Utility of Anti-DFS70 Antibodies to Predict Connective Tissue Disease in Patients Originally Presenting with Idiopathic Interstitial Pneumonia

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Keywords

Antibodies · Autoimmune diseases · Interstitial lung disease · Connective tissue disease

Abstract

Background: Anti-DFS70 antibodies, corresponding to the dense fine speckled antinuclear antibody (ANA) pattern in HEp-2 substrates, have been observed in chronic inflammatory conditions, cancer and in healthy individuals but in only a small percentage of patients with connective tissue diseases (CTD). **Objectives:** The study was aimed to investigate the possible role of Anti-DFS70 antibodies to distinguish CTD associated interstitial lung disease (CTD-ILD) from idiopathic interstitial pneumonia (IIP) and to explore potential correlations between anti-DFS70 antibodies and clinical parameters. **Methods:** Serum samples were collected from 49 healthy controls (HC), 35 scleroderma-ILD (SSc-ILD) patients as negative controls for anti-DFS70 antibody, and 260 patients with the initial diagnosis IIP including 100 nonspecific interstitial pneumonia (NSIP) and 160 idiopathic pulmonary fibrosis (IPF) patients. ANA pattern was identified by indirect

immunofluorescence on HEp-2 cells and anti-DFS70 antibodies were measured in serum by ELISA. **Results:** Serum anti-DFS70 antibodies were less frequently seen in ILD and SSc-ILD patients compared to HCs. Thirty-seven patients (34 initial idiopathic NSIP and 3 initial IPF patients) developed CTD during 24 months of follow-up, most of them combined with ANA positivity and anti-DFS70 antibody negativity. Anti-DFS70 antibody positivity was not significantly different between CTD-ILD and idiopathic ILD. **Conclusions:** The frequency of serum anti-DFS70 antibody is markedly decreased in patients with ILDs. Anti-DFS70 antibodies may be useful to predict CTD development in ILD patients.

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Introduction

Connective tissue diseases (CTDs) are characterized by autoimmune phenomena and immune-mediated organ dysfunction [1]. The lung is a vulnerable target of autoimmune mediated injury in patients with CTD because of its abundant vasculature and large content of

connective tissue. Interstitial lung disease (ILD) is a common pulmonary involvement, which has been shown to occur in all kinds of CTDs, especially in patients with systemic sclerosis (scleroderma [SSc]) [2, 3].

When ILDs occur in association with CTDs, they may present before, during or after the full manifestation of a given CTD [4]. If presenting before, ILDs may be the only manifestation and precede the extrapulmonary CTD manifestation by several months, sometimes by more than 5 years [5, 6]. Since the radiological and histological characteristics of CTD-ILDs are similar to their idiopathic counterparts [7, 8], autoimmune screening is recommended in all patients with suspected idiopathic interstitial pneumonia (IIP). The serologic evaluation should include the measurement of rheumatoid factor, anti-cyclic citrullinated peptide, and antinuclear antibody (ANA) screening [9].

ANAs are not specific for CTDs, since approximately 20% of apparently healthy individuals have a positive ANA test [10]. The limited specificity has been one of the most important drawbacks of the HEp-2 indirect immunofluorescence (IIF) assay as an ANA screening test for CTD [11]. The main target of the positive ANA pattern seen in healthy individuals is the dense fine speckled 70 antibody (anti-DFS70 antibody) [10, 11]. Anti-DFS70 antibodies are predominantly IgG and recognize a protein that can be visualized by IIF microscopy as dense fine speckles in the nucleoplasm of cells in interphase, typically excluding the nucleolus, with increased staining intensity of condensed mitotic chromosomes [12]. They have been reported in patients with a variety of chronic inflammatory conditions, in cancer patients, and also in apparently healthy individuals [10, 13, 14]. In contrast, the prevalence of anti-DFS70 antibodies in CTD patients is rather low [15, 16].

Dellavance et al. [17] evaluated over 13,000 ANA positive samples of a general clinical laboratory by IIF and immunoblot and found that anti-DFS70 autoantibodies were seen in 37% of samples and more commonly associated with non autoimmune conditions than with systemic autoimmune diseases. Interestingly, in regard to the long-term outcome, after an average follow-up of 4 years, none of the healthy individuals with isolated anti-DFS70 antibody reactivity later developed systemic autoimmune diseases [11].

