

## Etiology, Risk Factors, and Biomarkers in Systemic Sclerosis with Interstitial Lung Disease

Dinesh Khanna<sup>1</sup>, Donald P. Tashkin<sup>2</sup>, Christopher P. Denton<sup>3</sup>, Elisabetta A. Renzoni<sup>4,5</sup>, Sujal R. Desai<sup>6,7</sup>, and John Varga<sup>8</sup>

<sup>1</sup>Division of Rheumatology, Department of Internal Medicine, University of Michigan Scleroderma Program, Ann Arbor, Michigan; <sup>2</sup>Department of Medicine, David Geffen School of Medicine at UCLA, University of California Los Angeles, Los Angeles, California; <sup>3</sup>University College London Centre for Rheumatology and Connective Tissue Diseases, Royal Free Hospital, London, United Kingdom; <sup>4</sup>Interstitial Lung Disease Unit and <sup>5</sup>National Institute for Health Research Clinical Research Facility, Royal Brompton Hospital, London, United Kingdom; <sup>6</sup>National Heart and Lung Institute, Imperial College London, London, United Kingdom; <sup>7</sup>Department of Radiology, Royal Brompton & Harefield National Health Services Foundation Trust Hospital, London, United Kingdom; and <sup>8</sup>Northwestern Scleroderma Program, Feinberg School of Medicine, Chicago, Illinois

### Abstract

Systemic sclerosis (SSc) is a complex, multiorgan, autoimmune disease. Lung fibrosis occurs in ~80% of patients with SSc; 25% to 30% develop progressive interstitial lung disease (ILD). The pathogenesis of fibrosis in SSc-associated ILD (SSc-ILD) involves cellular injury, activation/differentiation of mesenchymal cells, and morphological/biological changes in epithelial/endothelial cells. Risk factors for progressive SSc-ILD include older age, male sex, degree of lung involvement on baseline high-resolution computed tomography imaging, reduced DL<sub>CO</sub>, and reduced FVC. SSc-ILD does not share the genetic risk architecture observed in idiopathic pulmonary fibrosis (IPF), with key risk factors yet to be identified. Presence of anti-Scl-70 antibodies and absence of anti-centromere antibodies indicate increased likelihood of progressive ILD. Elevated levels of serum Krebs von den Lungen-6 and C-reactive protein are both associated with SSc-ILD severity and predict

SSc-ILD progression. A promising prognostic indicator is serum chemokine (C-C motif) ligand 18. SSc-ILD shares similarities with IPF, although clear differences exist. Histologically, a nonspecific interstitial pneumonia pattern is commonly observed in SSc-ILD, whereas IPF is defined by usual interstitial pneumonia. The course of SSc-ILD is variable, ranging from minor, stable disease to a progressive course, whereas all patients with IPF experience progression of disease. Although appropriately treated patients with SSc-ILD have better chances of stabilization and survival, a relentlessly progressive course, akin to IPF, is seen in a minority. Better understanding of cellular and molecular pathogenesis, genetic risk, and distinctive features of SSc-ILD and identification of robust prognostic biomarkers are needed for optimal disease management.

**Keywords:** systemic sclerosis; interstitial lung diseases; autoimmune diseases; risk factors; biomarkers

Systemic sclerosis (SSc) is a complex autoimmune disease with a range of manifestations, including vasculopathy, Raynaud's phenomenon, immune

dysfunction, and fibrosis of the skin and internal organs (1–3). It is a rare disease, with an estimated global prevalence of 3 to 24 per 100,000 (4). Diagnostic criteria for

SSc were published jointly by the European League against Rheumatism and the American College of Rheumatology in 2013, with a scoring system based on a

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Correspondence and requests for reprints should be addressed to Dinesh Khanna, M.D., Division of Rheumatology/Department of Internal Medicine, University of Michigan Scleroderma Program, Suite 7C27, 300 North Ingalls Street, SPC 5422, Ann Arbor, MI 48109. E-mail: [khannad@med.umich.edu](mailto:khannad@med.umich.edu).

