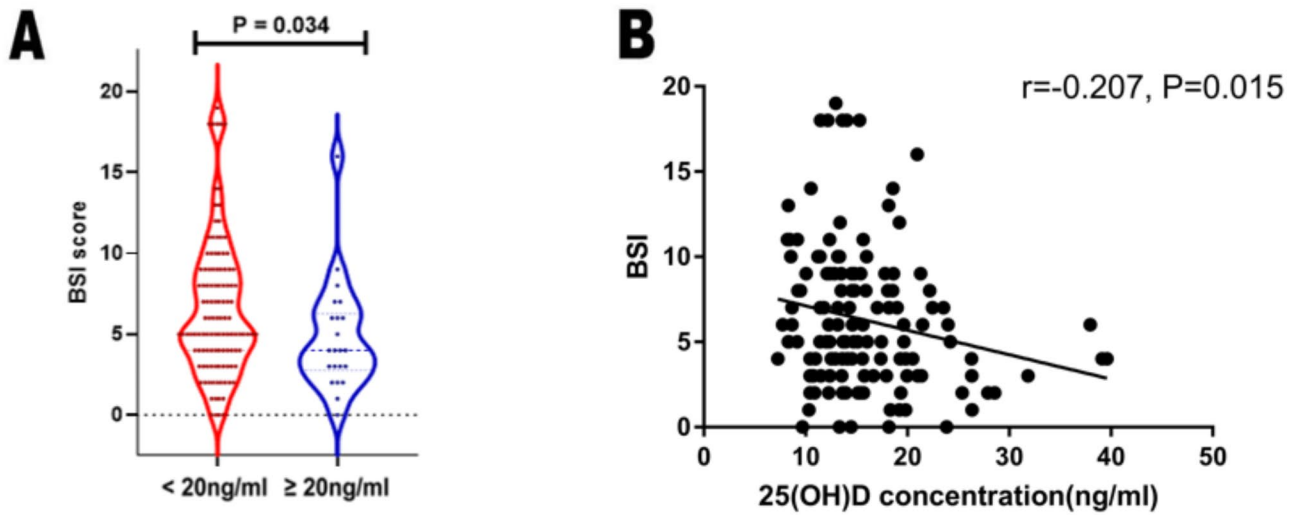


Fig. 3 The relationship between BSI score and vitamin D levels. (**A**) The BSI score in the vitamin D deficiency group and the non-deficiency group. The Mann-Whitney U-test was applied; $P = 0.034$ (**B**) The relationship between serum 25(OH)D concentration and BSI score in bronchiectasis patients. Spearman correlation analysis was applied; r=-0.207; $P = 0.015$

deficiency (OR (95%CI):2.993(1.086,8.251), $P = 0.034$) were independent risk factors for *P. aeruginosa* colonization. Patients with vitamin D deficiency had a 2.9-fold higher risk of *P. aeruginosa* colonization compared to those without deficiency (Fig. 4).

Elevated serum IL-1β levels in vitamin D deficient patients

To further investigate the relationship between vitamin D levels and inflammatory markers in patients with bronchiectasis, we compared the serum levels of key inflammatory cytokines TNF-α and IL-1β based on collected laboratory results. We found no significant difference in TNF-α concentration between the vitamin D deficiency and non-deficiency groups (27.2(13.33–55.78) vs. 38(24.05–79.85), $P = 0.054$) (Fig. 5A). However, serum IL-1β levels were significantly higher in the vitamin D deficiency group (9.90(2.00-59.58) ng/mL) compared to the non-deficient group (3.00(2.00–11.00) ng/mL, $P = 0.047$) (Fig. 5B).

