Vitamin D deficiency in connective tissue disease-associated interstitial lung disease

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Abstract

Objective

To determine and compare the prevalence of vitamin D deficiency in patients with connective tissue disease-associated interstitial lung disease (CTD-ILD).

Methods

The level of vitamin D was determined by the serum levels of $1,25(OH)_2D_3$. We evaluated 144 patients in our study, including 53 subjects in the CTD-ILD group and 91 subjects in the CTD group without ILD. CTD was diagnosed following the American College of Rheumatology criteria, and ILD was diagnosed by high-resolution computed tomography. Patients with other known causes of ILD and other pulmonary diseases were excluded. Vitamin D deficiency level was <20 ng/ml. This is a retrospective study.

Results

Serum vitamin D levels were significantly lower in CTD-ILD patients ($p<0.0001$). Vitamin D deficiency was lower in the CTD-ILD group (mean±SD: 11.5±4.1 ng/ml) than in the control group (13.9±4.8 ng/ml, $p=0.004$). The CTD-ILD group was older ($p=0.002$), had higher levels of fibrinogen ($p=0.028$) and positive anti-CCP ($p=0.026$), faster ESR ($p=0.001$), lower serum levels of serum calcium ($p=0.002$), and more immunosuppressive therapies ($p=0.011$). Decreased serum albumin and higher positive antinuclear antibodies (ANA) were associated with reduced vitamin D levels in the vitamin D subgroups. When the odds ratio was adjusted for CTD-ILD, vitamin D deficiency was also a risk factor for CTD-ILD, whereas serum levels of calcium was a protective factor for CTD-ILD.

Conclusion

Serum vitamin D deficiency is associated with CTD-ILD and is a risk factor. Therefore, vitamin D may play a role in the pathogenesis of CTD-ILD.

Key words

vitamin D deficiency, rheumatic diseases, connective tissue disease, interstitial lung disease, risk factor

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Introduction

Interstitial lung disease (ILD) is a common complication of connective tissue disease (CTD) and is usually associated with significant morbidity and mortality (1). Pulmonary symptoms may be the first and only manifestation of previously unrecognised CTD (2). The clinical presentation of CTD-ILD is usually nonspecific and range from an incidental radiographic finding, rash, cough, and arthritis. However, sometimes disease progression is rapid, leading to respiratory failure and even death. Proper diagnosis is based on clinical symptoms as well as physiology, radiography, laboratory, and pathology data. High-resolution computed tomography (HRCT) is usually reliably used to diagnose the disease (3, 4). ILD may occur in all types of CTD, including systemic lupus erythematosus, rheumatoid arthritis (RA), Sjögren's syndrome, systemic sclerosis, polymyositis/dermatomyositis, overlap connective tissue disease, undifferentiated connective tissue disease, and Anti-neutrophil cytoplasmic antibodies-associated vasculitis (5). The incidence of CTD associated with ILD varies, and studies have found that the median survival rate for CTD-ILD was approximately 6.5 years, and the mortality rate due to CTD attributable to ILD was 123.6 per 1000 person years. However, to date, effective guidelines and therapies to assist the clinician are lacking, emphasising the importance of appropriate and synthetic clinical management (6, 7).

The vitamin D analogue 1,25-dihydroxycholecalciferol (1,25$(OH)_2D_3$) can be measured by serology and a complicated physical transformation. 1,25$(OH)_2D_3$ is a steroid hormone and the last metabolic product of vitamin D. This hormone has an endocrine role in target organs by regulating skeletal development and calcium homeostasis. In addition, clinical studies have indicated that vitamin D deficiency and insufficiency is a potential risk factor for several chronic diseases, including osteoporosis, autoimmune diseases, chronic obstructive pulmonary disease, asthma, and pulmonary tuberculosis. Several studies demonstrated that lower serum levels of vitamin D were significantly associated with CTD, and thus vitamin D has a pivotal role in the maintenance of immune homeostasis, and the pronounced immunosuppressive function of vitamin D deficiency may increase the prevalence of certain autoimmune diseases (8-14). Many studies have confirmed that vitamin D can reduce cytokines by T lymphocytes and immunoglobulin production by B lymphocytes via expression of the vitamin D receptor, which is considered the primary immune cell target of vitamin D (15-17). Furthermore, a study reported that vitamin D deficiency was associated with idiopathic pulmonary fibrosis and could cause deficits in lung function, particularly among patients with CTD (18). It is well known that the mortality rate and challenge of diagnosis and treatment to CTD-ILD are higher than CTD without ILD, and vitamin D deficiency is deleterious in CTD or idiopathic pulmonary fibrosis. Therefore, this study aimed to evaluate the serum vitamin D levels in patients with CTD-ILD and patients with CTD without ILD, to assess whether vitamin D deficiency in CTD patients has clinical significance to the diagnosis and treatment of CTD-ILD.

