Vitamin D Deficiency and Reduced Lung Function in Connective Tissue-Associated Interstitial Lung Diseases

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Background: Vitamin D is a steroid hormone with pleiotropic effects including immune system modulation, lung tissue remodeling, and bone health. Vitamin D deficiency has been implicated in the development of autoimmune diseases. We sought to evaluate the prevalence of vitamin D deficiency in a cohort of patients with interstitial lung disease (ILD) and hypothesized that vitamin D deficiency would be associated with an underlying connective tissue disease (CTD) and reduced lung function.

Methods: Patients in the University of Cincinnati ILD Center database were evaluated for serum 25-hydroxyvitamin D levels as part of a standardized protocol. Regression analysis evaluated associations between 25-hydroxyvitamin D levels and other variables.

Results: One hundred eighteen subjects were included (67 with CTD-ILD, 51 with other forms of ILD). The overall prevalence of vitamin D deficiency and insufficiency in the study population was 38% and 59%, respectively. Those with CTD-ILD were more likely to have vitamin D deficiency (52% vs 20%, $P < .0001$) and insufficiency (79% vs 31%, $P < .0001$) than other forms of ILD. Diminished FVC was associated with lower 25-hydroxyvitamin D₃ levels ($P = .01$). The association between vitamin D insufficiency and CTD-ILD persisted (OR, 11.8; $P < .0001$) after adjustment for potential confounders. Among subjects with CTD-ILD, reduced 25-hydroxyvitamin D₃ levels were strongly associated with reduced lung function (FVC, $P = .015$; diffusing capacity for carbon monoxide, $P = .004$).

Conclusions: There is a high prevalence of vitamin D deficiency in patients with ILD, particularly those with CTD-ILD, and it is associated with reduced lung function. Vitamin D may have a role in the pathogenesis of CTD-ILD.

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Abbreviations: 6MWT = 6-min walk test; CTD = connective tissue disease; DLCO = diffusing capacity for carbon monoxide; HRCT = high-resolution CT; ILD = interstitial lung disease; RA = rheumatoid arthritis; SLE = systemic lupus erythematosus; Th = T helper; UCTD = undifferentiated connective tissue disease

In addition to its essential role in calcium homeostasis, vitamin D has many nonskeletal effects that are important in health and disease.¹ In animal models, vitamin D has been studied as a modifiable environmental factor² in a wide array of autoimmune diseases, including connective tissue diseases (CTDs).^{3–7} Epidemiologic evidence also supports an association between vitamin D and autoimmune disorder susceptibility and severity.^{8–10} In systemic lupus erythematosus (SLE), for example, up to two-thirds of patients are vitamin D deficient, and one in five have critically low levels of 25-hydroxyvitamin D₃, the form of vitamin D commonly measured in the serum.^{10,11}

Disease activity in SLE has been associated with vitamin D level,¹² and vitamin D supplementation has led to attenuation of some disease manifestations in experimental models.³ Patients with undifferentiated connective tissue disease (UCTD),¹³ rheumatoid arthritis (RA),¹⁴ fibromyalgia,^{9,15} and general rheumatologic populations¹⁶ have also been shown to have lower serum 25-hydroxyvitamin D₃ when compared with healthy control subjects, even after controlling for activity level and dietary intake. These epidemiologic and clinical associations suggest that vitamin D may be involved in the pathogenesis and end-organ dysfunction of these autoimmune disorders.

Lung involvement is common in CTD with prevalence estimates of up to 80%,¹⁷ with diffuse interstitial lung disease (ILD) being the most common pulmonary manifestation. The impact of pulmonary involvement is underscored by the fact that it is now the leading cause of death in several CTDs.¹⁷⁻²⁰ Corticosteroids are a mainstay of treatment regimens in patients with CTD-ILD,^{21,22} and the detrimental effects of long-term usage on bone health are well documented.²³ In SLE, vitamin D insufficiency was associated with cumulative corticosteroid exposure,²⁴ and the interplay of chronic inflammation and low vitamin D levels has been causally implicated in low bone mineral density in these patients.²⁵ In subjects with asthma, it has recently been reported that reduced vitamin D levels are associated with impaired steroid responsiveness.²⁶ Thus, the presence of hypovitaminosis D may be particularly relevant for patients with CTD-ILD, who are often treated with corticosteroids.