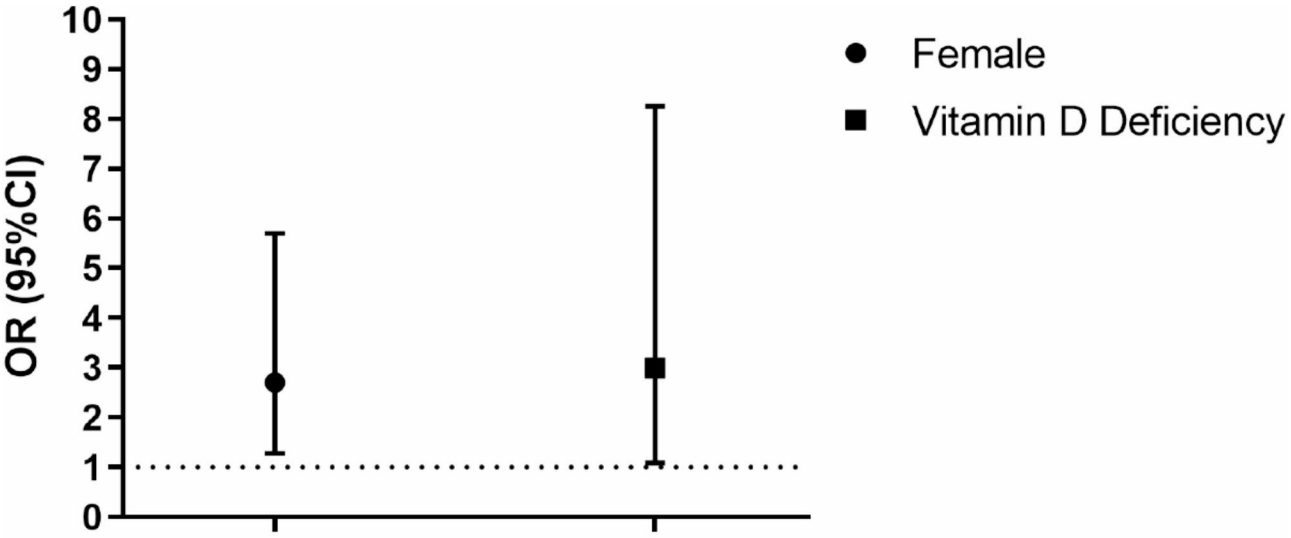
1,25D suppresses IL-1β secretion in PAO1-infected BMDMs

Vitamin D deficiency has been associated with increased IL-1β secretion, leading us to explore whether vitamin D levels influence *P.* aeruginosa colonization through modulation of the inflammatory response. Antimicrobial assay showed that ethanol or 1,25D had no significant direct inhibitory effect on the proliferation of *P. aeruginosa* within 24 h. Therefore, in subsequent experiments, 1,25D was pre stimulated for 24 h (Fig. 6A, Figure S2). In vitro experiments with BMDMs revealed that pretreatment with 1,25D for 24 h inhibited phagocytosis of PAO1, as evidenced by the CFU assay. However, this treatment had no measurable effect on cell death, as indicated by the LDH release assay (Fig. 6B–C). Furthermore, 1,25D significantly reduced the production of IL-1β and TNF-α in PAO1-infected macrophages, while leaving IL-18 and IL-6 levels unaffected (Fig. 6D–G). These findings indicate that 1,25D mitigates inflammation during *P. aeruginosa* infection by regulating IL-1β production.

Discussion

Our study found a negative correlation between BSI scores and vitamin D levels, with a higher rate of *P. aeruginosa* colonization observed in the vitamin D-deficient group. Vitamin D deficiency was identified as a significant risk factor for *P. aeruginosa* colonization among patients with bronchiectasis. Additionally, IL-1βsecretion was elevated in the vitamin D-deficient group compared to the non-deficient group. Further, our in vitro experiments demonstrated that 1,25D can modestly inhibit *P. aeruginosa* phagocytosis in BMDMs and reduce IL-1β secretion. In summary, these results suggest that vitamin D supports a balanced immune response, potentially influencing the intracellular survival of *P. aeruginosa* and attenuating excessive *P. aeruginosa*-induced inflammatory responses.