Patients and methods

Study subjects

The subjects who met the American College of Rheumatology criteria for CTD were included in the analysis. The patients included in the study had complete HRCT and, among these, the patients whose HRCT met the interstitial pneumonia criteria were defined as the CTD-ILD group (3, 22-25). All HRCT scans were reviewed by two independent physicians from the Department of Radiology and Rheumatology of Affiliated Hospital of Chongqing Medical University, Chongqing, China, and the evaluated signs were consolidation, ground glass opacities, traction bronchiectasis, irregular linear opacities, subpleural curvilinear shadows, and honeycombing. All eligible patients with CTD-ILD or CTD without ILD were recruited between January 2016 and December 2016 from the Department of Rheumatology at the Second Affiliated Hospital of Chongqing Medical University. Patients with other

known causes of ILD and environmental exposure such as chronic obstructive pulmonary disease, asthma, pulmonary tuberculosis, and infection, were excluded. The clinical data were obtained from medical records when the serum samples were obtained. In the current study, we retrospectively analysed 144 patients with CTD, among all patients, 53 cases were diagnosed as CTD-ILD, and HRCT without lung involvement was classified as CTD without ILD. All patients were informed of the objectives of the study and gave written consent for their voluntary participation and the anonymous use of their personal data in statistical analyses. This is a retrospective and non-interventional study that used anonymised data sets, ethics approval was not required by local law.

Data collection

Clinical data, including detailed patient history, signs, and laboratory findings, were obtained from patients' medical records during the first clinical consultation.

Vitamin D measurement

The vitamin D status was evaluated by measurement of the serum levels of $1,25(OH)_2D_3$ by liquid chromatography-tandem mass spectrometry at the Department of nuclear medicine. The serum of Vitamin D in patient group was obtained random throughout of the year. By convention, vitamin D levels were defined as follows: serum 25(OH)D levels ≥75 ng/ml (≥30 ng/ml) indicated vitamin sufficiency, ≥50–75 nmol/l (≥20–30 ng/ml) indicated insufficiency, and <50 nmol/l (<20 ng/ml) indicated deficiency.

Statistical analysis

Patient characteristics, clinical symptoms and signs, and serologic test results were reported as mean ± standard deviation or as frequency counts and percentages. The data were analysed using analysis of variance, chi-square test or Fisher exact as appropriate. Logistic regression analysis was conducted to evaluate the association between relatively significant variables and serum concentrations of $1,25(OH)_2D_3$. All statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS 22.0, Chicago, IL, USA). *P*-values less than 0.05 were considered statistically significant.

Table I. Baseline demographic and clinical characteristics between patients with CTD-ILD and patients with CTD but without ILD.

Variable	Overall (n=144)	CTD-ILD (n=53)	CTD without ILD (n=91)	*p*-value
Age (years), mean (SD)	58.8 (16.0)	63.8 (12.5)	55.9 (17.2)	0.002*
Disease duration (years), mean (SD)	6.9 (8.9)	6.87 (7.8)	6.9 (9.5)	NS
Sex ratio female/all, n (%)	111 (77.1)	39 (73.6)	72 (79.2)	NS
Smoking status, n (%)	20 (13.9)	7 (13.2)	13 (14.3)	NS
ADL, mean (SD)	92.5 (15.6)	90.5 (22.4)	94.3 (9.6)	NS
Medication use history				
Corticosteroids, n (%)	35 (24.3)	16 (30.2)	19 (20.9)	NS
Vitamin D supplementation, n (%)	32 (22.3)	14 (26.4)	18 (19.8)	NS
Immunosuppressive therapy, n (%)	31 (21.5)	18 (34.0)	13 (14.3)	0.011*
Symptoms and signs				
Dry eyes/dry mouth, n (%)	47 (32.6)	15 (28.3)	32 (35.2)	NS
Joint pain/swelling, n (%)	121 (84.0)	43 (81.1)	78 (85.7)	NS
Rash, n (%)	21 (14.6)	6 (11.3)	15 (16.5)	NS
Raynaud's phenomenon, n (%)	13 (9.0)	8 (15.1)	5 (5.5)	NS
Sensitivity to light, n (%)	3 (2.1)	2 (3.8)	1 (1.1)	NS
Hand/mouth ulcers, n (%)	18 (12.5)	5 (9.4)	13 (14.3)	NS
Morning stiffness, n (%)	66 (47.8)	25 (47.2)	41 (45.1)	NS
Proximal muscle weakness, n (%)	22 (15.3)	10 (18.9)	12 (13.2)	NS
Cough and expectoration, n (%)	11 (7.6)	8 (15.1)	3 (3.3)	0.019*
Rheumatic disease				
Rheumatoid arthritis, n (%)	74 (51.4)	29 (54.7)	45 (49.5)	NS
Polymyositis/dermatomyositis, n (%)	3 (2.1)	2 (3.8)	1 (1.1)	NS
Systemic lupus erythematous, n (%)	10 (6.9)	1 (1.9)	9 (9.9)	NS
Sjögren's syndrome, n (%)	15 (10.4)	2 (3.8)	13 (14.3)	0.046*
Overlap CTD, n (%)	33 (22.9)	16 (30.2)	17 (18.7)	NS
Undifferentiated CTD, n(%)	4 (2.8%)	1 (1.9%)	3 (3.3%)	NS
ANCA-associated vasculitis, n (%)	2 (1.4%)	1 (1.9%)	1 (1.1%)	NS
Systemic sclerosis, n (%)	3 (2.1%)	1 (1.9%)	2 (2.2%)	NS