The pulmonary, bone, and autoimmune actions of vitamin D are of interest in the setting of CTD, given the significant role ILD can play in the lives of these patients. However, there is no available information in the literature regarding the prevalence of vitamin D deficiency among patients with diffuse parenchymal lung disease or whether reduced levels are associated with end-organ dysfunction. For this study, we examined the prevalence of vitamin D deficiency and insufficiency in a cohort of patients with ILD and hypothesized that 25-hydroxyvitamin D₃ levels would be associated with the presence of an underlying CTD diagnosis. Further, we sought to determine if serum 25-hydroxyvitamin D₃ levels were associated with impaired lung function as measured by pulmonary function tests and the 6-min walk test (6MWT).

Study Subjects, Clinical Evaluation, and Radiographic Evaluation

Consecutive patients seen in the University of Cincinnati Interstitial Lung Disease Clinic were enrolled in a longitudinal database and evaluated for serum 25-hydroxyvitamin D₃ levels as part of a standardized evaluation protocol between October 2008 and January 2010. Subjects enrolled in the database underwent a detailed questionnaire evaluating symptom and exposure history, past and current medication usage, functional status, family history, and comorbidities. Medical records of all patients in the database were reviewed for information regarding sex, ethnicity, smoking status, physical examination findings, use of supplemental oxygen, pulmonary function tests, 6-min walk distance, high-resolution CT (HRCT) scan findings, serologic tests, and pathologic studies. All testing was ordered for the clinical evaluation of the patient in a standardized fashion and was not performed specifically for the purposes of this study. Prebronchodilator lung function tests and the 6MWT were performed at/around the time of enrollment according to published guidelines and interpreted according to reference values.²⁷⁻²⁹ BMI, kg/m² was calculated from measured height and weight. HRCT scan interpretation was uniformly performed by an expert specializing in ILD (B. W. K.). Patients were assigned a final ILD diagnosis through a multidisciplinary clinical-radiologic-pathologic consensus conference with input from pulmonologists, rheumatologists, radiologists, and pathologists, as has been previously described.³⁰ This process was performed independently of this study and without knowledge of vitamin D levels. Patients classified as having CTD-ILD met pre-specified criteria according to the American College of Rheumatology criteria for diagnosis of the following conditions: RA, SLE, scleroderma, polymyositis/dermatomyositis, mixed CTD, Wegener granulomatosis, and Sjögren disease. The diagnosis of UCTD was based on previously published criteria.^{31,32} Patients enrolled in the database lacking radiographic or clinical evidence of ILD were excluded from the study. Patients without serum 25-hydroxyvitamin D₃ levels obtained during the study period were also excluded (*n* = 38). All subjects were enrolled into a University of Cincinnati institutional review board-approved protocol (07091806) investigating the natural history of ILD. Informed consent was obtained from all subjects at the time of the initial visit.

Vitamin D Measurement

Serum 25-hydroxyvitamin D₃ concentrations were assayed by liquid chromatography-tandem mass spectrometry at Esoterix Endocrinology Laboratories (Calabasas Hills, California). By convention, vitamin D insufficiency and deficiency were defined as serum 25-hydroxyvitamin D₃ values <30 ng/mL and 20 ng/mL, respectively.¹

Statistical Analysis

Comparisons between groups, CTD-ILD vs other forms of ILD, were made using the Student *t* test, χ^2 test, or Fisher exact test as appropriate. Associations with 25-hydroxyvitamin D₃ level were investigated with Student *t* test, Pearson correlation, analysis of variance, χ^2 test, or Fisher exact test as appropriate. In the primary analysis, patients with unclassifiable ILD (*n* = 8) were grouped with the non-CTD related ILD group. The impact of this grouping was tested in sensitivity analyses, in which these patients were excluded. In addition, we performed a sensitivity analysis excluding subjects with UCTD. All *P* values corresponded to two-sided tests and statistical significance was defined as a *P* value

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of $<.05$. Variables found to be associated at a P value of $<.05$ in the unadjusted analysis and those considered important a priori were considered for inclusion in multivariate regression models. Before inclusion, Pearson correlation coefficients between continuous covariates were evaluated to avoid collinearity. The final multivariate models were chosen using stepwise backward deletion. All analyses were performed with STATA statistical software version 9.2 (Stata Corp; College Station, Texas).

RESULTS

Subjects

One hundred eighteen patients were included in the study (67 with CTD-ILD and 51 with other forms of ILD). Clinical characteristics of the cohort and comparisons between patients with CTD-ILD and other forms of ILD are shown in Table 1. The distribution of patients with ILDs not associated with CTD was the following: idiopathic interstitial pneumonias ($n = 22$), granulomatous diseases ($n = 16$), unclassifiable ILD ($n = 8$), and miscellaneous forms of ILD ($n = 5$). The CTD-ILD subjects were more likely to be women, younger, and have taken corticosteroids (all $P < .001$). In addition, they had lower pulmonary function as measured by FVC ($P = .01$) and were more likely to have undergone surgical lung biopsy as part of their diagnosis ($P = .04$).