Previous studies have shown that vitamin D has multiple immunomodulatory effects and that vitamin D deficiency is associated with an increased risk of respiratory infections [29, 35, 36]. Our study also found a higher incidence of vitamin D deficiency in Chinese patients with bronchiectasis compared to the study of James D Chalmers, which reported a 50% deficiency rate in bronchiectasis patients [28]. BSI scores were negatively correlated with vitamin D concentrations, which means that vitamin D status is related to the severity of bronchiectasis. The results of the study are consistent with the study of Chalmers et al. [28], who reported that the prevalence of



Variables	OR (95%CI)	P value
Female	2.7(1.278,5.703)	0.009
Vitamin D deficiency	2.993(1.086,8.251)	0.034

Fig. 4 The risk factors associated with *P. aeruginosa* colonization in bronchiectasis patients, Multivariate logistic regression analyses were applied. OR, odds ratio; CI, confidence interval

vitamin D deficiency was higher in patients with bronchiectasis than in controls, and these patients had a higher frequency of exacerbations. Additionally, A case-control study involving 130 Turkish patients with bronchiectasis a high prevalence of vitamin D deficiency, which was significantly associated with decreased forced vital capacity [37]. Vitamin D deficiency can reduce phagocytosis of macrophages [38], which may contribute to severe colonization in bronchiectasis patients. Our study found an association between vitamin D deficiency and *P. aeruginosa* colonization, which may reflect an aspect of the overall health status of the patients, rather than solely the effect of vitamin D levels. Vitamin D deficiency may be linked to poor disease control and suboptimal nutritional status, both of which contribute to an increased risk of infection [39]. Therefore, vitamin D deficiency may serve as an indicator of poor disease control, rather than being the sole causal factor.

Importantly, we identified vitamin D deficiency as a risk factor for *P. aeruginosa* colonization, consistent with studies linking vitamin D deficiency and an increased risk of lung infections. We also examined cytokine secretion and found elevated levels of IL-1β in vitamin D-deficient patients. In in vitro experiments, 1,25D treatment moderately inhibited *P. aeruginosa* phagocytosis and reduced IL-1β secretion in macrophages. Previous studies have shown that *P. aeruginosa* activates NLRC4 inflammasomes through flagella and type III secretion system (T3SS), leading to caspase-1 activation and downstream IL-1β and IL-18 signaling [40, 41]. IL-1β plays a key role in inflammatory infiltration and tissue damage, contributing to disease exacerbation [42]. For example, Taylor S. Cohen et al. established that in an acute *P. aeruginosa* lung infection mouse model, IL-1β induced inflammatory responses that increased apoptosis and mortality, while mice lacking the IL-1β receptor showed reduced lung damage [43]. Studies have confirmed that vitamin D can inhibit inflammatory responses by downregulating cytokines and chemokines that promote lung tissue destruction in bronchiectasis [44]. In cystic fibrosis studies, vitamin D treatment significantly reduced IL-8 levels, a chemokine that attracts neutrophils [45]. Our findings suggest that vitamin D supplementation could modulate the inflammatory response by downregulating pro-inflammatory cytokines such as IL-1β, potentially offering a therapeutic strategy for mitigating *P.*