The aim of the current study was to investigate the frequency of serum anti-DFS70 antibodies and a possible correlation with ANA status in patients with idiopathic pulmonary fibrosis (IPF) and idiopathic non-

specific interstitial pneumonia (NSIP). Additionally, we investigated the potential role of anti-DFS70 antibody to distinguish between CTD-ILD and IIP and to predict CTD development in patients diagnosed initially as IIP.

Methods

Disposition of the Patients

We retrospectively studied 260 ILD patients initially diagnosed as IPF ($n = 160$) or idiopathic NSIP ($n = 100$) seen in our institution between April 2007 and August 2013.

Two groups of controls were included: the healthy control (HC) group composed of 49 blood donors and 35 SSc-ILD patients who served as negative controls as having ANA positivity with specific subtypes other than anti-DFS70 [18].

The patients with IPF and NSIP were diagnosed based on clinical, radiographic, and histopathologic findings according to the ATS/ERS criteria of 2011 and 2013 respectively [9, 19]. The SSc patients were diagnosed based on the 2013 classification criteria [20]. Out of 260 patients, 64 (24.6%) ILD patients had histological confirmation, 37 with IPF and 27 with NSIP.

During follow-up of the IPF and NSIP patients, the diagnosis of CTD was made by rheumatologists according to current criteria for the respective disease [20–23]. All patients had a follow-up time of at least 2 years after blood sampling.

ANA and Anti-DFS70 Antibody Laboratory Assays

Serum samples were obtained at time of first evaluation and were stored at -80°C until analysis. LDH was routinely measured in the serological laboratory of our institution.

The ANA-IIF assay was performed on HEp-2 cells (Euroimmun AG, BIOCHIPS TITERPLANE®-Technique, EUROPLUS®, ANA-Global Test, FA1510-2010-1, Lübeck, Germany). ANA were considered positive with a titer above 1:80.

The concentration of serum anti-DFS70 antibody at baseline was measured using a commercially available ELISA kit (Medical and Biological Laboratories Co., Ltd., Fujioka City, Japan). The ELISA was performed according to the manufacturer's instructions (https://www.mblintl.com/assets/7808_DFS70_200902_4final.pdf). The cut-off for positivity was set at 400 U/ml in this study, calculated as twice the standard deviation above the average of the negative control group (35 SSc-ILD patients).

Pulmonary Function Tests and Blood Gas Analysis

Measurements were performed at the time of the serum sample collection. Pulmonary function tests included forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), total lung capacity (TLC), and diffusing capacity of the lung for carbon monoxide (DLco). Values were expressed as percentages of predicted normal values. Blood gas analysis included partial pressure of oxygen in arterial blood (PaO_2), arterial carbon dioxide tension (PaCO_2), arterial oxygen saturation (SaO_2), and alveolar-arterial oxygen gradient (AaDO_2).

Statistics

Parametric data were presented as mean $\pm$ SEM. Student's t test was used to compare continuous variables between 2 groups; chi-

Table 1. Demographics and patients' characteristics of the studied groups

Variables	NSIP (n = 100)	IPF (n = 160)	SSc-ILD (n = 35)	HC (n = 49)	p values
Demographics					
Age, years	65±1	68±0.7	57±2	34±2	<0.05 NSIP vs. IPF <0.01 all vs. HC; IPF and SNIP vs. SSc-ILD
Gender					
Male/female, n	47/53	128/32	9/26	36/13	<0.05 NSIP vs. IPF <0.01 NSIP and SSc-ILD vs. HC <0.001 NSIP vs. IPF and IPF vs. SSc-ILD
Smoking status [‡]					
Current/ex/non, n	2/39/29	5/100/30	1/7/23	7/4/22	
BMI, kg/m ²	28±0.4	28±0.5	27±1	–	
Pulmonary function					
FVC, % pred.	70±2	70±1	78±5	–	
FEV ₁ , % pred.	69±2	72±1	71±5	–	
DLco, % pred.	48±4	42±1	55±4	–	<0.01 IPF vs. SSc-ILD
TLC, % pred.	68±2	67±1	78±4	–	<0.01 IPF and NSIP vs. SSc-ILD
Blood gas analysis					
PaO ₂ , mm Hg	74±1	72±1	75±3	–	
PaCO ₂ , mm Hg	37±0.4	38±0.3	37±1	–	
AaDO ₂ , mm Hg	29±1	30±1	27±3	–	
SaO ₂ , %	95±0.3	95±0.2	96±0.6	–	
BAL tot cells, %	8±7	7±9	5±4	–	
Macrophages	62±24	72±22	75±19	–	
Lymphocytes	17±15	9±9	18±13	–	<0.05 IPF vs. NSIP and SSc-ILD
Neutrophils	11.5±11	10±17	9±10	–	
Eosinophils	4.5±3	3±2	0.4±1	–	
Unless otherwise indicated, values are expressed as mean ± SEM.					
[‡] Smoking status was not available in all patients.					
BAL, bronchoalveolar lavage; BMI, body mass index.					