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range of possible signs, symptoms, and autoantibodies (5).

Lung fibrosis occurs in up to approximately 80% of patients with SSc, with varying prevalence depending on ascertainment methods, and 25% to 30% of patients develop progressive interstitial lung disease (ILD) (2). In a large international cohort study, 35% of SSc-related deaths were attributed to pulmonary fibrosis, making it the leading cause of mortality in this patient population (6). The course of SSc-associated ILD (SSc-ILD) is highly variable; some patients have limited or stable lung involvement, whereas in others, lung disease progresses inexorably. Because of the largely irreversible and potentially progressive nature of ILD, it is important that diagnostic tests are performed early, so that treatment can be initiated with minimal delay.

In this article, we review SSc-ILD with a focus on pathogenesis, risk factors, and patient characteristics associated with the condition, with a view to identifying patients most at risk for the disease and its progression. We also highlight similarities and differences between SSc-ILD and idiopathic pulmonary fibrosis (IPF), the most frequent and deadly of the idiopathic ILDs.

## Pathogenesis

The architectural disruption and collagen-rich extracellular matrix (ECM) in SSc-ILD results from the interaction of cells in the epithelial, endothelial, and interstitial compartments with components of the innate and adaptive immune system and the ECM, after chronic microinjuries in the lung. The first step in the pathological process is believed to comprise repetitive endothelial and epithelial cell injury. This leads to activation of the innate and adaptive immune system, recruitment and activation of fibroblasts, and differentiation of fibroblasts to a myofibroblast phenotype (7), with accumulation of ECM and development of fibrosis (8). Apoptosis is triggered in some epithelial cells, and others undergo epithelial–mesenchymal transition (7). Many of the phenotypic changes occurring in respiratory epithelial cells in the context of fibrosis remain unknown and require further study. Cells undergoing epithelial–mesenchymal transition exhibit profound morphological and biological

changes, such as loss of polarity, increased capacity for migration, increased production of ECM components, and increased resistance to apoptosis (7). Resistance to apoptosis is also characteristic of certain myofibroblasts, which may contribute to the rate and extent of fibrosis (7) in SSc-ILD.

A plausible model of pathogenesis for parenchymal lung involvement in connective tissue disease, which consolidates current evidence on SSc-ILD pathology and describes initial alveolar epithelial and endothelial injuries that are triggered by environmental factors, pathogens, or inflammation, is shown in Figure 1 (9). The latter event results in damage to the lung tissue and initiation of repair pathways including the recruitment of fibroblasts and myofibroblasts; close anatomical and functional interactions between alveolar epithelial and endothelial compartments result in recruitment of circulating cellular components and mediators, such as platelets and progenitor cells. In this model, myofibroblasts are key profibrotic cells that persist in affected lung tissue; the extent of their persistence determines the pattern and type of fibrotic reaction. Interplay of myofibroblasts with the ECM via matricellular proteins, such as integrins and microfibrils, together with soluble factors, such as connective tissue growth factor, drive the fibrotic process. The degree of irreversible architectural disruption likely determines the progression or reversibility of the lung condition (9).

TGF- $\beta$  (transforming growth factor- $\beta$ ) is believed to be one of the key factors in the process of fibrosis. It has been implicated in ECM accumulation and the regulation of immune response (7, 8). Injured cells secrete TGF- $\beta$ , which leads to the recruitment of immune cells, including macrophages, which in turn release more TGF- $\beta$  (7). Increased expression of genes regulated by TGF- $\beta$  has been confirmed in patients with progressive lung fibrosis (10). Type 2 helper T cells that secrete IL (e.g., IL-4 and IL-13) are also believed to play a role in the development of fibrosis (8). Moreover, levels of thrombin are increased in the lungs of patients with SSc-ILD (7), probably as a consequence of cellular injury. In addition to its role in the coagulation cascade, thrombin may contribute to fibrosis by increasing proliferation of fibroblasts in response to

fibrinogen and facilitating differentiation of fibroblasts into myofibroblasts (7). The Wnt/ $\beta$ -catenin pathway has been implicated in the activation of fibroblasts and in pulmonary tissue remodeling (7).