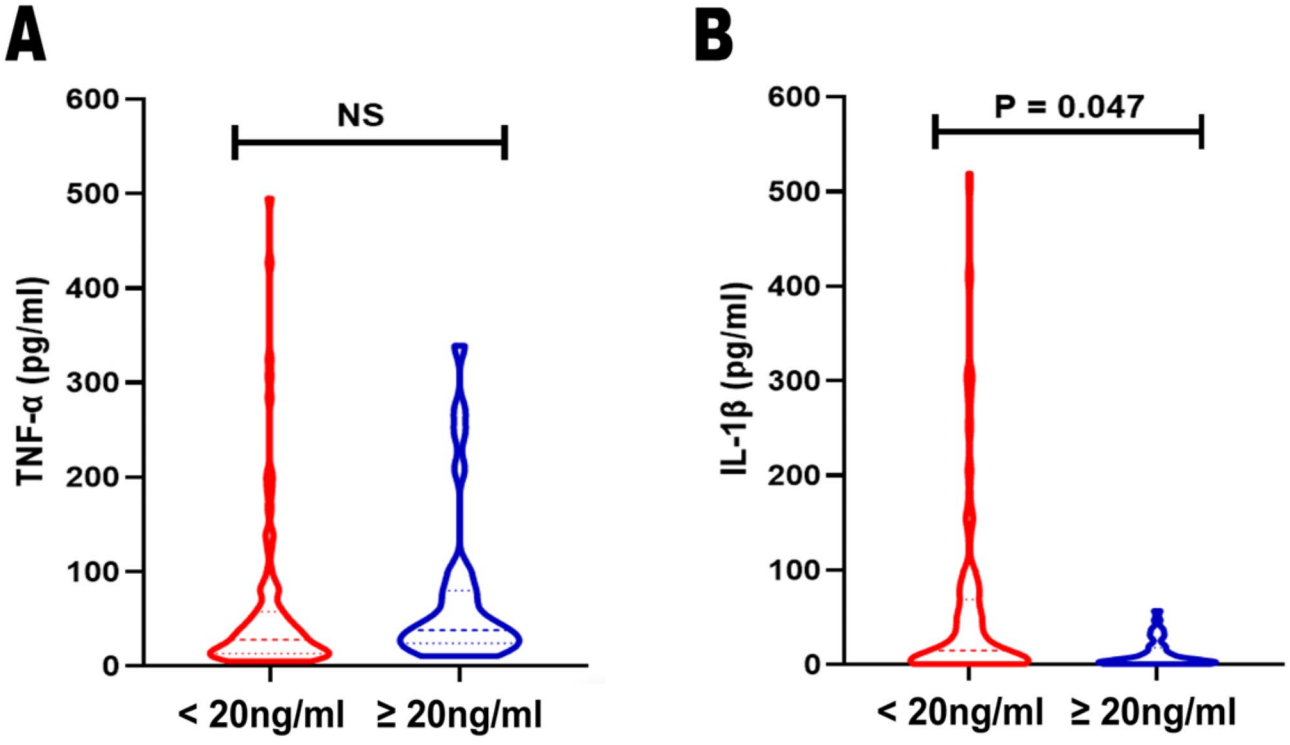


Fig. 5 The levels of cytokines in bronchiectasis patients. (**A**) The level of TNF-α in vitamin D deficiency(*N* = 92) and non-deficiency group(*N* = 28), NS, not significant; (**B**) The level of IL-1β in vitamin D deficiency(*N* = 138) and non-deficiency group(*N* = 33) The Mann-Whitney U-test was applied. *P* value was showed in figure