$p<0.05$ was considered significant.
NS: no statistical significance; ADL: Activity of Daily Living Scale.

Results

Subject characteristics

The baseline characteristics of the study participants are shown in Table I. One hundred forty-four patients were included in the study, including 53 patients with CTD-ILD and 91 patients with CTD without ILD. Overall, the mean age was 58.8±16.0 years, the mean age of the CTD-ILD group was 63.8±12.5 years and significantly higher than that of the group with CTD without ILD (55.9±17.2 years; (p=0.002). The CTD-ILD group included 39 (73.6%) women whereas the control group included 72 (79.2%) women. There were no sex differences between the groups (p=0.538). The overall mean disease duration was 6.9±8.9 years, but there was no difference in duration between the CTD-ILD and the control group (6.8±7.8 and 6.9±9.5 years, respectively). The ADL (Activity of Daily Living Scale) was no difference as well. The rate of history of use of immunosuppressive therapy in the CTD-ILD group were significantly higher (p=0.011) than those in the control group. However, no statistically significant differences in the distribution of baseline characteristics were observed between the two groups except cough and expectoration (p=0.019). The incidence of RA was non-significantly higher in the CTD-ILD group (54.7%) and CTD group without ILD (49.5%).

Table II shows the laboratory data for the two groups. The overall mean level of $25(OH)_2D_3$ was 16.2±7.2 ng/ml, 12.9±6.0 ng/ml in the CTD-ILD group, and 18.2±7.2 ng/ml in the CTD group without ILD. The serum $25(OH)_2D3$ levels were significantly lower in patients with CTD-ILD (p<0.0001) compared with the CTD group. The mean vitamin D level in the CTD-ILD group

with vitamin D insufficiency (<30 ng/ml) was significantly lower than that in the CTD group without ILD (12.6±5.5 ng/ml and 17.0±5.8 ng/ml, respectively) but similar to that of patients with vitamin deficiency (<20 ng/ml) (11.5±4.1 ng/ml in the CTD-ILD group and 13.9±4.8 ng/ml in the CTD group without ILD, respectively). The serum calcium levels were significantly lower in the CTD-ILD group compared with the control (p=0.002). The positive rate of detection of the auto-antibodies antinuclear antibody (ANA), anti-cyclic citrullinated peptide (anti-CCP) was higher in the CTD-ILD group (p<0.05) compared with the group with CTD without ILD. Similarly, among inflammatory markers, a significant correlation was observed between the two groups. The CTD-ILD group had higher fibrinogen (4.4±1.5 g/l, p=0.028) and faster ESR (51.0±31.7 mm/s, p=0.001), whereas the serum levels of IL-6 and D-dimer were not significantly different between the two groups.

Table II. Serum of 25-hydroxyvitamin D3 and other laboratory data compared with CTD-ILD and CTD without IL.