square test was done for categorical variables. One-way ANOVA and least significant difference or Dunnett's post hoc tests were performed for multiple comparisons. A logistic regression was performed to assess whether ANA+/antiDFS70– was independently associated with the development of CTD by using backward conditional method regression with Hosmer-Lemeshow goodness of fit test. *p* values lower than 0.05 were considered statistically significant. SPSS 17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Results

Characteristics of Studied Subjects

The demographic, functional, and laboratory characteristics of the studied subjects are shown in Table 1. Comorbidities in the studied ILD subjects are shown in

the online supplemental Table 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000496483). During 24 months of follow-up, 37 of 260 ILD patients (14%) developed CTD (3 initial IPF and 34 initial idiopathic NSIP patients). Of these 37 patients, 22 developed rheumatoid arthritis (RA), 7 SSc, 4 antisynthetase syndrome, 2 Sjögren's syndrome, 1 mixed CTD, 1 undifferentiated CTD. All the 3 CTD-UIP patients developed RA.

Acute exacerbation occurred in 47 patients, 34 with IPF and 13 with NSIP.

ANA and Anti-DFS70 Antibody at Baseline

ANA was positive in 47% of NSIP patients compared to 32% of IPF patients (*p* < 0.05). In both the NSIP and the IPF patients, ANA positivity was significantly lower

Table 2. Prevalence of ANA and anti-DFS70 antibody in studied groups

Diagnosis	ANA (+), <i>n</i> (%)	Anti-DFS70 (+), <i>n</i> (%)	<i>p</i> value (between disease groups)
ILD			
Initial classification			
NSIP (<i>n</i> = 100)	47 (47)	12 (12)	<0.05 NSIP vs. all groups
IPF (<i>n</i> = 160)	51 (32)	31 (19)	<0.001 IPF vs. HC and SSc-ILD
Reclassification after 24 months			
IIP (<i>n</i> = 223)	73 (33)	37 (17)	<0.001 IIP vs. CTD-ILD, SSc-ILD and HC (ANA) <0.01 vs. HC (DFS-70)
CTD-ILD (<i>n</i> = 37)	25 (68)	6 (16)	<0.05 vs. HC
SSc-ILD (<i>n</i> = 35)	29 (83)	2 (6)	<0.01 vs. HC
HC (<i>n</i> = 49)	0 (0)	17 (35)	

than in the SSc-ILD patients ($p < 0.001$; Table 2). No effect of gender, age, and smoking status on ANA positivity was observed (data not shown).

Anti-DFS70 antibodies were positive in 12% of NSIP patients and 19% of IPF patients ($p > 0.05$). Anti-DFS70 antibody positivity was significantly lower in both the NSIP and IPF groups than in HC ($p < 0.05$, respectively), and tended to be higher than in SSc-ILD ($p > 0.05$, respectively; Table 2). In the total individuals of ILD patients, the prevalence of anti-DFS70 antibodies was not different between men and women (17 vs. 15%, $p > 0.05$) and appeared to be stable across 3 age groups (17% in ≤ 60 years, 16% in 61–70 years, and 17% in more than 70 years, $p > 0.05$).

ANA and Anti-DFS70 Antibody Positivity According to Development of CTD

After a follow-up time of 24 months, of the 260 patients initially diagnosed as idiopathic ILD, 223 remained as IIP, and 37 developed CTD (Table 2). The prevalence of ANA positivity in the final IIP group was significantly lower than in the final CTD-ILD group (33 vs. 68% $p < 0.05$; Table 2). Within the NSIP group, ANA were positive in 35% iNSIP patients, which was a significantly lower percentage than that in CTD-NSIP (71%) and SSc-ILD (83%) patients ($p < 0.001$, respectively). The ANA positive percentage in IPF-UIP (32%) was also significantly lower than that in SSc-ILD ($p < 0.001$), but similar to CTD-UIP (33%, $p > 0.05$; Fig. 1). Although the difference between CTD-UIP and SSc-ILD was significant, when considering the small number of CTD-UIP ($n = 3$), the result is questionable.