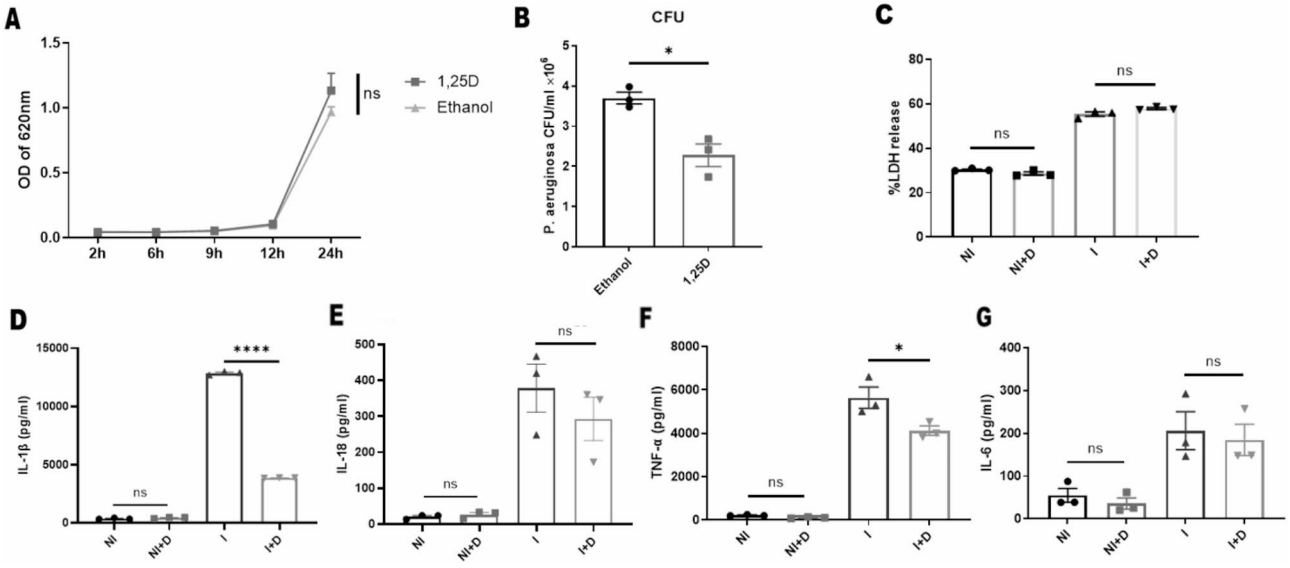


Fig. 6 1,25D decreased the secretion of IL-1β in BMDMs. (**A**) Antimicrobial assay of PAO1 incubated with or without 100nM 1,25D/ethanol for 24 h, *N* = 3; (**B**) CFU assay of pre-treated with 1,25D/ethanol in BMDMs infected by PAO1, *N* = 3; (**C**) Release of LDH as assessed by cytotoxicity assay in uninfected or PAO1-infected BMDMs. *N* = 3. (NI, uninfected cells; NI + D, uninfected cells pre-treated with 1,25D; I, PAO1-infected cells; I + D, PAO1-infected pre-treated with 1,25D). (**D-G**) Protein secretion of IL-1β(**D**)/IL-18(E)/TNF-α(**F**)/IL-6(G) as assessed by ELISA in uninfected or PAO1-infected BMDMs. *N* = 3. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001 as determined by Student's t-test between two groups (**A, B**) and ONE WAY ANOVA across more than two groups (**C-G**)

aeruginosa-induced lung inflammation in bronchiectasis. Our results utilized multivariate regression analysis, which revealed that female gender is a risk factor for *P. aeruginosa* colonization. This finding is consistent with previous studies, which the proportion of females among patients with bronchiectasis and *P. aeruginosa* colonization reach 72.6% and 60.1% [46, 47]. This suggests that gender may play a role in disease severity and colonization risk. Therefore, gender should be considered in the future management and treatment strategies for patients with bronchiectasis.

Our study has several limitations. First, it was conducted at a single center with inpatients only. In the future, it will be necessary to collaborate with multiple hospitals and include outpatients to obtain more comprehensive data. Second, our findings show that bronchiectasis patients with Vitamin D deficiency exhibit higher BSI scores, and that Vitamin D deficiency is associated with *P. aeruginosa* colonization. However, no significant differences were observed in other components of the BSI, which suggests that Vitamin D levels may influence specific aspects of disease severity rather than providing a broad reflection of overall disease burden. Further research is necessary to elucidate the precise role of Vitamin D in bronchiectasis. Additionally, further investigation is needed to determine whether Vitamin D supplementation could serve as a potential therapeutic strategy, which could be validated through in vivo animal models or human-derived tissues.

In conclusion, we demonstrated that vitamin D deficiency is a significant risk factor for *P. aeruginosa* colonization in bronchiectasis patients, potentially through the inhibition of IL-1β secretion. Supplementation with 1,25D may offer protective benefits against *P. aeruginosa* exposure in bronchiectasis patients.

Abbreviations

P. aeruginosa	*Pseudomonas aeruginosa*
1,25D	1,25-dihydroxyvitamin D
COPD	Chronic obstructive pulmonary disease
IL	Interleukin
ELISA	Enzyme-linked immunosorbent assay
CFU	Colony-forming unit
LPS	Lipopolysaccharides
T3SS	Type III secretion system

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12890-025-03548-6.