Laboratory data	Overall (n=144)	CTD-ILD (n=53)	CTD without ILD (n=91)	p-value
25-hydroxyvitamin D3 (ng/ml), mean (SD)	16.2 (7.2)	12.9 (6.0)	18.2 (7.2)	<0.0001*
Below sufficiency (<30 ng/ml)				
Percentage, n (%)	137 (95.1)	52 (98.1)	85 (93.4)	NS
Mean (SD), ng/ml	15.3 (6.1)	12.6 (5.5)	17.0 (5.8)	<0.0001*
Deficiency (<20 ng/ml)				
Percentage, n (%)	106 (73.6)	48 (90.5)	58 (63.7)	<0.0001*
Mean (SD), ng/ml	12.8 (4.1)	11.5 (4.1)	13.9 (4.8)	0.004*
Serum creatinine (mg/dl), mean (SD)	60.4 (23.0)	59.1 (15.4)	61.1 (26.5)	NS
Serum calcium (mg/dl), mean (SD)	2.2 (0.13)	2.16 (0.13)	2.2 (0.12)	0.002*
Serum phosphorus (mg/dl), mean (SD)	1.2 (0.20)	1.2 (0.18)	1.2 (0.21)	NS
ANA positive, n (%)	81 (56.3)	36 (67.9)	45 (49.5)	0.037*
RF positive, n (%)	95 (66.0)	37 (69.8)	58 (63.7)	NS
Anti-CCP positive, n (%)	69 (47.9)	32 (60.4)	37 (40.7)	0.026*
Anti-Ro-52 positive, n (%)	51 (35.4)	19 (35.8)	32 (35.2)	NS
Anti-Sm positive, n (%)	7 (4.9)	1 (1.9)	6 (6.6)	**
Anti-Jo-1 positive, n (%)	5 (3.5)	5 (9.4)	0 (0.0)	**
Anti-SSA positive, n (%)	48 (33.3)	16 (30.2)	32 (35.2)	NS
Anti-SSB positive, n (%)	16 (11.1)	5 (9.4)	11 (12.4)	NS
Inflammatory markers, mean (SD)				
D-dimmer (mg/l)	0.3 (0.6)	0.4 (0.6)	0.3 (0.6)	NS
Fib (g/l)	4.0 (1.3)	4.4 (1.5)	3.9 (1.2)	0.028*
IL-6 (pg/ml)	26.9 (41.9)	31.3 (46.6)	22.6 (38.5)	NS
CRP (mg/l)	14.3 (21.2)	17.1 (23.6)	12.6 (19.2)	NS
ESR (mm/s)	39.9 (28.8)	51.0 (31.7)	33.4 (24.9)	0.001*

ANA: antinuclear antibodies; anti-SSA: anti-Sjögren's syndrome-related antigen A; anti-SSB: anti-Sjögren's syndrome-related antigen B: anti-CENPB: anti-centromere protein B; anti-Ro52: anti-Ro52 antibody; anti-Sm: anti smith antibody; RF: rheumatoid factor; anti-CCP: anti-cyclic citrullinated peptide antibody; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; Fib: fibrinogen; IL-6: interleukin-6. p<0.05 was considered significant, NS: absence of significant differences.

Serological examination and clinical manifestations in the vitamin D subgroups

The vitamin D groups were divided into three subgroups: >30 ng/ml (vitamin sufficiency), ≤21–29 ng/ml (vitamin insufficiency), and <20 ng/ml (vitamin deficiency) (Table III). Serological markers and clinical manifestations were evaluated in these vitamin D subgroups. No correlation was observed between the presence of autoantibodies and clinical manifestations in the three subgroups, except for ALB and ANA: the increase in serum ALB levels were positively correlated with an increase in vitamin D levels (p=0.044). In addition, the level of positive ANA increased as the levels of vitamin D decreased (p=0.042). There was a non-significant positive correlation between ESR and low vitamin D levels. Furthermore, we found no association between the different levels of vitamin D and clinical manifestations.

Serum levels of 1,25(OH)$_2$D$_3$ in the study groups

As the group of patients with RA is clearly superior to that of the other CTD, we divide RA patients from the other CTD. We evaluated the levels of 1,25(OH)$_2$D$_3$ in the two groups from Table IV compared with the average levels. A statistically significant difference in vitamin D levels was found in patients diagnosed with RA and other CTD respectively, RA-ILD *vs*. RA without ILD (14.4±6.1 ng/ml, n=29 *vs*. 19.3±8.8 ng/ml, n=45, p=0.007), other CTD-ILD *vs*. other CTD without ILD (11.0±5.4 ng/ml, n=24 *vs*. 17.0±5.0 ng/ml, n=46, p<0.0001).

Adjusted odds ratio for CTD-ILD

On the basis of the analysis shown in Tables I and II, we used the relatively significant variables to determine independent predictors by logistical regression (Table V). The analysis indicated that vitamin D deficiency was a risk factor for CTD-ILD after adjusting for other variables at baseline (adjusted odds ratio (OR) [95% CI]: 8.09 (2.28–28.69), p=0.002), including immunosuppressive therapy (adjusted OR [95% CI]: 6.41 (2.00–20.48), p=0.002), positive anti-CCP (adjusted OR [95% CI]: 3.07 (1.19–7.91), p=0.052), positive ANA (adjusted OR [95% CI]: 4.90 (1.78–13.46), p=0.006), age (adjusted OR [95% CI]: 1.08 (1.04–1.12), p=0.000), which were strong risk factors for CTD-ILD. Conversely, serum calcium (adjusted OR [95% CI]: 0.015 (0.00–0.73), p=0.015) were protective factors for CTD-ILD.