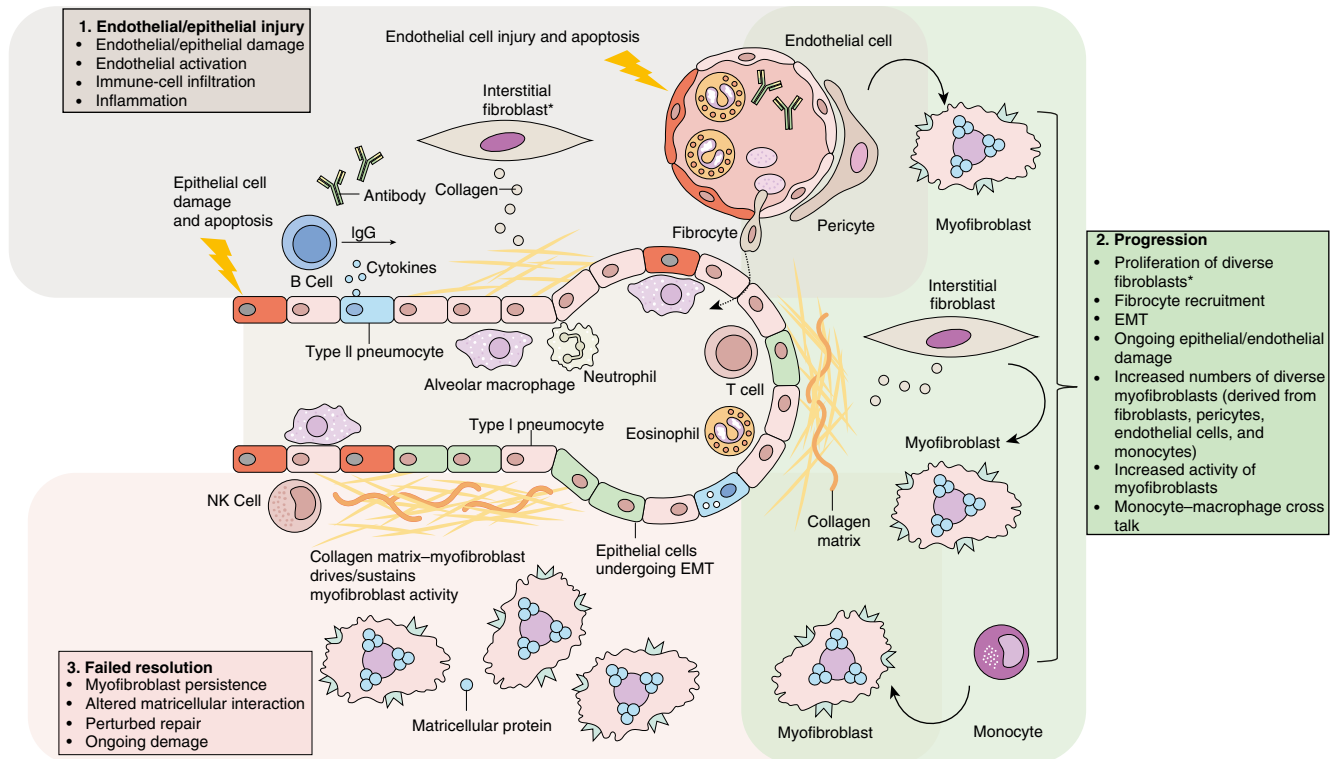
Elements involved in the pathogenesis of SSc, such as IL-6 and M2-like macrophages, may also contribute to the development of SSc-ILD, especially early in the disease (11–13). Increases in macrophage polarization, elevated C-reactive protein, and serum IL-6 levels have been associated with the progression of early SSc-ILD (10, 12, 14).