Vitamin D deficiency and insufficiency were highly prevalent in the cohort (Table 2), as 58% were vitamin D insufficient (25-hydroxyvitamin $D_3 < 30$ ng/mL) and 38% were deficient (25-hydroxyvitamin $D_3 < 20$ ng/mL). The mean 25-hydroxyvitamin D_3 level was significantly lower for those with CTD-ILD as compared with other forms of ILD (including granulomatous diseases, idiopathic interstitial pneumonias,

and miscellaneous forms of ILD) (20.8 vs 33.1 ng/mL, $P < .0001$) (Table 2). For the entire cohort, blacks had lower 25-hydroxyvitamin D_3 levels than whites/Asians (17.2 vs 28.9 ng/mL, $P = .0001$). An inverse association was also seen between BMI and 25-hydroxyvitamin D_3 levels; 25-hydroxyvitamin D_3 levels declined with increasing BMI ($P = .009$).

Notably, 25-hydroxyvitamin D_3 levels were not significantly lower for underweight patients ($P = .9$) or those with subjective reports of weight loss ($P = .9$) (data not shown). There was no observed association between 25-hydroxyvitamin D_3 levels and sex, smoking status, or season drawn ($P = .08, .94, .13$, respectively, data not shown). Notably, there was also no statistically significant association between prednisone use and 25-hydroxyvitamin D_3 levels (mean of 25 ng/mL on steroids vs 28 ng/mL not on steroids, $P = .30$). A minority of subjects were receiving vitamin D supplementation at the time of testing ($n = 16, 14\%$); however, there was no observed association between supplementation and 25-hydroxyvitamin D_3 levels (mean 31 ng/mL [95% CI, 21.6-40.1] on supplementation [$n = 16$] vs 26 ng/mL [95% CI, 22.8-28.3] for those not on supplementation [$n = 99, P = .17$]).

Vitamin D Levels and CTD-ILD

Of those with CTD-ILD, 80% had vitamin D insufficiency, and more than one-half were vitamin D deficient (Table 2). The distribution of specific CTDs in the population was the following: UCTD ($n = 28$), scleroderma ($n = 12$), RA ($n = 10$), polymyositis/dermatomyositis ($n = 7$), Sjögren disease ($n = 4$), Sjögren disease/SLE ($n = 2$), SLE ($n = 1$), mixed CTD ($n = 1$), and Wegener granulomatosis ($n = 2$). In sensitivity

Table 1—Demographics and Lung Function of All Subjects in ILD Cohort

Variable	Overall (N = 118)	CTD-ILD (n = 67)	Non-CTD Forms of ILD (n = 51)	P Value ^a
Age, y, mean $\pm$ SD	59.6 $\pm$ 1.4	55.4 $\pm$ 14.7	65.0 $\pm$ 12.9	.0003
Men, No. (%)	39 (33.1)	13 (19.4)	26 (50.1)	< .0001
Race, white, No. (%)	90 (76.3)	47 (70.2)	43 (84.3)	.08
Symptom duration prior to evaluation, d, mean $\pm$ SD	1,534 $\pm$ 1,455	1,430 $\pm$ 1,377	1,672 $\pm$ 1,554	.37
BMI, mean $\pm$ SD)	32.3 $\pm$ 9.2	33.5 $\pm$ 10.3	30.7 $\pm$ 7.3	.10
Smoking status, No. (%)				
Nonsmoker	48 (40.7)	29 (43.3)	19 (37.2)	.57
Current or former smoker	70 (59.3)	38 (56.7)	32 (62.8)	
Medication use history, No. (%)				
Corticosteroids	65 (55.1)	48 (71.6)	17 (33.3)	< .0001
Vitamin D supplementation	16 (13.9)	7 (10.8)	9 (18)	.29
Diuretic usage	27 (23.9)	12 (18.5)	15 (31.3)	.13
Lung function				
FVC, mean % predicted $\pm$ SD	75.3 (19.2)	71.2 (20.5)	80.7 (15.9)	.01
DLCO, mean % predicted $\pm$ SD	55.9 (22.2)	57.5 (24.0)	53.7 (19.6)	.4
6MWT distance, ft, mean $\pm$ SD	1,001 (471)	1,053 (417)	928 (537)	.22
Diagnosis including lung biopsy, No. (%)	48 (41)	33 (49.3)	15 (30)	.04

6MWT = 6-min walk test; CTD = connective tissue disease; DLCO = diffusing capacity for carbon monoxide; ILD = interstitial lung disease.

