Risk factors for pulmonary interstitial fibrosis in patients with systemic sclerosis

Ji Yang
Zhongshan Hospital Fudan University

Xiangxiang Cui
Department of Dermatology,Zhongshan Hospital, Fudan University, Shanghai, China. Department of Dermatology, Shanghai Children's Hospital, Shanghai, China

Ming Li (li.ming@zs-hospital.sh.cn)
Department of Dermatology, Zhongshan Hospital, Fudan University, Shanghai, China

Research

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Abstract

Background

Pulmonary interstitial fibrosis (PIF) is a frequent manifestation of systemic sclerosis (SSc). However, there is a lack of good clinical indicators for predicting PIF. Here, we evaluated autoantibodies and clinical phenotypes to predict the involvement of PIF in SSc.

Methods

Peripheral blood was collected from the 86 SSc patients enrolled in this study to detect autoantibodies. PIF, lung function, and heart function were analyzed by lung high-resolution computed tomography, pulmonary function test, and standard transthoracic echocardiography, respectively. The correlation between autoantibodies, clinical phenotype, and internal organ involvement were summarized.

Results

PIF occurred in 68.4% anti-SCL-70 antibody positive SSc patients (p = 0.0045), 59.8% anti-centromere antibody negative SSc patients (p = 0.0013), and 71.4% anti-SSA antibody positive SSc patients (p = 0.0107). PIF occurred in 94.1% anti-SCL-70 antibody and anti-SSA antibody double positive SSc patients, which was higher than in anti-SCL-70 antibody single positive patients, p = 0.0452. More SSc patients with digital ulcers had PIF (73.5%) than SSc patients without digital ulcers (p = 0.0008).

Conclusion

These data suggest that SSc patients with double positive for anti-SCL-70 and anti-SSA antibodies are more prone to develop PIF. In addition, digital ulcers are an important and convenient predictor for PIF in SSc patients.

Background

Systemic sclerosis (SSc) is a chronic, autoimmune connective tissue disease characterized by small vessel vasculopathy, autoantibodies production, and fibroblast proliferation that leads to increased extracellular matrix deposition and fibrosis [1, 2]. Skin fibrosis can manifest as thickening of the fingers, hand, or skin of the entire body, and Raynaud's phenomenon is also a hallmark of the disease [2].

The clinical presentation of SSc varies, with symptoms presenting in the skin and cardiovascular, gastrointestinal, musculoskeletal, and pulmonary systems. SSc patients have increased risks for developing serious internal organ involvement such as PIF, pulmonary hypertension, heart failure, sclerosisrenal crisis, and severe gastrointestinal involvement [2–5]. Thus, it is necessary and urgent to

diagnose and treat the disease and affected internal organs as early as possible. However, this is a difficult task, because reliable predictors or biomarkers for organ involvement are still missing in clinical practice. At present, the general strategy is to screen SSc patients regularly for internal organ involvement using high-resolution computed tomography (HRCT), transthoracic echocardiography, and pulmonary function tests[5–8]. However, detection of internal organ involvement is relatively late in the disease process using these clinical tests. For this reason, there is an urgent need to identify and validate early predictive factors for SSc disease worsening[8]. Anti-SCL-70 antibody and anti-centromere antibody are the hallmark antibodies of SSc and can be used for the diagnosis of SSc, and anti-SCL-70 antibody positive is related to PIF in SSc patients [1, 9]. Anti-SSA antibody and anti-SSB antibody could also be detected in SSc patients [10, 11]. But the positive rate of these antibodies in Chinese SSc population is not clear. Raynaud's phenomenon and digital ulcers are common in SSc patients, whereas the incidence is not clear in Chinese SSc patients. In addition, the predictive value of these antibodies and clinical phenotypes on the occurrence of PIF is not clear.

In this study, 86 SSc patients were enrolled and the clinical features, autoantibodies, and internal organ involvement were studied. The potential predicators associated with internal organ involvement are systematically analyzed and summarized. The results showed that SSc patients with anti-SCL-70 antibody positive are prone to develop PIF. We further discovered that SSc patients that were double positive for anti-SCL-70 and anti-SSA antibodies have a higher incidence of PIF than anti-SCL-70 antibody alone positive patients do. In addition, digital ulcer is also an effective predictive factor for SSc patients with PIF. Thus, these factors could be used as an early prediction index for lung involvement in SSc patients.