## Genetics and Epigenetics

SSc-ILD has been associated with a number of HLA-dependent genes and non-HLA genes (see Tables E1 and E2 in the online supplement) (15). After the analyses of at least 200 patients with SSc-ILD, only two variants conferred an odds ratio of at least 2.0 with statistical significance: *HLA-DRB1\*3* (Han Chinese population) and *CTGF rs6918698* (GG genotype; UK population) (15).

Despite the number of reported associations, genetic biomarkers relevant to the risk of ILD in patients with SSc are yet to be established with certainty (15). Many of the individual studies reporting associations of genetic variants with SSc-ILD have been small, and follow-up studies of specific associations are either lacking or have reported conflicting data. Therefore, a concerted effort is needed, involving large numbers of patients of different ethnicities, to establish more definite genetic risk factors for SSc-ILD and its progression.

A few studies have investigated the epigenetics of SSc-ILD (7). Epigenetic factors that may play a role in the pathogenesis of SSc-ILD include CpG methylation, which is related to increased DNA methyltransferase expression in fibroblasts. Increased DNA methyltransferase expression may affect the activities of nitric oxide synthase or the collagen transcription suppression factor Fli1 (Friend leukemia virus integration 1). Fli1 appears to play a role in protecting against ILD, by upregulating the expression of genes, including *autoimmune regulator* and *CXCL13* (7, 16). A genome-wide study of genes in peripheral blood mononuclear cells identified four methylation-regulated genes (*F2R*, *FYN*, *PAG1*, and *PRKCH*) as



**Figure 1.** Cellular pathogenesis of fibrotic lung injury in systemic sclerosis. \*Including SPINT2hi, MFAP5hi, and few WIF1hi fibroblasts. EMT = epithelial-mesenchymal transition; NK cell = natural killer T cell.

being underexpressed in patients with SSc-ILD versus patients with SSc and no ILD (17). Significantly increased expression of the *XRCC4 DNA repair* gene was reported in patients with SSc with versus without ILD (18). Micro-RNA (miRNA) expression has also been assessed in animal models and in lung tissue and peripheral blood mononuclear cells derived from patients with SSc-ILD. Studies have shown that increased expression of *miR-155* is associated with worsened lung function and increased lung fibrosis (19).

### Risk Factors for the Development and Progression of SSc-ILD

Risk factors associated with progressive ILD among patients with SSc include diffuse cutaneous SSc, male sex, African American race, and the presence of anti-Scl-70 antibodies, also known as antitopoisomerase I antibodies or ATA, discussed previously in the section on genetics and epigenetics (20–22). Other indices of SSc-ILD severity have also been associated with progressive disease,

including the extent of disease on high-resolution computed tomography (HRCT) imaging, reduced DL<sub>CO</sub> (% predicted), and decreased FVC (% predicted) (23, 24).

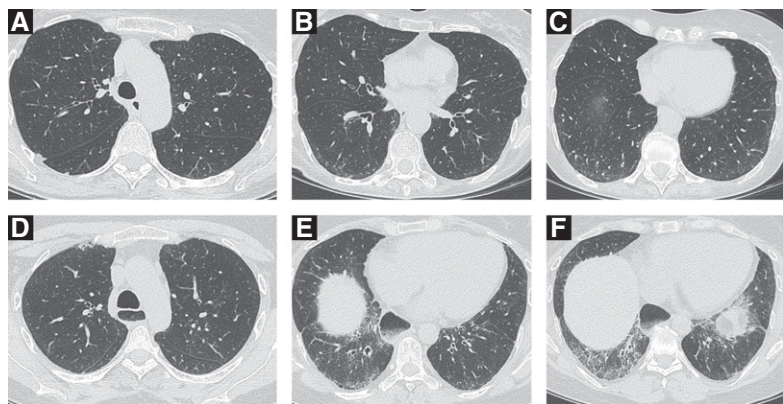
Similarly, risk factors for mortality in SSc-ILD include older age, male sex, extent of disease on HRCT imaging, lower FVC, and lower DL<sub>CO</sub> (23). Several models, including the Composite Physiologic Index; Interstitial Lung Disease–Gender, Age, Physiology Index; du Bois index; and modified du Bois index, have been reported to help predict mortality in patients with SSc-ILD (25). These models are based on readily available clinical details, such as age, sex, and FVC. HRCT imaging is routinely performed at most centers, and the findings can be integrated with pulmonary function test results as per the Limited/Extensive Staging System developed by Goh and colleagues for SSc-ILD (26). This staging system, which is based on the visual estimation of extent of disease on HRCT and, as necessary, integrated with FVC (% predicted), appears to predict the patient’s risk of mortality more accurately than either of the component variables when used in isolation (26). This validated staging system proposes the rapid