The prevalence of anti-DFS70 antibody positivity in the IIP group (17%) was significantly lower than in HCs

Fig. 1. Prevalence of ANA positivity according to CTD development. Data expressed as percentage.

(35%, $p < 0.05$) but not significantly different from CTD-ILD (16%, $p > 0.05$; Table 2). Anti-DFS70 antibodies were positive in 11% iNSIP patients, significantly less than in HC ($p < 0.05$) but not significantly different from CTD-NSIP (15%) and SSc-ILD (6%), $p > 0.05$ respectively. The anti-DFS70 antibody positive percentage of IPF-UIP (19%) was also significantly lower than HC ($p < 0.05$) and similar to CTD-UIP (33%, $p > 0.05$) but higher than that in SSc-ILD ($p = 0.06$; Fig. 2).

Fig. 2. Prevalence of anti-DFS70 antibody positivity according to CTD development. Data expressed as percentage.

Relationship between ANA and Anti-DFS70 Antibodies

Among the ANA positive ILD patients, 20% were anti-DFS70 antibody positive. This was not significantly different from the ANA negative individuals who were positive in 14%.

At baseline, among the ANA positive ILD patients, the prevalence of anti-DFS70 antibody positivity in IPF (25%) was significantly higher than that in SSc-ILD (3%, $p < 0.05$), and was also higher, but not significantly, than in NSIP (15%, $p > 0.05$). Among the ANA negative subjects, HC had the highest percentage of anti-DFS70 antibody positivity (35%), significantly higher than NSIP or IPF (15 and 17%, $p < 0.05$ respectively; Fig. 3).

After follow-up, among the ANA positive subjects, the prevalence of anti-DFS70 antibody positivity in IIP (22%) tended to be higher than in CTD-ILD (16%, $p > 0.05$). Among the ANA negative subjects, the prevalence of anti-DFS70 antibody positivity was similar in IIP and in CTD-ILD (14% and 17%, $p > 0.05$ respectively; Fig. 3).

Frequency of CTD Development According to ANA and Anti-DFS70 Antibody Status

Among the 260 ILD patients, 98 (38%) were ANA positive at baseline. The frequency of CTD development was significantly higher in those ILD patients who were ANA positive (25/98, 26%) than ANA negative (12/162, 7%), $p < 0.001$. The ANA titres were not different between the IIP patients who developed CTD and those who did not.

Among the IIP patients, 129 out of 223 (58%) were ANA (-)/DFS70 (-). This was a significantly higher percentage than that in CTD-ILD patients (10/37, 27%), $p < 0.001$. Conversely, 21 of 37 CTD-ILD patients (57%) were ANA (+)/DFS70 (-), a significantly higher proportion than that in IIP patients (57/223, 26%), $p < 0.001$ (Table 3).

A logistic regression was performed to assess whether ANA+/antiDFS70- status was independently associated with the development of CTD-ILD (online suppl. Table 2). We included age, gender, smoking status, HRCT pattern and BAL lymphocytes as covariates, since patients who developed CTD in follow-up had significantly higher BAL lymphocytes at baseline than those who did not (21 ± 5 vs. 12 ± 9 , $p = 0.003$).

ANA+/antiDFS70- status and NSIP HRCT pattern were both significantly associated with CTD development (online suppl. Table 1). According to this model based on ANA+/antiDFS70- status and NSIP HRCT pattern, 89.4% of patients who developed CTD over time could be correctly classified.

Correlations between Anti-DFS70 Antibody Status and Clinical/Laboratory Parameters

In NSIP patients, there were no differences in clinical characteristics between the NSIP groups according to anti-DFS70 antibody status except for a significantly lower arterial PaCO₂ in anti-DFS70 (+) patients than in anti-DFS70 (-) patients (35 ± 0.8 vs. 38 ± 0.4 mm Hg, $p < 0.05$).

In IPF patients, serum LDH was significantly lower in anti-DFS70 antibody (+) than in anti-DFS70 antibody (-) patients (252 ± 10 vs. 286 ± 6 , $p < 0.05$; online suppl. Table 3). In regard to pulmonary function parameters, DLco (%pred.) was significantly higher in anti-DFS70 antibody (+) than that in anti-DFS70 antibody (-) IPF patients (48 ± 3 vs. 41 ± 1 , $p < 0.05$), and the AaDO₂ was significantly lower in anti-DFS70 antibody (+) than that in anti-DFS70 antibody (-) IPF patients (26 ± 2 vs. 31 ± 1 , $p < 0.05$; online suppl. Table 3).