Materials And Methods

SSc patients

This study was approved by the Ethical Committee of Zhongshan Hospital, Fudan University, Shanghai, People's Republic of China. Eight-six adult patients (77 women and 9 men, mean age 46.3±12.7 years) with a diagnosis of SSc, based on the 1980 American Rheumatology Association classification criteria for SSc [12], were consecutively included in the study. All patients enrolled in the study after giving informed and written consents. All SSc patients were referred to the Department of Dermatology, Zhongshan Hospital, Fudan University, Shanghai, China. Among the 86 SSc patients, 33 patients had diffuse type, 46 patients had limited type, 2 patients had SSc and dermatomyositis overlap, 4 patients had SSc and systemic lupus erythematous (SLE) overlap, and 1 patient had SSc, SLE, and dermatomyositis overlap. The average course of SSc was 70.8±78.5 months with reference from onset of Raynaud' phenomenon.

Autoantibodies detection

The fasting peripheral blood samples were obtained from all patients at 6:00 am. Serum levels of anti-SCL-70 antibody, anti-centromere antibody, anti-SSA antibody, and anti-SSB antibody were detected using the EUROLINE ANA profile kit according to the manufacturer's instruction (EUROLINE, Lübeck, Germany). The results were read using EUROBlotMaster (Lübeck, Germany).

Pulmonary interstitial fibrosis analysis

PIF was analyzed using HRCT (128-slice spiral CT scanner, Siemens AG, Erlangen, Germany) in Zhongshan Hospital, Fudan University. The PIF was analyzed and verified by two independent radiologists.

Pulmonary arterial systolic pressure detection

Pulmonary arterial systolic pressure was detected using standard transthoracic echocardiography using a Philips iE33 system (Philips Medical Systems, Bothell, WA, USA) in Zhongshan Hospital, Fudan University. Pulmonary hypertension (PH) was defined as >40 mmHg as measured by power Doppler heart ultrasound examination according to previous reports [3, 13].

Pulmonaryfunction tests

Pulmonary function tests were performed by trained and certified operators using standard techniques (Jaeger pulmonary test system; Würzburg, Germany). Carbon monoxide diffusing capacity (DLco) level was used in this study. DLco between 80%~120% was defined as normal and less than 80% was defined as decreased, as in previous reports [13].

Statistical analyses

Results were expressed as means ± standard deviation (SD). Comparisons between groups were performed using Chi-square test analysis for categorical variables. A $p<0.05$ was considered statistically significant. Statistical analysis was performed using GraphPad Prism Version 6.0c.

Results

The autoantibodies positive rates in SSc patients

Eighty-six patients with SSc were enrolled in this study, including 46 patients with limited SSc, 33 patients with diffuse SSc, and 7 patients with overlap syndrome. We assessed the incidence of four antibodies implicated in SSc: anti-SCL-70, anti-centromere, anti-SSA, and anti-SSB (Table 1). In our study population, 44.2% SSc patients (38/86 patients) were anti-SCL-70 antibody positive, including limited (18/38 patients, 47%) and diffuse (19/38 patients, 50%) SSc. Patients positive for anti-centromere antibody (19/86 patients, 22.1%) presented primarily with limited SSc (15/19 patients, 79%). 32.6% SSc patients (28/86 patients) were anti-SSA antibody positive, including limited (14/27 patients, 52%) and diffuse (11/28 patients, 40%) SSc, and 3 overlap syndrome patients. Only 8 SSc patients (9.3%) were anti-SSB

antibody positive (Table 1). In our patient population, 1 patient was positive for both anti-SCL-70 and anti-centromere antibody, 17 patients were positive for both anti-SCL-70 and anti-SSA antibody, and 7 patients were positive for both anti-SCL-70 and anti-SSB antibody (data not shown).

Next, we assessed the incidence of the four antibodies according to disease classification (Table 2). We found that only anti-centromere antibody was significantly different between limited (32.6% positive) and diffuse (6.45% positive) SSc disease classifications (p=0.0052). Anti-SCL-70, and anti-SSB antibodies were more prevalent in diffuse than limited disease states, but the differences were not statistically significant.

The clinical phenotype and internal organ involvement of SSc patients

Forty-three SSc patients (50.0%) had lung involvement, manifested as PIF, and 44 SSc patients (51.1%) had impaired lung function, manifested as decreased DLco (Table 3). In contrast, few patients (15/86 patients, 17.4%) had PH detected by cardiac ultrasound. Raynaud's phenomenon was present in 81.4% of SSc patients (70/86 patients) as the first manifestation of SSc. The average incidence of Raynaud's phenomenon occurred approximately 49.9 months before the patient was diagnosed with SSc. The other 16 SSc patients presented with skin swelling and hardening as the first manifestations. Finally, 34 SSc patients (39.5%) had digital ulcers (Table 3).