identification of limited or extensive lung disease using HRCT on the basis of a disease extent threshold of 20%. In cases in which disease extent remains indeterminate on HRCT, FVC is used to classify lung disease as either limited or extensive on the basis of an FVC threshold of 70%. This system represents a practical means of integrating HRCT extent and functional severity in routine prognostic evaluation (26). HRCT images from patients with SSc-ILD are provided in Figures 2, 3, and 4 to demonstrate examples of ILD with limited, indeterminate, and extensive disease on CT imaging, according to the Goh and colleagues 20% threshold (26). Stratification of patients using this system has been shown to be predictive of both progression-free survival and mortality.

The 6-minute-walk test has also been demonstrated to be an independent predictor of mortality in SSc-ILD. Certain blood biomarkers may also be used to predict the risk of disease progression (27, 28), although they are not routinely used in clinical practice.

In the SLS (Scleroderma Lung Study) I (NCT00004563) and II (NCT00883129), higher baseline skin score, older age, and a

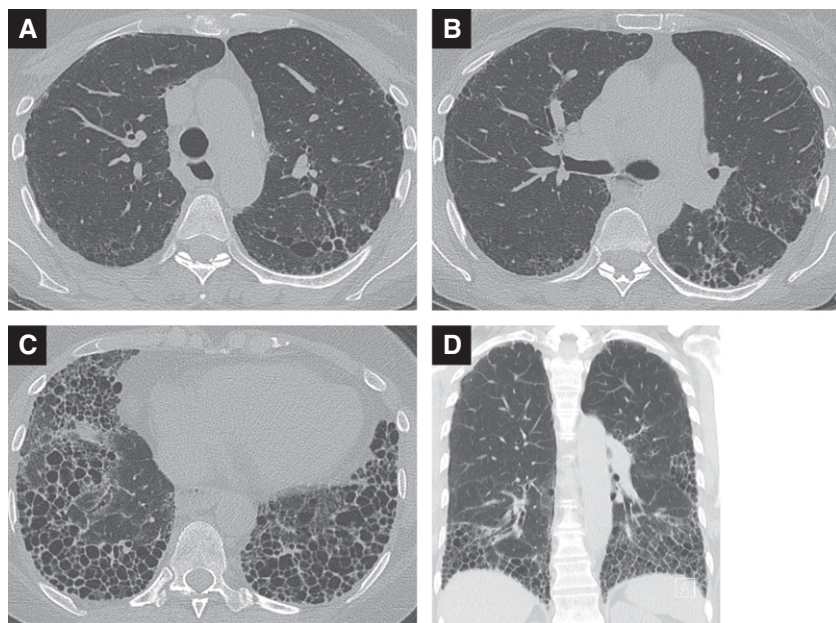




**Figure 2.** (A–C) Limited disease (<20% extent) on high-resolution computed tomography (HRCT) imaging in a 72-year-old female nonsmoker. HRCT images at the level of (A) the aortic arch show no convincing interstitial lung disease (ILD), and (B and C) very limited subpleural ground-glass opacification. (D–F) ILD of “indeterminate” extent on HRCT imaging in a 46-year-old female nonsmoker with systemic sclerosis. (A–D) The upper zones show minor reticulation, (E) just below the level of the right hemidiaphragm, and (F) the costophrenic recesses demonstrating reticulation, ground-glass opacification, and traction bronchiectasis/bronchiolectasis. The morphologic features are in keeping with a fibrotic nonspecific interstitial pneumonia pattern. Disease extent on HRCT imaging with regard to the 20% threshold is difficult to gauge (i.e., “indeterminate” according to the Goh staging); FVC in this patient was 60% predicted, thereby indicating “extensive” ILD. Note the marked esophageal dilatation containing food residue.