^aCTD-ILD vs non-CTD forms of ILD.

Table 2—Laboratory and Radiographic Data for Study Participants

Variable	Overall	CTD-ILD	Non-CTD-Related ILD	P Value ^a
Serum 25-hydroxyvitamin D ₃ levels				
Mean ± SD, ng/mL	26.1 ± 14.3	20.8 ± 11.0	33.1 ± 15.3	< .0001
Deficient < 20 ng/dL, No. (%)	45 (38.1)	35 (52.2)	10 (19.6)	< .0001
Insufficient < 30 ng/dL, No. (%)	69 (58.5)	53 (79.1)	16 (31.4)	< .0001
Laboratory data				
ANA positive, No. (%)	34 (30.1)	29 (45.3)	5 (10.2)	< .0001
RF positive, No. (%)	32 (28.1)	26 (40)	6 (12.2)	.001
ESR, mm/s, mean ± SD	26.2 ± 24.5	29.7 ± 25.8	21.3 ± 21.9	.08
Creatinine, mg/dL, mean ± SD	1.0 ± 0.5	1.0 ± 0.7	1.1 ± 0.3	.55
Serum calcium, mg/dL, mean ± SD	9.4 ± 0.5	9.4 ± 0.5	9.3 ± 0.6	.5
Serum phosphorus, mg/dL, mean ± SD	3.4 ± 0.5	3.3 ± 0.5	3.4 ± 0.6	.6
HRCT scan findings				
Ground glass opacity, No. (%)	75 (64.1)	50 (74.6)	25 (50)	.007
Reticular opacities, No. (%)	71 (60.7)	36 (53.7)	35 (70)	.09
Honeycombing, No. (%)	19 (16.2)	8 (11.9)	11 (22)	.2

ANA = antinuclear antibody; ESR = erythrocyte sedimentation rate; HRCT = high-resolution CT; RF = rheumatoid factor. See Table 1 legend for expansion of other abbreviations.

^aCTD-ILD vs non-CTD forms of ILD.

analyses, when the UCTD subjects were removed, the observed association between CTD and vitamin D insufficiency remained (OR, 6.3; CI, 2.5-16; $P < .001$; data not shown). Reduced mean 25-hydroxyvitamin D₃ levels were consistent across all subsets of CTD-ILD (data not shown).

Although vitamin D deficiency/insufficiency was still highly prevalent in patients with ILDs not associated with CTD, mean serum 25-hydroxyvitamin D₃ levels were significantly higher in this group ($P \leq .0001$) (Table 2). These groups all had higher mean 25-hydroxyvitamin D₃ than those with CTD-ILD (Fig 1).

Vitamin D Levels and Lung Function

Across the entire cohort, there was a significant association between lower percent predicted FVC and reduced serum 25-hydroxyvitamin D₃ levels ($R = 0.31$, $P = .01$). In contrast, there was no statistically significant association observed between lower 25-hydroxyvitamin D₃ levels and percent predicted diffusing capacity for carbon monoxide (DLCO) ($R = 0.13$, $P = .18$), percent predicted total lung capacity ($R = 0.17$, $P = .09$), and 6MWT distance ($R = 0.08$, $P = .47$) (data not shown). However, when the analysis was restricted to those with CTD-ILD, there was a

FIGURE 1. Histogram of serum vitamin D (25(OH)D₃, ng/mL) by patient subgroup (analysis of variance [ANOVA], $P < .00001$). 25(OH)D₃ = 25-hydroxyvitamin D₃; CTD-ILD = connective tissue disease-associated interstitial lung disease; granulomatous disease = sarcoidosis and hypersensitivity pneumonitis; IIP = idiopathic interstitial pneumonias; Misc = miscellaneous ILD.

strong inverse association between serum vitamin D insufficiency and FVC, as well as DLCO (OR, 0.42; $P = .015$ and OR, 0.33; $P = .004$ respectively) (Fig 2, Table 3).