Correlation between autoantibodies, digital ulcers, and pulmonary interstitial fibrosis

Our results showed that 68.4% SSc patients that were anti-SCL-70 antibody positive had PIF (26/38 patients), which was significantly more than SSc patients that were anti-SCL-70 antibody negative had (17/48 patients, 35.4%, p=0.0045) (Table 4). SSc patients that were anti-centromere antibody positive had less PIF (3/16 patients, 15.8%) than SSc patients that were anti-centromere antibody negative (40/67 patients, 59.8%, p=0.0013) (Table 4). SSc patients that were anti-SSA antibody positive with PIF (20/28 patients, 71.4%) had a higher incidence than SSc patients that were anti-SSA antibody negative (23/58 patients, 39.7%, p=0.0107) (Table 4). The presence of anti-SSB antibody (7/8 patients) did not associate with PIF (p=1.0000) (Table 4). Interestingly, 94.1% SSc patients with double positive for anti-SCL-70 and anti-SSA antibodies (16/17 patients) had PIF, which was a significantly higher incidence rate than anti-SCL-70 antibody single positive patients (p=0.0452) (Table 5). Digital ulcers were significantly prone to develop PIF (25/34 patients, 73.5%, p=0.0008), whereas those without digital ulcers tended to less develop PIF (Table 4). Our data did not find a significant correlation between PIF and PH (p=1.0000) (Table 4).

Discussion

The manifestations of SSc are fibrosis of the skin and internal organs, but the pathogenesis of SSc is complex and unclear. Currently, the main view is that there is a close and complex relationship between autoimmune abnormalities, increased collagen synthesis, and vascular injuries [14]. Our previous studies

and other reports indicate the immune cells may be an important bridge linking the increased collagen synthesis with vascular injuries in SSc [15–18].

PIF is the most common internal organ fibrosis in SSc [5, 8, 19, 20]. In this study, we identified PIF in approximately 50% of SSc patients by HRCT. Lung fibrosis is often associated with ventilatory dysfunction, which can manifest as restrictive ventilatory dysfunction [8, 19, 21]. DLco is a reflection of carbon monoxide transfer from the alveoli to the blood. The fibrosis of the alveolar membrane leads to increased thickness, which prevents carbon monoxide diffusion and leads to a DLco decrease [21]. In this study, 51.1% of the whole cohort SSc patients had a DLco decrease, suggesting that PIF affects the ventilatory function of the lung in SSc patients. PH is associated with autoimmune inflammatory injury of the vascular wall, lung interstitial fibrosis, and thrombosis [22, 23]. Our data showed that only 17.4% SSc patients had PH, but here we did not find significant correlation between PH and PIF, these results was similar with previous reports [22].

Arteriole thickening, lumen narrowing, and vascular stretch and contraction dysfunction might cause Raynaud's phenomenon, which can result in vascular occlusion and fingertip necrosis in severe cases [24, 25]. Our study showed that 81.4% SSc patients presented with Raynaud's phenomenon as the first clinical manifestation. Occurrence of Raynaud's phenomenon may precede the skin sclerosis by several months or years [2]. Our data showed that patients developed a swelling and hardening of the skin and were diagnosed with systemic sclerosis approximately 49.9 months after the first time of Raynaud's phenomenon.

The main skin manifestations of SSc are swelling, hardening, and then atrophy [2]. The skin rash can be directly assessed in a clinic, whereas the early symptoms of PIF are hidden and lack specific and sensitive indexes. HRCT is a reliable method for discovering PIF, but when the fibrosis that can be detected by HRCT is relative delayed for proper treatment. If we can predict the occurrence of PIF through convenient indexes, it will greatly enhance clinical practice. If the onset of PIF can be detected by clinical phenotype or laboratory test, we can intervene early and prevent further fibrosis progress. Digital ulcers are prevalent and easy to observe in clinical practice [26]. Previous studies have found an association between the presence of digital ulcers and more severe disease such as more extensive skin involvement in the SSc diffuse cutaneous subset, PH, and lung interstitial fibrosis [3, 26, 27]. Here, we found that 34 cases of SSc patients (39.5%) had digital ulcers and 73.5% SSc patients with digital ulcers had PIF, which was more than SSc patients without digital ulcers had. These data indicated that digital ulcers could be used an effective predictive factor for high PIF risk.