We did not observe any association between DFS-70 antibodies and BAL differential cell count. In particular, DSF-70 negative IPF patients had the same BAL lymphocytes as DSF-70 positive patients (10.5 ± 9 vs. $9.5 \pm 9\%$ respectively, $p = 0.66$; online suppl. Table 3). Similarly, whole blood cellular subpopulations did not vary according to anti-DFS70 antibody status (data not shown). Finally, we did not observe any association between DFS-70 status and comorbidities (online suppl. Table 1) or with acute exacerbation of ILD ($p = 0.093$).

Fig. 3. Frequency of anti-DFS70 antibody positivity according to ANA status at baseline (a) and at end of follow-up (b). Data expressed as percentage (%).

Discussion

In the current study, we found that serum anti-DFS70 antibodies are less prevalent in ILD patients than in HCs. During follow-up, ANA positive ILD patients with negative anti-DFS70 antibody developed CTD more frequently than those who were anti-DFS70 positive. In IPF, the patients with anti-DFS70 antibody positivity seemed to have less impaired pulmonary function. To the best of our knowledge, this is the first study that investigated the role of anti-DFS70 antibodies in ILD patients.

In the 1960s, Turner-Warwick was one of the first to describe that ANA are positive in nearly 50% of ILD patients [24]. In our study, positive ANAs were observed in 47% of NSIP and 32% of IPF patients. The observed frequency is consistent with the existing literature [25]. In our CTD-ILD patients, ANA positivity was seen in 68% which is relatively low for a CTD cohort. This may be due to the composition of our CTD-ILD population, with 59% being RA patients. RA is in general not an ANA-associated disease, since ANA positivity has been reported in only up to 30% of patients with RA [26]. Despite the fact that the prevalence of ANAs has been reported to be higher in healthy women than that in healthy men [27, 28] and higher in elderly than in younger healthy individuals [29, 30], in the ILD subjects of our study we did not observe these associations.

Anti-DFS70 antibodies have been investigated in only a few lung disorders. In a cohort of atopic dermatitis pa-

Table 3. Association of ANA and anti-DFS70 antibody status with the development of CTD, assessed 24 months after initial evaluation

	IIP (n = 223), n (%)	CTD-ILD (n = 37), n (%)	p value*
ANA (+)/DFS70 (+)	16 (7)	4 (11)	
ANA (+)/DFS70 (-)	57 (26)	21 (57)*	<0.001
ANA (-)/DFS70 (+)	21 (9)	2 (5)	
ANA (-)/DFS70 (-)	129 (58)	10 (27)*	<0.001

* Calculated by using chi-square test.

tients, 16% of the patients with asthma were DFS70 positive compared with 30% of those with atopic dermatitis only and 0% of healthy subjects [31]. Yamada et al. [38] found that in patients with panuveitis, including sarcoidosis patients, anti-DFS70 positivity was seen in 67% of patients versus 22% of HCs.

In some studies, anti-DFS70 antibodies were more prevalent or had higher titers in females than in males [10, 31]. Our study, in agreement with others, did not find such gender difference [32]. Variations in cohort collection and disease composition may explain the discrepancy. An age dependency was shown in a study of 918 healthy individuals, where more than 90% of the DFS positive subjects were younger than 50 years [11]. How-

ever, this was not confirmed in another study [32]. Also in our study, the status of anti-DFS70 antibodies showed no correlation with age.

The prevalence of anti-DFS70 antibodies has been reported to vary, but consistent findings are that they are present in healthy ANA positive individuals even when ANA reach moderate or high titers, and that they are rarely found in patients with CTD [17, 33, 34]. Fitch-Rogalsky et al. [35] investigated 643 patients with a positive ANA and found that 15% were anti-DFS70 antibody positive and 91% of these did not have an autoimmune disease. In a study of routine ANA screening DFS and traditional fine speckled patterns were the major ANA-IIF patterns, and 61% of DFS positivity was seen in non autoimmune conditions [17]. Our study confirms this for patients with ILDs. Among the ANA positive ILD patients, anti-DFS70 antibody positivity was seen in 22% of IIP patients, compared to 16% of the patients who developed CTD. This suggests that anti-DFS70 antibody positivity decreases the likelihood of a CTD-ILD. The regression analysis confirmed the association between the presence of ANA positivity and DFS70 negativity together with an NSIP pattern at baseline and the development of CTD during follow up. These variables combined were able to identify 85.8% of patients who developed CTD. Whether this approach can be routinely used to identify patients at risk for CTD development among those with ILD needs further validation.