Discussion

Although CTD-ILD severely affects the patient's the quality of life, no controlled clinical trial data are available to guide management decisions. Moreover, the treatment of CTD-ILD is a challenge because the available therapeutic options are scarce (1). The pathogenesis of CTD changes in the presence of ILD. In addition, the clinical manifestations and radiography findings limit the diagnosis, and HRCT is the most powerful tool for the diagnosis of ILD in patients with CTD. Our

Table III. Comparison of baseline demographic and clinical characteristics according to the vitamin D subgroups.

Variable	Vitamin D level (ng/ml)			
	<20 ng/ml (n=106) deficiency	≥20–30 ng/ml (n=31) insufficiency	≥30 ng/ml (n=7) sufficiency	*p*-value
Age (years), mean (SD)	57.4 (14.8)	57.3 (12.1)	57.3 (10.9)	NS
Serum calcium (mg/dl), mean (SD)	2.2 (0.1)	2.2 (0.1)	2.26 (0.9)	NS
D-dimmer (mg/l), mean (SD)	0.3 (0.5)	0.4 (0.8)	0.6 (1.2)	NS
Fib (g/l), mean (SD)	4.12 (1.2)	4.16 (1.5)	3.4 (2.1)	NS
ALB (mg/l), mean (SD)	38.0 (5.0)	39.9 (4.5)	41.5 (3.7)	0.044*
IL-6 (pg /ml), mean (SD)	26.8 (38.9)	30.1 (54.2)	13.6 (17.2)	NS
CRP (mg/l), mean (SD)	12.7 (15.8)	18.4 (30.8)	19.9 (37.8)	NS
ESR (mm/s), mean (SD)	41.0 (28.1)	37.8 (31.9)	31.3 (26.8)	NS
ANA positive, n (%)	66 (45.8)	13 (9.0)	2 (1.4)	0.045*
RF positive, n (%)	68 (47.2)	22 (15.2)	5 (3.5)	NS
Anti-CCP positive, n (%)	52 (36.1)	14 (9.7)	3 (2.1)	NS
Anti-Sm positive, n (%)	6 (4.17)	2 (1.39)	0 (0)	**
Dry eyes/dry mouth, n (%)	31 (21.5)	15 (10.4)	1 (0.69)	**
Rash, n (%)	14 (9.72)	7 (4.86)	1 (0.69)	**
Raynaud's phenomenon, n (%)	12 (8.33)	1 (0.69)	0 (0)	**
Morning stiffness, n (%)	49 (34.0)	13 (9.0)	4 (2.8)	NS
Sensitivity to light, n (%)	3 (2.08)	0 (0)	0 (0)	**
Proximal muscle weakness, n (%)	19 (13.2)	3 (2.1)	0 (0)	**
Joint pain/swelling, n (%)	89 (61.8)	27 (16.7)	6 (4.2)	NS

$p<0.05$ was considered significant; NS: absence of significant differences.
Refer to the legend of Table II for the abbreviations.

Table IV. Vitamin D status according to the subgroups in RA and other CTD.

	RA-ILD (n=29)	RA without ILD (n=45)	*p*-value
25-hydroxyvitamin D3 (ng/ml), mean (SD)	14.4 ± 6.1	19.3 ± 8.8	0.007*
	Other CTD-ILD (n=24)	Other CTD without ILD (n=46)	*p*-value
25-hydroxyvitamin D3 (ng/ml), mean (SD)	11.06 ± 5.4	17.0 ± 5.0	<0.0001*

*Statistical significance.

Table V. Logistic regression analysis for the CTD-ILD with adjustment for various factors.

	Odds ratio (95% CI)	*p*-value
Vitamin D deficiency	8.09 (2.28–28.69)	0.002*
Immunosuppressor use	6.41 (2.00–20.48)	0.002*
Anti-CCP positive	3.07 (1.19–7.91)	0.052*
ANA	4.90 (1.78–13.46)	0.006*
Age	1.08 (1.04–1.12)	0.000*
Serum calcium	0.015 (0.00–0.73)	0.032*

Refer to the legend of Table II for the abbreviations. *Statistical significance.

results indicated differences in baseline characteristics between CTD-ILD and CTD without ILD: patients with CTD-ILD were older, but gender and disease duration were similar between the groups, which agrees with the results of previous studies that patients presented with lung fibrosis at the first medical consultation (4). After adjusting the OR for CTD-ILD, the CTD-ILD group was older, used more immunosuppressive drugs, had increased levels of positive ANA and positive anti-CCP, and lower serum levels of calcium, and all these conditions are risk factors for CTD-ILD. Therefore, HRCT is urgent in cases in which the patients with CTD present these characteristics. However, the analysis clinical manifestations indicated that only cough was significantly different between the two groups, demonstrating that the evaluation of symptoms does not contribute significantly to the diagnosis of ILD.