decline in FVC and  $DL_{CO}$  over 2 years were independently associated with an increased risk of mortality (29). A decline in the FVC and the  $DL_{CO}$  over 2 years was a better

predictor of mortality than the baseline FVC and  $DL_{CO}$  (29). In a long-term study of the prognostic significance of pulmonary function test changes, the strongest 1-year



**Figure 3.** High-resolution computed tomography images in a 58-year-old woman with systemic sclerosis who never smoked;  $DL_{CO}$  32% predicted and FVC 76% predicted. Axial images at (A) the level of the aortic arch, (B) the carina, and (C) the lower lobes demonstrate extensive disease (>20% extent by visual estimation) and (D) coronal reconstruction. There is marked honeycombing, particularly in the lower lobes, indicating a usual interstitial pneumonia pattern. The coronal image shows striking lower zone preponderance of disease.

predictor of future mortality in patients with SSc-ILD was a composite endpoint defined either by a decline from baseline in FVC of  $\geq 10\%$  or a decline of 5% to 9% in FVC with a decrease in  $DL_{CO}$  of  $\geq 15\%$  (30). Thus, short-term changes in measurements of SSc-ILD progression appear to have important implications regarding long-term outcomes. The overlap between risk factors for ILD progression and for increased mortality is unsurprising.

Treatment of SSc-ILD is beyond the scope of this review; however, several landmark studies have indicated that some treatments may be able to stabilize or slow down disease progression and, therefore, improve patient outcomes. All these trials focused on patients with clinically meaningful ILD, defined as a combination of moderate to severe ILD on HRCT and abnormal pulmonary physiology with symptoms. SLS I showed that 12 months of treatment of SSc-ILD with cyclophosphamide (CYC) improved FVC % predicted by 2.53% versus placebo ( $P < 0.03$ ). A modest benefit was also reported in TLC, dyspnea, skin thickening, and health-related quality of life (31, 32). SLS II was a 24-month study comparing 2-year treatment with mycophenolate mofetil (MMF) with 1 year of treatment with CYC followed by 1 year of placebo in patients with SSc-ILD. The two treatment approaches showed similar efficacy in terms of FVC % predicted (mean improvement of 2.19% and 2.88%, respectively) at 24 months. However, MMF treatment was reported to be better tolerated (e.g., lower rates of leucopenia and thrombocytopenia) (33). The Fibrosing Alveolitis in Scleroderma Trial was a randomized, placebo-controlled study of low-dose prednisolone and six-monthly doses of intravenous CYC and oral azathioprine. Compared with placebo, study intervention showed a nonsignificant trend toward improving FVC (treatment difference, 4.19%;  $P = 0.08$ ) (34). Recently, nintedanib became the first U.S. Food and Drug Administration–approved treatment for SSc-ILD; it is indicated for slowing the rate of decline in pulmonary function in patients with SSc-associated ILD on the basis of the results of the phase III, randomized, double-blind placebo-controlled SENSICIS (Safety and Efficacy of Nintedanib in Systemic Sclerosis) trial (35). Primary endpoint analysis in the SENSICIS trial showed that the adjusted annual rate of