Supplementary Material 1: **Figure S1**. Boxplot of serum 25(OH)D concentration by season (seasons in Shanghai were categorized as: February to April as spring; May to July as summer; August to October as autumn; November to January as winter).

Supplementary Material 2: **Figure S2**. CFU assay of PAO1 incubated with or without 100nM 1,25D/ethanol across multiple time points, N = 3.

Acknowledgements

The authors thank our patients the medical workers who have contributed to the study.

Author contributions

YH Wen, RX Dai, and H Yang: Conceptualization, Methodology, Investigation, Data Curation, Writing - Review & Editing. JL Lin, RJ Tao: Conceptualization, Data Curation, Software, Writing - Original Draft. L Yang: Conceptualization, Data Curation, Software. JF Xu: Conceptualization, Supervision, Funding acquisition, Writing - Review & Editing. HW Lu: Conceptualization, Supervision, Writing - Review & Editing. All authors reviewed the manuscript.

Funding

This work was supported by the Innovation Program of Shanghai Municipal Education Commission (202101070007-E00097); Program of Shanghai Municipal Science and Technology Commission (21DZ2201800), Program of Shanghai Shenkang Development Center (SHDC12023110).

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study protocol was approved by ethics committees of Shanghai Pulmonary Hospital. The approval number of the study is K15-169. Our study was conducted in accordance with the Declaration of Helsinki. Our study included components exempt from informed consent, as certain data were collected retrospectively. The data collection process ensured patient confidentiality and anonymity. Clinical trial number: not applicable. The animal experiments were approved by the Institutional Animal Care and Use Committee of Tongji University (Number: TJBE00120101). This study was conducted following the ARRIVE guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 22 November 2024 / Accepted: 3 February 2025
Published online: 14 February 2025

References

1. Pasteur MC, Bilton D, Hill AT. British Thoracic Society Bronchiectasis non CFGG. British thoracic society guideline for non-CF bronchiectasis. Thorax. 2010;65(Suppl 1):i1–58.
2. Long MB, Chotirmall SH, Shteinberg M, Chalmers JD. Rethinking bronchiectasis as an inflammatory disease. Lancet Respir Med. 2024.
3. Choi H, McShane PJ, Aliberti S, Chalmers JD. Bronchiectasis management in adults: state of the art and future directions. Eur Respir J. 2024;63(6).
4. Lommatzsch ST. Infection prevention and chronic disease management in cystic fibrosis and noncystic fibrosis bronchiectasis. Ther Adv Respir Dis. 2020;14:1753466620905272.
5. McShane PJ, Naureckas ET, Tino G, Strek ME. Non-cystic fibrosis bronchiectasis. Am J Respir Crit Care Med. 2013;188(6):647–56.
6. Flume PA, Chalmers JD, Olivier KN. Advances in bronchiectasis: endotyping, genetics, microbiome, and disease heterogeneity. Lancet. 2018;392(10150):880–90.
7. Aksamit TR, O'Donnell AE, Barker A, Olivier KN, Winthrop KL, Daniels MLA, et al. Adult patients with bronchiectasis: a First look at the US Bronchiectasis Research Registry. Chest. 2017;151(5):982–92.
8. Chalmers JD, Polverino E, Crichton ML, Ringshausen FC, De Soyza A, Vendrell M, et al. Bronchiectasis in Europe: data on disease characteristics from the European bronchiectasis registry (EMBARC). Lancet Respir Med. 2023;11(7):637–49.