Adjusted Models

Variables found to be associated with vitamin D insufficiency in the unadjusted analysis in addition to those considered important a priori (age, corticosteroid usage, race, and season drawn) were included as predictors in a multivariate linear regression model (with 25-hydroxyvitamin D₃ levels as a continuous variable) and a multivariate logistic regression model (looking at categorical vitamin D insufficiency) (Tables 4, 5). In the adjusted analysis, linear regression models showed that patients with an underlying CTD diagnosis had mean 25-hydroxyvitamin D₃ levels 11 ng/mL less than those with other forms of ILD, even after adjustment for other potentially confounding variables ($P < .0001$;

Table 3—Factors Associated With Vitamin D Insufficiency Among Subjects With CTD-ILD (Unadjusted Analysis)

Factor	OR	95% CI	P Value
FVC predicted (per SD)	0.42	0.21-0.84	.015
DLCO predicted (per SD)	0.33	0.16-0.70	.004
BMI (per SD)	2.16	0.93-5.02	.07
Age (per SD)	0.61	0.32-1.14	.12
Corticosteroid use	1.55	0.44-5.41	.49
Male	0.85	0.20-3.64	.83
Race (black) ^a	7.26	0.88-60	.07
6MWT distance (per SD)	0.67	0.30-1.51	.34
Spring/summer season ^b	0.83	0.25-2.69	.75
Ground glass opacities on CT scan	4.3	1.23-15	.02

Vitamin D insufficiency defined as vitamin D₃ levels < 30 ng/mL. All comparisons used logistic regression. See Table 1 legend for expansion of abbreviations.

^aReference is white/Asian.

^bFor measurements April through September compared with October through March.

FIGURE 2. A, Plot of serum vitamin D (25(OH)D₃, ng/mL) by FVC tertiles (first $< 64\%$ predicted, second = 64% – 83% predicted, third $> 83\%$ predicted) among subjects with CTD-ILD (ANOVA, $P = .045$). B, Plot of serum vitamin D (25(OH)D₃, ng/mL) by diffusing capacity for carbon monoxide tertiles (first $< 45\%$ predicted, second = 45% – 58% predicted, third $> 58\%$ predicted) among subjects with CTD-ILD. (ANOVA, $P = .032$). DLCO = diffusing capacity for carbon monoxide. See Figure 1 legend for expansion of the other abbreviations.

Table 4). The presence of CTD-ILD was a strong independent predictor of vitamin D insufficiency (OR, 11.8; CI, 3.5-40.6; $P < .0001$) (Table 5). Black race was also strongly associated with vitamin D insufficiency (OR, 12.9; CI, 2.6-64; $P = .002$). In sensitivity analyses, when the UCTD subjects were removed, the observed association between CTD and vitamin D insufficiency remained (OR, 10.0; CI, 2.4-41; $P = .001$; data not shown).

DISCUSSION

We demonstrated that vitamin D deficiency and insufficiency are highly prevalent in a cohort of patients with ILD and are associated with the presence of an underlying CTD independent of other measurable confounders in this patient population. Furthermore, among those subjects with CTD-ILD, reduced 25-hydroxyvitamin D₃ levels were strongly associated with reduced lung function. These findings have important implications for important ILD comorbidities, such as osteoporosis and opportunistic infections, and possibly the underlying fibrogenic process. Underscoring the potential impact on bone health is the observance that 55% of our cohort had taken corticosteroids prior to 25-hydroxyvitamin D₃ measurement and thus were at high risk for bone demineralization. Beyond the impact on bone health, the strong association of vitamin D deficiency with the presence of CTD seen in this and other studies suggests a possible pathogenic role of vitamin D in autoimmune disorders, which frequently have life-threatening manifestations in the lung.

It has been suggested that corticosteroid usage reduces 25-hydroxyvitamin D₃ levels through increased

Table 4—Predictors of Serum Vitamin D Level in Entire Cohort Using Multivariate Linear Regression Analysis

Predictor	Modeled Effect		
	Estimate (Coefficient)	95% CI	P Value
CTD-ILD	−10.6	−16.3 to −5.0	<.0001
Race (black)	−12.6	−18.9 to −6.3	<.0001
BMI	−0.46	−0.74 to −0.18	.002
Age	−0.1	−0.31 to 0.12	.38

Analysis adjusted for presence of CTD, race, BMI, age, FVC % predicted, season of measurement, and whether taking vitamin D supplementation. See Table 1 legend for expansion of abbreviations.

consumption.^{33,34} As expected, those with CTD-ILD in our study were more likely to have received corticosteroids, which could potentially confound the relationship between 25-hydroxyvitamin D₃ levels and CTD-ILD. However, we did not observe a statistically significant association between 25-hydroxyvitamin D₃ levels and prednisone use, and any effect appeared to be modest (ie, those on prednisone had levels only 3 ng/mL less.) Indeed, the observed association of CTD-ILD with hypovitaminosis D was unchanged after adjusting for corticosteroid usage in multivariate models. This suggests the association of CTD-ILD and hypovitaminosis D cannot be explained by corticosteroid usage.