Vitamin D deficiency was significantly associated with CTD, especially when combined with ILD (19). The mean level of vitamin D in the CTD-ILD group was 12.9±6.0 ng/ml, indicating strong deficiency. The difference persisted after classifying the patients into subgroups. As is known to all, daily activity is related to vitamin D absorption, CTD-ILD patients maybe has less daily activity and less exposed to sunlights, but we do not see statistical significance in the ADL between the two groups when the serum level of $1,25(OH)_2D_3$ were obtained. Single factor analysis indicated significant differences between the groups, and the correlation between vitamin D deficiency and CTD-ILD persisted after adjusting for single risk factors. As reported in other studies, in patients with CTD, the highest incidence is RA, so does in patients with CTD-ILD, the comparison of the mean vitamin D levels in RA-ILD *vs*. RA without ILD, other CTD-ILD *vs*. other CTD without ILD indicated that the patients with ILD had significantly lower levels of vitamin D.

Consistent with our findings, verifying the assertion we mentioned above. The CTD-ILD group had faster ESR, higher positive ANA, and higher FIB, suggesting that ILD may worsen the inflammatory processes of the disease. Vitamin D plays crucial functions in calcium metabolism, our study indicated that lower serum calcium levels were lower in the CTD-ILD group accompany with lower vitamin D (p=0.002). Calcium levels may play a significant role in the autoimmune disease at the same time (20).

Our results demonstrated that positive anti-CCP was higher in the CTD-ILD group (p=0.022). Positive anti-CCP remained a risk factor after adjusting for CTD-ILD (adjusted OR [95% CI]: 3.070 (1.19–7.91), p=0.052). Anti-CCP has been shown to play a crucial role in the diagnosis of RA, maybe our result correlated with the highest incidence

of RA in CTD. However, recent studies proposed that the lungs might be an extra-articular mucosal site for initiating associated organ immunity, and the environment and genes may trigger local inflammation and induce autoantigen expression, which may lead to the generation of anti-CCP in the lungs. Moreover, positive anti-CCP aggravates lung damage, which in turn contributes to the development of ILD (21-23).

Our results indicated that decreased serum ALB and higher positive ANA were associated with reduced vitamin D levels in the vitamin D subgroups. A clinical trial involving more than 3,000 patients showed that lower vitamin D levels were associated with specific clinical manifestations in some cases of CTD (24). However, we did not observe a correlation between clinical manifestations and different vitamin D concentrations, probably because of the small sample size.

Vitamin D deficiency increases rheumatic activity. Moreover, previous studies on immune mechanisms of lung disease reported worse lung function in animal models, and vitamin D deficiency upregulated the expression of inflammatory molecules, stimulates extracellular matrix deposition and induce renin-angiotensin system activation, leading to changes in lung structure and inflammation after an insult, therefore, vitamin D deficiency may lead to pulmonary fibrosis. Lung epithelial cells could express VDR, vitamin D could inhibit the expression of epithelial cell markers and significantly inhibit TGF-β1 stimulation of alpha-smooth muscle actin expression and polymerisation via VDR. Impaired TGF-β-induced increasing of Collagen I and fibronectin (25-27). Another study indicated a significant association between lower percentage of predicted forced vital capacity, carbon monoxide diffusing capacity, and reduced serum vitamin D levels in patients with CTD-ILD (18). However, few studies have evaluated the correlation between lower vitamin D levels and CTD-ILD.

In addition, current mainstay therapies for CTD-ILD include immunosuppressive and corticosteroid therapies, together with the increased use of biological agents, and occasional lung transplantation in some centres. However, the effect of these therapies differs among patients, some therapies are ineffective, and some might even be toxic (28-31). Each investigator persisted in its own views in the treatment effect of vitamin D. At present, uncontrolled studies suggest that vitamin D supplementation may be useful to prevent the development of autoimmune diseases and reduce the severity of pre-existing diseases. One study described the feasibility of supplementation with vitamin D from the aspects of mechanism, and the efficacy of supplementation with small doses of vitamin D has been described (32-34). Animal experiments also indicated that vitamin D supplementation attenuated mice pulmonary fibrosis (19, 29), confirming the beneficial effect of therapeutic vitamin D supplementation in some rheumatic diseases (35). Notwithstanding, multiple-database randomised controlled trials and CTD outcomes are lacking, let alone CTD-ILD outcomes. Our results indicated that vitamin D supplementation might be a good therapeutic candidate for the control of autoimmune processes in rheumatic diseases by reducing the activity of inflammation and an effective treatment to improve the prognosis of patients with CTD-ILD. Furthermore, the monitoring of serum vitamin D levels might provide insights about the risk factors for patients with underlying CTD that progress into CTD-ILD, but this hypothesis needs confirmation.