9. Wang H, Ji XB, Mao B, Li CW, Lu HW, Xu JF. Pseudomonas aeruginosa isolation in patients with non-cystic fibrosis bronchiectasis: a retrospective study. BMJ Open. 2018;8(3):e014613.
10. Wang N, Qu JM, Xu JF. Bronchiectasis Management in China, what we can learn from European Respiratory Society Guidelines. Chin Med J (Engl). 2018;131(16):1891–3.
11. Wang LL, Lu HW, Li LL, Gao YH, Xu YH, Li HX, et al. Pseudomonas aeruginosa isolation is an important predictor for recurrent hemoptysis after bronchial artery embolization in patients with idiopathic bronchiectasis: a multicenter cohort study. Respir Res. 2023;24(1):84.
12. Finch S, McDonnell MJ, Abo-Leyah H, Aliberti S, Chalmers JD. A comprehensive analysis of the impact of Pseudomonas aeruginosa colonization on prognosis in Adult Bronchiectasis. Ann Am Thorac Soc. 2015;12(11):1602–11.
13. Hewison M. An update on vitamin D and human immunity. Clin Endocrinol. 2012;76(3):315–25.
14. Skrobot A, Demkow U, Wachowska M. Immunomodulatory Role of Vitamin D: A Review. Advances in experimental medicine and biology. 2018.
15. Amrein K, Scherkl M, Hoffmann M, Neuwersch-Sommeregger S, Köstenberger M, Tmava Berisha A, et al. Vitamin D deficiency 2.0: an update on the current status worldwide. Eur J Clin Nutr. 2020;74(11):1498–513.
16. Yu S, Fang H, Han J, Cheng X, Xia L, Li S, et al. The high prevalence of hypovitaminosis D in China: a multicenter vitamin D status survey. Medicine. 2015;94(8):e585.
17. Shaheen SO, Jameson KA, Robinson SM, Boucher BJ, Syddall HE, Sayer AA, et al. Relationship of vitamin D status to adult lung function and COPD. Thorax. 2011;66(8):692–8.
18. Sanket S, Madireddi J, Stanley W, Sura P, Prabhu M. Relation between Vitamin D Deficiency and Severity of Chronic Obstructive Pulmonary Disease-A Case Control Study. J Clin Diagn Research: JCDR. 2016;10(1):OC16–9.
19. Korn S, Hubner M, Jung M, Blettner M, Buhl R. Severe and uncontrolled adult asthma is associated with vitamin D insufficiency and deficiency. Respir Res. 2013;14:25.
20. Iftikhar R, Kamran SM, Qadir A, Haider E, Bin Usman H. Vitamin D deficiency in patients with tuberculosis. J Coll Physicians Surgeons–Pakistan: JCPSP. 2013;23(10):780–3.
21. Talat N, Perry S, Parsonnet J, Dawood G, Hussain R. Vitamin d deficiency and tuberculosis progression. Emerg Infect Dis. 2010;16(5):853–5.
22. Kunisaki KM, Niewoehner DE, Connett JE, Network CCR. Vitamin D levels and risk of acute exacerbations of chronic obstructive pulmonary disease: a prospective cohort study. Am J Respir Crit Care Med. 2012;185(3):286–90.
23. Loukou I, Moustaki M, Sardeli O, Plyta M, Douros K. Association of vitamin D status with lung function measurements in children and adolescents with cystic fibrosis. Pediatr Pulmonol. 2020;55(6):1375–80.
24. Ganmaa D, Uyanga B, Zhou X, Gantsetseg G, Delgerekh B, Enkhmaa D, et al. Vitamin D Supplements for Prevention of Tuberculosis Infection and disease. N Engl J Med. 2020;383(4):359–68.
25. Mitchell F. Vitamin-D and COVID-19: do deficient risk a poorer outcome? Lancet Diabetes Endocrinol. 2020;8(7):570.
26. Sartini M, Del Puente F, Oliva M, Carbone A, Bobbio N, Schinca E et al. Preventive vitamin D supplementation and risk for COVID-19 infection: a systematic review and Meta-analysis. Nutrients. 2024;16(5).
27. Ferri S, Crimi C, Heffler E, Campisi R, Noto A, Crimi N. Vitamin D and disease severity in bronchiectasis. Respir Med. 2019;148:1–5.