The immunoregulatory role of 1,25-(OH)₂D, the biologically active form of vitamin D, provides biologic plausibility for a pathogenic role of hypovitaminosis D in the development of autoimmune diseases and end-organ dysfunction, such as ILD. All cells of the adaptive immune system express vitamin D receptors and are sensitive to the action of 1,25-(OH)₂D.³⁵ High levels of 1,25-(OH)₂D are potent inhibitors of dendritic cell maturation with lower expression of major histocompatibility complex class 2 molecules, down-regulation of costimulatory molecules, and lower production of proinflammatory cytokines.^{36,37} In several mouse models, 1,25-(OH)₂D drives the adaptive immune system from a T helper (Th) 1/Th17 response toward a Th2 and regulatory T-cell response, suggesting the potential for beneficial effects on the occurrence and progression of Th1-mediated autoimmune

Table 5—Predictors of Vitamin D Insufficiency in Entire Cohort Using Multivariate Logistic Regression Analysis

Predictor	OR	95% CI	P Value
CTD-ILD	11.8	3.46-40.6	<.0001
Race (black)	12.9	2.62-63.7	.002
BMI (per SD)	2.59	1.22-5.50	.97
Age (per SD)	1.45	0.75-2.82	.27

Vitamin D insufficiency defined as vitamin D₃ levels < 30 ng/mL. Analysis adjusted for presence of CTD, race, BMI, age, FVC % predicted, season of measurement, and whether taking vitamin D supplementation. See Table 1 legend for expansion of abbreviations.

diseases in humans.³⁸ The immune system of vitamin D receptor-deficient mice is grossly normal but shows increased sensitivity to autoimmune diseases, such as inflammatory bowel disease or type 1 diabetes, after exposure to predisposing factors.³⁹ Furthermore, laboratory evidence suggests vitamin D might play a role in regulating autoantibody production by B cells, inhibiting the ongoing proliferation of activated B cells and inducing their apoptosis.⁴⁰ A common theme in the immunomodulatory functions of vitamin D is that higher levels are immunosuppressive, which is consistent with a potential role for hypovitaminosis D in the pathogenesis of autoimmune disorders.

Vitamin D has also recently been implicated in the development of lung disease. In patients with COPD, National Health and Nutrition Examination Survey III showed an association between FEV₁ and 25-hydroxyvitamin D₃, even when adjusted for activity level.⁴¹ Despite this apparent relationship, a pathogenic relationship between low 25-hydroxyvitamin D₃ levels and COPD has yet to be established.³⁵ In asthma, reduced 25-hydroxyvitamin D₃ levels are also associated with impaired lung function, increased airway hyperresponsiveness, and reduced glucocorticoid response.²⁶ To our knowledge, our study is the first to document a high prevalence of hypovitaminosis D in patients with diffuse parenchymal lung disease. As in these recent studies of airway-centered diseases of the lung, we found a strong association between lung function and vitamin D level in subjects with ILD, particularly among those with CTD-ILD. In the laboratory, 25-hydroxyvitamin D₃ levels are reduced in rats with bleomycin-induced lung fibrosis.⁴² Further, vitamin D appears to inhibit the profibrotic effects of transforming growth factor β in lung fibroblasts and epithelial cells, and may blunt epithelial-to-mesenchymal transition.⁴³ The specific mechanisms for autoimmune parenchymal lung injury and how tissue vitamin D levels modulate its occurrence need to be further investigated.

Our study has limitations that merit discussion. First, although our study included a relatively large population by ILD cohort standards, it was only performed at a single tertiary center. Consistent with referral patterns to our center, we had a higher proportion of patients with CTD-ILD than may be seen in other institutions or in a population of patients with ILD in the general public. Given the potential influence of ambient sun exposure on serum vitamin D levels, it is possible that other centers from warmer climates may not observe such a high prevalence of hypovitaminosis D. We attempted to mitigate the influence of sun exposure during the analysis phase by adjusting for season of measurement. Next, as our study was cross-sectional in design, we did not evaluate whether vitamin D supplementation is associated

with any improved clinical outcomes. Prospective controlled interventional studies are needed to determine if vitamin D supplementation can ameliorate symptoms and improve outcomes in patients with CTD-ILD.