This study has several limitations. First, serum vitamin D level was affected by many risk factors, including aging, female sex, race, winter season, renal function, use of vitamin D supplements, and corticosteroids. Second, a lot of factors can lead to ILD, environmental factors, such as vitamin D deficiency should have a minor role in the pathophysiology of the disease. Furthermore, a vitamin D supplement group was not included in our study and, for this reason, the outcomes might be affected by the sample size.

In conclusion, this study demonstrated that serum vitamin D deficiency occurs in CTD-ILD and that the patients with CTD with low levels vitamin D should be alert about the development of ILD. Vitamin D deficiency may be an environmental trigger for disease onset and not a consequence of rheumatic disease, and future studies should test the hypothesis that vitamin D supplementation may be used in the treatment of CTD-ILD. Randomised controlled studies that use large samples and multiple therapeutic agents are also needed.

References

1. FISCHER A, DU BOIS R: Interstitial lung disease in connective tissue disorders. *Lancet* 2012; 380: 689-98.
2. HU Y, WANG LS, WEI YR *et al*.: Clinical characteristics of connective tissue disease-associated interstitial lung disease in 1,044 Chinese patients. *Chest* 2016; 149: 201-8.
3. RAGHU G, COLLARD HR, EGAN JJ *et al*.: An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med* 2011; 183: 788-824.
4. ANTIN-OZERKIS D, RUBINOWITZ A, EVANS J, HOMER RJ, MATTHAY RA: Interstitial lung disease in the connective tissue diseases. *Clin Chest Med* 2012; 33: 123-49.
5. BRYSON T, SUNDARAM B, KHANNA D, KAZEROONI EA: Connective tissue disease-associated interstitial pneumonia and idiopathic interstitial pneumonia: similarity and difference. *Semin Ultrasound CT MR* 2014; 35: 29-38.
6. DEMORUELLE MK, MITTOO S, SOLOMON JJ: Connective tissue disease-related interstitial lung disease. *Best Pract Res Clin Rheumatol* 2016; 30: 39-52.
7. NAVARATNAM V, ALI N, SMITH CJ, MCKEEVER T, FOGARTY A, HUBBARD RB: Does the presence of connective tissue disease modify survival in patients with pulmonary fibrosis? *Respir Med* 2011; 105: 1925-30.
8. CUTOLO M, PLEBANI M, SHOENFELD Y, ADORINI L, TINCANI A: Vitamin D deficiency in undifferentiated connective tissue disease. *Vitam Horm* 2011; 86: 327-51.
9. DALL'ARA F, CUTOLO M, ANDREOLI L, TINCANI A, PAOLINO S: Vitamin D and systemic lupus erythematous: a review of immunological and clinical aspects. *Clin Exp Rheumatol* 2018; 36: 153-62.
10. RUIZ-IRASTORZA G, EGURBIDE MV, OLIVARES N, MARTINEZ-BERRIOTXOA A, AGUIRRE C: Vitamin D deficiency in systemic lupus erythematosus: prevalence, predictors and clinical consequences. *Rheumatology* (Oxford) 2008; 47: 920-3.
11. PATEL S, FARRAGHER T, BERRY J, BUNN D, SILMAN A, SYMMONS D: Association between serum vitamin D metabolite levels and disease activity in patients with early inflammatory polyarthritis. *Arthritis Rheum* 2007; 56: 2143-9.
12. KAMEN DL, COOPER GS, BOUALI H, SHAFTMAN SR, HOLLIS BW, GILKESON GS:

Vitamin D deficiency in systemic lupus erythematosus. *Autoimmun Rev* 2006; 5: 114-7.
13. ERTEN S, SAHIN A, ALTUNOGLU A, GEMCIOGLU E, KOCA C: Comparison of plasma vitamin D levels in patients with Sjögren's syndrome and healthy subjects. *Int J Rheum Dis* 2015; 18: 70-5.
14. STEEN VD, MEDSGER TA: Changes in causes of death in systemic sclerosis, 1972-2002. *Ann Rheum Dis* 2007; 66: 940-4.
15. VAN ETTEN E, DECALLONNE B, MATHIEU C: 1,25-dihydroxycholecalciferol: endocrinology meets the immune system. *Proc Nutr Soc* 2002; 61: 375-80.
16. LEMIRE J: 1,25-Dihydroxyvitamin D3 – a hormone with immunomodulatory properties. *Z Rheumatol* 2000; 59 (Suppl. 1): 24-7.
17. CUTOLO M, PIZZORNI C, SULLI A: Vitamin D endocrine system involvement in autoimmune rheumatic diseases. *Autoimmun Rev* 2011; 11: 84-7.
18. HAGAMAN JT, PANOS RJ, MCCORMACK FX *et al*.: Vitamin D deficiency and reduced lung function in connective tissue-associated interstitial lung diseases. *Chest* 2011; 139: 353-60.
19. VASILE M, CORINALDESI C, ANTINOZZI C, CRESCIOLI C: Vitamin D in autoimmune rheumatic diseases: A view inside gender differences. *Pharmacol Res* 2017; 117: 228-41.
20. WATAD A, TIOSANO S, AZRIELANT S *et al*.: Low levels of calcium or vitamin D - which is more important in systemic lupus erythematosus patients? An extensive data analysis. *Clin Exp Rheumatol* 2017; 35: 108-12.
21. PAKPOOR J, PAKPOOR J: Vitamin d deficiency and systemic lupus erythematosus: cause or consequence? *Oman Med J* 2013; 28: 295.
22. CHATZIDIONISYOU A, CATRINA AI: The lung in rheumatoid arthritis, cause or consequence? *Curr Opin Rheumatol* 2016; 28: 76-82.
23. YTTERBERG AJ, JOSHUA V, REYNISDOTTIR G *et al*.: Shared immunological targets in the lungs and joints of patients with rheumatoid arthritis: identification and validation. *Ann Rheum Dis* 2015; 74: 1772-7.
24. AGMON-LEVIN N, THEODOR E, SEGAL RM, SHOENFELD Y: Vitamin D in systemic and organ-specific autoimmune diseases. *Clin Rev Allergy Immunol* 2013; 45: 256-66.
25. RAMIREZ AM, WONGTRAKOOL C, WELCH T, STEINMEYER A, ZUGEL U, ROMAN J: Vitamin D inhibition of pro-fibrotic effects of transforming growth factor beta1 in lung fibroblasts and epithelial cells. *J Steroid Biochem Mol Biol* 2010; 118: 142-50.
26. FOONG RE, BOSCO A, JONES AC *et al*.: The effects of in utero vitamin D deficiency on airway smooth muscle mass and lung function. *Am J Respir Cell Mol Biol* 2015; 53: 664-75.
27. ZOSKY GR, BERRY LJ, ELLIOT JG, JAMES AL, GORMAN S, HART PH: Vitamin D deficiency causes deficits in lung function and alters lung structure. *Am J Respir Crit Care Med* 2011; 183: 1336-43.
28. CASTELINO FV, VARGA J: Interstitial lung disease in connective tissue diseases: evolving concepts of pathogenesis and management. *Arthritis Res Ther* 2010; 12: 213.
29. MATHAI SC, DANOFF SK: Management of interstitial lung disease associated with connective tissue disease. *BMJ* 2016; 352: h6819.
30. OLDHAM JM, LEE C, VALENZI E *et al*.: Azathioprine response in patients with fibrotic connective tissue disease-associated interstitial lung disease. *Respir Med* 2016; 121: 117-22.
31. WITT LJ, DEMCHUK C, CURRAN JJ, STREK ME: Benefit of adjunctive tacrolimus in connective tissue disease-interstitial lung disease. *Pulm Pharmacol Ther* 2016; 36: 46-52.
32. VOJINOVIC J, TINCANI A, SULLI A *et al*.:European multicentre pilot survey to assess vitamin D status in rheumatoid arthritis patients and early development of a new Patient Reported Outcome questionnaire (D-PRO). *Autoimmun Rev* 2017; 16: 548-54.
33. VARENNA M, MANARA M, CANTATORE FP *et al*.: Determinants and effects of vitamin D supplementation on serum 25-hydroxyvitamin D levels in patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2012; 30: 714-9.
34. ZHANG Z, YU X, FANG X *et al*.: Preventive effects of vitamin D treatment on bleomycin-induced pulmonary fibrosis. *Sci Rep* 2015; 5: 17638.
35. ANDJELKOVIC Z, VOJINOVIC J, PEJNOVIC N *et al*.: Disease modifying and immunomodulatory effects of high dose 1 alpha (OH) D3 in rheumatoid arthritis patients. *Clin Exp Rheumatol* 1999; 17: 453-6.