28. Chalmers JD, McHugh BJ, Docherty C, Govan JR, Hill AT. Vitamin-D deficiency is associated with chronic bacterial colonisation and disease severity in bronchiectasis. Thorax. 2013;68(1):39–47.
29. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357(3):266–81.
30. Simoneau T, Bazzaz O, Sawicki GS, Gordon C. Vitamin D status in children with cystic fibrosis. Associations with inflammation and bacterial colonization. Ann Am Thorac Soc. 2014;11(2):205–10.
31. Reiff DB, Wells AU, Carr DH, Cole PJ, Hansell DM. CT findings in bronchiectasis: limited value in distinguishing between idiopathic and specific types. AJR Am J Roentgenol. 1995;165(2):261–7.
32. Chalmers JD, Goeminne P, Aliberti S, McDonnell MJ, Lonni S, Davidson J, et al. The bronchiectasis severity index. An international derivation and validation study. Am J Respir Crit Care Med. 2014;189(5):576–85.
33. Pineda-Torra I, Gage M, de Juan A, Pello OM. Isolation, culture, and polarization of murine bone marrow-derived and peritoneal macrophages. Methods Mol Biol. 2015;1339:101–9.
34. Foong RE, Zosky GR. Vitamin D deficiency and the lung: disease initiator or disease modifier? Nutrients. 2013;5(8):2880–900.
35. Jolliffe DA, Griffiths CJ, Martineau AR. Vitamin D in the prevention of acute respiratory infection: systematic review of clinical studies. J Steroid Biochem Mol Biol. 2013;136:321–9.
36. Nnoaham KE, Clarke A. Low serum vitamin D levels and tuberculosis: a systematic review and meta-analysis. Int J Epidemiol. 2008;37(1):113–9.
37. Niksarlıoğlu EY, Kılıç L, Bilici D, Yiğitbaş B, Uysal MA, Çamsarı G. Vitamin D Deficiency and Radiological findings in Adult Non-cystic Fibrosis Bronchiectasis. Turk Thorac J. 2020;21(2):87–92.
38. Parekh D, Patel JM, Scott A, Lax S, Dancer RC, D'Souza V, et al. Vitamin D Deficiency in Human and Murine Sepsis. Crit Care Med. 2017;45(2):282–9.
39. Marino LV, Harris A, Johnstone C, Friend A, Newell C, Miles EA, et al. Characterising the nutritional status of children with primary ciliary dyskinesia. Clin Nutr. 2019;38(5):2127–35.
40. Zhao Y, Yang J, Shi J, Gong YN, Lu Q, Xu H, et al. The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. Nature. 2011;477(7366):596–600.
41. Zhao Y, Shao F. The NAIP-NLRC4 inflammasome in innate immune detection of bacterial flagellin and type III secretion apparatus. Immunol Rev. 2015;265(1):85–102.
42. Liu Y, Xie YZ, Shi YH, Yang L, Chen XY, Wang LW, et al. Airway acidification impaired host defense against Pseudomonas aeruginosa infection by promoting type 1 interferon β response. Emerg Microbes Infect. 2022;11(1):2132–46.
43. Cohen TS, Prince AS. Activation of inflammasome signaling mediates pathology of acute P. Aeruginosa pneumonia. J Clin Investig. 2013;123(4):1630–7.
44. Bartley J, Garrett J, Grant CC, Camargo CA. Jr. Could vitamin d have a potential anti-inflammatory and anti-infective role in bronchiectasis? Curr Infect Dis Rep. 2013;15(2):148–57.
45. McNally P, Coughlan C, Bergsson G, Doyle M, Taggart C, Adorini L, et al. Vitamin D receptor agonists inhibit pro-inflammatory cytokine production from the respiratory epithelium in cystic fibrosis. J Cyst Fibros. 2011;10(6):428–34.
46. Kwok WC, Ho JCM, Tam TCC, Ip MSM, Lam DCL. Risk factors for Pseudomonas aeruginosa colonization in non-cystic fibrosis bronchiectasis and clinical implications. Respir Res. 2021;22(1):132.
47. Martinez-García MA, Oscullo G, Posadas T, Zaldivar E, Villa C, Dobarganes Y, et al. Pseudomonas aeruginosa and lung function decline in patients with bronchiectasis. Clin Microbiol Infect. 2021;27(3):428–34.

Publisher's note