Autoantibody testing can be used not only for the diagnosis of SSc, but also for predicting the severity of the disease and internal organ damage [28]. Here, we found patients with anti-SCL-70 antibody positive results were more likely to develop PIF, which is consistent with previous reports [9]. Anti-SSA antibody is mainly detected in subacute cutaneous lupus erythematosus and Sjogren syndrome and is associated with photosensitivity and congenital heart block [29, 30]. Here, we found that 32.6% SSc patients were anti-

SSA antibody positive. Among these patients, 71.4% anti-SSA positive SSc patients had PIF, which is more than anti-SSA antibody negative SSc patients had (39.7%). These data indicated that anti-SSA antibody is a prognostic risk factor for PIF in SSc patients. Further analysis showed that the incidence of PIF in SSc patients that were double positive for anti-SSA antibody and anti-SCL-70 antibody was 91%, which was higher than that for anti-SCL-70 antibody positive alone. Therefore, SSc patients with double positive for anti-SSA antibody and anti-SCL-70 antibody can be identified in the clinic and referred for regular HRCT follow-up. Early detection of PIF and early intervention can prevent further ventilatory dysfunction and secondary cardiovascular system injuries.

Conclusion

In conclusion, our data show that SSc patients with digital ulcers or double positive for anti-SSA antibody and anti-SCL-70 antibody are more likely to develop PIF. Thus, these factors could be used as predictors for the occurrence of PIF.

Abbreviations

SSc
systemic sclerosis
PIF
pulmonary interstitial fibrosis
HRCT
high-resolution computed tomography
DLco
carbon monoxide diffusing capacity
PAH
pulmonary arterial hypertension

Declarations

Ethics approval and consent to participate

This study was approved by the Ethical Committee of Zhongshan Hospital, Fudan University, Shanghai, People's Republic of China.

Consent for publication

Written informed consent was obtained from all men and women who participated in the study.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors contributed to one or more of the following aspects of the manuscript: conception and design, acquisition, analysis and interpretation of data, drafting and revising the article. Dr. Li had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study conception and design: Ji Yang, Xiangxiang Cui, Ming Li; Acquisition of data: Ji Yang, Xiangxiang Cui, Ming Li; Analysis and interpretation of data: Ji Yang, Ming Li.

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Tables

Table 1. The positive rate of autoantibodies in SSc patients

Gender	Classification	Anti-SCL-70 antibody	Anti-centromere antibody	Anti-SSA antibody	Anti-SSB antibody
Male: 9 Female: 77	Limited: 46 Diffuse: 33 Overlap□7	44.2% (38/86)	22.1% (19/86)	32.6% (28/86)	2.1% (8/86)

Table 2. The positive rate of autoantibodies in different types of SSc patients

Classification	Anti-SCL-70 antibody	Anti-centromere antibody	Anti-SSA antibody	Anti-SSB antibody
Limited: 46	39.1% (18/46)	32.6% (15/46)	30.4% (14/46)	6.5% (3/46)
Diffuse: 33	57.6% (19/33)	6.06% (2/33)	33.3% (11/33)	12.1% (4/33)
	P=0.1166	P=0.0052	P=0.8104	P=0.4432

Table 3. The visceral involvement and clinical phenotypes in SSc patients

PIF	PAH	DLCO	Raynaud's phenomenon as first manifestation	Raynaud's time before diagnosis	Digital ulcers
50.0% (43/86)	17.4% (15/86)	51.1% (44/86)	81.4% (70/86)	49.9±64.1months	39.5% (34/86)

PIF：Pulmonary interstitial fibrosis；PAH：Pulmonary arterial hypertension; DLco: Carbon monoxide diffusing capacity

Table 4. Associations between autoantibodies, skin rash phenotypes and internal organ involvement

	PIF (+)	PIF (-)	Total	Incidence rate
Anti-SCL-70 antibody (+)	26	12	38	68.4%
Anti-SCL-70 antibody (-)	17	31	48	35.4%
	P=0.0045			
Anti-centromere antibody (+)	3	16	19	15.8%
Anti-centromere antibody (-)	40	27	67	59.8%
	P=0.0013			
Anti-SSA antibody (+)	20	8	28	71.4%
Anti-SSA antibody (-)	23	35	58	39.7%
	P=0.0107			
Digital ulcers (+)	25	9	34	73.5%
Digital ulcers (-)	18	34	52	34.6%
	P=0.008			
Anti-SSB antibody (+)	7	8	15	87.5%
Anti-SSB antibody (-)	36	35	71	50.7%
	P=1.0000			
PAH (+)	7	8	15	46.7%
PAH (-)	36	35	71	50.7%
	P=1.0000			

PIF：Pulmonary interstitial fibrosis；PAH：Pulmonary arterial hypertension

Table 5. Pulmonary interstitial fibrosis difference between anti-SCL-70 and anti-SSA antibody double positive and anti-SCL-70 antibody single positive

	PIF (+)	PIF (-)	Total	Incidence rate
Anti-SCL-70 antibody and Anti-SSA antibody (+)	16	1	17	94.1%
Anti-SCL-70 antibody (+)	26	12	38	68.4%
	P=0.0452			