To avoid the low specificity of ANA-IIF test as screening test for systemic autoimmune rheumatic disease, subsequent immunoassays for specified antibodies are usually done, but there is no consistency in the recommendations for the use of autoantibodies and diagnostic algorithms in different serological laboratories [36]. It has been recommended to include Ro/SSA, La/SSB, Jo-1, Scl-70, Th/To, RNA-Polymerase III, Sm, dsDNA (SLE) and CCP in the serological examination of ILD patients [37]. Since by now more than 150 different autoantibodies have been discovered, in consideration of feasibility and economic costs, it is impossible to include all CTD associated ANAs into ANA screening ELISA tests. Therefore, it would be useful to have a biomarker, which would exclude CTD in patients with ILD and positive ANA serology but without symptoms or signs of CTD. Such a biomarker could result in considerable cost-savings by adding accuracy to the ANA screening test. As such, Anti-DFS70 antibody is one of the best candidates, since it seems to be helpful in discriminating between subjects with and those without autoimmune disease among ANA positive patients.

The clinical and biological significance of anti-DFS70 autoantibody is still puzzling. The higher prevalence of anti-DFS70 antibodies in healthy subjects than in autoimmune diseases supports the hypothesis that these autoantibodies can serve as protective or natural autoantibodies [33]. On the other hand, since the antigen could possibly trigger the elicitation and accumulation of autoantibodies, the presence of anti-DFS70 antibodies in apparently healthy individuals may be indicative of an undetected chronic inflammatory response. In the current study, we found that in IPF patients, the anti-DFS70 antibody negative patients showed higher levels of LDH. A strong trend, though statistically not significant due to the small number of patients with anti-DFS70 antibody positivity, was also seen in NSIP patients. LDH is a non-specific marker of tissue injury widely used to monitor the course of acute lung injury, indicating that a higher degree of tissue injury is possibly associated with a reduction of anti-DFS70 antibodies. Whether anti-DFS70 antibody has a pathogenic role or is merely a bystander in the pathogenesis of ILDs is unclear at present.

Our study has several limitations. First, we did not include healthy subjects with ANA positivity as controls; therefore, we cannot draw any conclusion on the presence of DFS-70 antibodies in healthy individuals. Our controls were blood donors therefore been screened for autoimmune diseases. Second, the exact ANA-IIF pattern as well as ANA subtypes was not available in all individuals, so that it is not possible to investigate further correlations with DFS-70 antibodies in CTD-ILD patients. Second, the number of CTD-UIP patients was very small ($n = 3$) because few IPF patients developed CTD. Third, measurement of DFS70 levels in BALF/tissue was not performed and should be done in further studies. Comparing lung tissue expression of DFS70 versus circulating levels of anti-DFS70 antibodies would be important to explore the potential function of this antigen-antibody system in the pathogenesis of ILDs. Finally, we did not perform a longitudinal analysis to correlate DFS-70 status and lung function tests over time or ILD outcome; this should be considered in future studies.

In conclusion, the prevalence of anti-DFS70 antibody was highest in healthy subjects and decreased in patients with ILDs and SSc-ILD. Most of the ILD patients who developed CTD during follow-up were shown to be characterized by ANA positivity combined with anti-DFS70 antibody negativity in the presence of an NSIP pattern on HRCT. The potential use of this antibody in the clinical routine to reclassify patients with initially diagnosed idiopathic ILD needs to be further investigated.

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None.

Statement of Ethics

Written informed consent was obtained from the ILD patients and controls. The study was approved by the local IRB (RLK 23.06.2015).

Financial Disclosure and Conflicts of Interest

The authors declare that they have no potential conflicts of interest to disclose.

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Authors' Contributions

Y.L. and F.B. contributed to the conception and design of the study, analysis and interpretation of the data, and drafting and finalization of the manuscript. E.B. contributed to the collection of samples. D.T. and J.G. contributed to interpretation of the data and drafting of the manuscript. M.K. contributed to the conception and design of the study and interpretation of the data. U.C. contributed to the conception of the study, interpretation of the data, statistical results, drafting and finalizing manuscript. All authors have read and approved of the final manuscript.

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