Vitamin D is increasingly recognized as an important mediator of immune function and lung health.²⁶ We have shown that there is a high prevalence of vitamin D deficiency and insufficiency in patients with ILD, particularly those with CTD, and that lower 25-hydroxyvitamin D₃ levels are associated with reduced lung function. Future study should investigate the underlying molecular mechanisms of this observed association and determine if supplementation with vitamin D is associated with improved clinical outcomes, including longitudinal changes in lung function as well as other systemic disease manifestations.

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REFERENCES

- Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357(3):266-281.
- DeLuca HF, Cantorna MT. Vitamin D: its role and uses in immunology. *FASEB J*. 2001;15(14):2579-2585.
- Lemire JM, Ince A, Takashima M. 1,25-Dihydroxyvitamin D₃ attenuates the expression of experimental murine lupus of MRL/l mice. *Autoimmunity*. 1992;12(2):143-148.
- Cantorna MT, Humpal-Winter J, DeLuca HF. Dietary calcium is a major factor in 1,25-dihydroxycholecalciferol suppression of experimental autoimmune encephalomyelitis in mice. *J Nutr*. 1999;129(11):1966-1971.
- Cantorna MT, Hayes CE, DeLuca HF. 1,25-Dihydroxycholecalciferol inhibits the progression of arthritis in murine models of human arthritis. *J Nutr*. 1998;128(1):68-72.
- Zella JB, McCary LC, DeLuca HF. Oral administration of 1,25-dihydroxyvitamin D₃ completely protects NOD mice from insulin-dependent diabetes mellitus. *Arch Biochem Biophys*. 2003;417(1):77-80.
- Cantorna MT, Munsick C, Bemiss C, Mahon BD. 1,25-Dihydroxycholecalciferol prevents and ameliorates symptoms of experimental murine inflammatory bowel disease. *J Nutr*. 2000;130(11):2648-2652.
- Arnson Y, Amital H, Shoenfeld Y. Vitamin D and autoimmunity: new aetiological and therapeutic considerations. *Ann Rheum Dis*. 2007;66(9):1137-1142.
- Huisman AM, White KP, Algra A, et al. Vitamin D levels in women with systemic lupus erythematosus and fibromyalgia. *J Rheumatol*. 2001;28(11):2535-2539.
- Kamen DL, Cooper GS, Bouali H, Shaftman SR, Hollis BW, Gilkeson GS. Vitamin D deficiency in systemic lupus erythematosus. *Autoimmun Rev*. 2006;5(2):114-117.
- Becker A, Fischer R, Schneider M. [Bone density and 25-OH vitamin D serum level in patients with systemic lupus erythematosus]. *Z Rheumatol*. 2001;60(5):352-358.
- Kamen D, Aranow C. Vitamin D in systemic lupus erythematosus. *Curr Opin Rheumatol*. 2008;20(5):532-537.
- Zold E, Szodoray P, Gaal J, et al. Vitamin D deficiency in undifferentiated connective tissue disease. *Arthritis Res Ther*. 2008;10(5):R123.
- Merlino LA, Curtis J, Mikuls TR, Cerhan JR, Criswell LA, Saag KG; Iowa Women's Health Study. Vitamin D intake is inversely associated with rheumatoid arthritis: results from the Iowa Women's Health Study. *Arthritis Rheum*. 2004;50(1):72-77.
- Armstrong DJ, Meenagh GK, Bickle I, Lee AS, Curran ES, Finch MB. Vitamin D deficiency is associated with anxiety and depression in fibromyalgia. *Clin Rheumatol*. 2007;26(4):551-554.
- Mouyis M, Ostor AJ, Crisp AJ, et al. Hypovitaminosis D among rheumatology outpatients in clinical practice. *Rheumatology (Oxford)*. 2008;47(9):1348-1351.
- Ferri C, Valentini G, Cozzi F, et al; Systemic Sclerosis Study Group of the Italian Society of Rheumatology (SIR-GSSSc). Systemic sclerosis: demographic, clinical, and serologic features and survival in 1,012 Italian patients. *Medicine (Baltimore)*. 2002;81(2):139-153.
- Hellmann DB, Petri M, Whiting-O'Keefe Q. Fatal infections in systemic lupus erythematosus: the role of opportunistic organisms. *Medicine (Baltimore)*. 1987;66(5):341-348.
- Yuhara T, Takemura H, Akama T, Suzuki H, Yamane K, Kashiwagi H. Predicting infection in hospitalized patients with systemic lupus erythematosus. *Intern Med*. 1996;35(8):629-636.
- Steen VD, Medsger TA. Changes in causes of death in systemic sclerosis, 1972-2002. *Ann Rheum Dis*. 2007;66(7):940-944.
- Strange C, Highland KB. Interstitial lung disease in the patient who has connective tissue disease. *Clin Chest Med*. 2004;25(3):549-559.
- Marder W, McCune WJ. Advances in immunosuppressive therapy. *Semin Respir Crit Care Med*. 2007;28(4):398-417.
- Caplan L, Saag KG. Glucocorticoids and the risk of osteoporosis. *Expert Opin Drug Saf*. 2009;8(1):33-47.
- Tolosa SM, Cole DE, Gladman DD, Ibanez D, Urowitz MB. Vitamin D insufficiency in a large female SLE cohort. *Lupus*. 2010;19(1):13-19.
- Alele JD, Kamen DL. The importance of inflammation and vitamin D status in SLE-associated osteoporosis. *Autoimmun Rev*. 2010;9(3):137-139.
- Sutherland ER, Goleva E, Jackson LP, Stevens AD, Leung DY. Vitamin D levels, lung function and steroid response in adult asthma. *Am J Respir Crit Care Med*. 2010;181(7):699-704.
- American Thoracic Society. Standardization of Spirometry, 1994 Update. *Am J Respir Crit Care Med*. 1995;152(3):1107-1136.
- Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med*. 1999;159(1):179-187.
- ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for

- the six-minute walk test. *Am J Respir Crit Care Med*. 2002;166(1):111-117.
30. Flaherty KR, King TE Jr, Raghu G, et al. Idiopathic interstitial pneumonia: what is the effect of a multidisciplinary approach to diagnosis? *Am J Respir Crit Care Med*. 2004;170(8):904-910.
 31. Kinder BW, Collard HR, Koth L, et al. Idiopathic nonspecific interstitial pneumonia: lung manifestation of undifferentiated connective tissue disease? *Am J Respir Crit Care Med*. 2007;176(7):691-697.
 32. Kinder BW, Shariat C, Collard HR, et al. Undifferentiated connective tissue disease-associated interstitial lung disease: changes in lung function. *Lung*. 2010;188(2):143-149.
 33. Zhou C, Assem M, Tay JC, et al. Steroid and xenobiotic receptor and vitamin D receptor crosstalk mediates CYP24 expression and drug-induced osteomalacia. *J Clin Invest*. 2006;116(6):1703-1712.
 34. Holick MF. Resurrection of vitamin D deficiency and rickets. *J Clin Invest*. 2006;116(8):2062-2072.
 35. Janssens W, Lehouck A, Carremans C, Bouillon R, Mathieu C, Decramer M. Vitamin D beyond bones in chronic obstructive pulmonary disease: time to act. *Am J Respir Crit Care Med*. 2009;179(8):630-636.
 36. van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. *J Steroid Biochem Mol Biol*. 2005;97(1-2):93-101.
 37. Baeke F, van Etten E, Gysemans C, Overbergh L, Mathieu C. Vitamin D signaling in immune-mediated disorders: Evolving insights and therapeutic opportunities. *Mol Aspects Med*. 2008;29(6):376-387.
 38. Daniel C, Sartory NA, Zahn N, Radeke HH, Stein JM. Immune modulatory treatment of trinitrobenzene sulfonic acid colitis with calcitriol is associated with a change of a T helper (Th) 1/Th17 to a Th2 and regulatory T cell profile. *J Pharmacol Exp Ther*. 2008;324(1):23-33.
 39. Bouillon R, Carmeliet G, Verlinden L, et al. Vitamin D and human health: lessons from vitamin D receptor null mice. *Endocr Rev*. 2008;29(6):726-776.
 40. Chen S, Sims GP, Chen XX, Gu YY, Chen S, Lipsky PE. Modulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differentiation. *J Immunol*. 2007;179(3):1634-1647.
 41. Black PN, Scragg R. Relationship between serum 25-hydroxyvitamin d and pulmonary function in the third national health and nutrition examination survey. *Chest*. 2005;128(6):3792-3798.
 42. Mert H, Yoruk I, Ertekin A, et al. Vitamin levels in lung tissue of rats with bleomycin induced pulmonary fibrosis. *J Nutr Sci Vitaminol (Tokyo)*. 2009;55(2):186-190.
 43. Ramirez AM, Wongtrakool C, Welch T, Steinmeyer A, Zügel 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(3):142-150.