**Figure 4.** Computed tomography imaging in a 52-year-old man, ex-smoker, with a DL<sub>CO</sub> of 22% and FVC 56% predicted. Axial images at (A) the level of the arch, (B) the pulmonary venous confluence, and (C) the costophrenic recesses show extensive (>20%) disease. There is predominant ground-glass opacification with fine reticulation, no honeycombing, but severe traction bronchiectasis. The computed tomography features are consistent with a fibrotic nonspecific interstitial pneumonia pattern. Note also the marked esophageal dilatation.

decline in FVC was 52.4 ml/yr in nintedanib-treated patients versus 93.3 ml/yr in placebo-treated patients (difference, 41.0 ml/yr; 95% confidence interval, 2.9–79.0 ml/yr; *P*=0.04) over a 1-year period in the total study population. Subgroups analyses reported that nintedanib reduced the progression of ILD irrespective of mycophenolate use at baseline. Statistical testing did not indicate heterogeneity in the treatment effect of nintedanib between those who were or were not receiving mycophenolate at baseline (*P*=0.45 for treatment-by-time-by-subgroup interaction). The absolute effect of nintedanib versus placebo in reducing the rate of decline in FVC was numerically lower in patients who were receiving mycophenolate at baseline compared with those who were not receiving mycophenolate at baseline (26.3 ml/yr vs. 55.4 ml/yr). The relative treatment effect of nintedanib was similar between these subgroups (40% and 46%, respectively) and consistent with that observed in the overall population (44%). No other significant clinical benefits were observed (36).

### Blood Serum and BAL Fluid Biomarkers

Blood serum or BAL fluid (BALF) biomarkers may be of value in diagnosing SSc-ILD and in prognostication. A number of potential biomarkers have been identified, which could be indicative of lung involvement in patients with SSc (Tables 1 and E3) (27). Autoantibodies are the only blood markers currently available in routine clinical practice (Tables 1 and E3). The presence of anti-Scl-70 antibodies and the absence of anticentromere antibodies in SSc indicate an increased likelihood of progressive ILD (20, 22, 37). Associations

of these antibodies with major histocompatibility complex II antigens support the genetic basis of SSc-ILD (37).

A number of biomarkers are being investigated in clinical research (Tables 1 and E3), although they are not currently available for use in routine clinical practice, with the exception of KL-6 (Krebs von den Lungen-6), which is available but only in Japan. Among biomarkers under clinical investigation, high plasma levels of KL-6 appear to be predictive of lung involvement and ILD progression in patients with SSc (23, 38, 39), including in SLS-II. Serum CCL18 (chemokine [C-C motif] ligand 18), a macrophage 2-derived protein that is chemotactic for a number of immune cells, has also been shown to be a good prognostic marker, even after adjustment for baseline ILD severity (40, 41). Analysis of serum CCL18 was able to differentiate the impact of tocilizumab versus placebo in SSc with early ILD on FVC% (14).

Serum levels of MMP7 (matrix metalloproteinase-7) are higher in patients with SSc-ILD versus SSc without ILD, and combined measurements of KL-6 and MMP7 have been suggested for identifying patients at risk of developing clinically significant ILD (27). Higher levels of MMP12 (matrix metalloproteinase-12) have been found in patients with SSc-ILD versus those without lung involvement; in the population with SSc-ILD, increased MMP12 levels appear to be associated with lower FVC (42). Data from two cohorts of patients with SSc showed that high plasma concentrations of CCL2 are predictive of ILD progression and shorter survival (43). Elevated acute-phase reactants, such as high plasma C-reactive protein levels, have been associated with an increased likelihood of progressive early SSc-ILD (44). Also, elevated serum IL-6 levels have been reported to be predictive of early disease

progression (specifically, declines in DL<sub>CO</sub> and FVC or death within 12 mo) in patients with SSc-ILD (12). However, IL-6 would provide only low specificity for diagnosing SSc-ILD, because its levels are elevated in a range of inflammatory diseases.

A proteome-wide analysis in SSc identified CXCL4 (chemokine [C-X-C motif] ligand 4) as the principal protein secreted by plasmacytoid dendritic cells (45). Plasmacytoid dendritic cells in the BALF are associated with the severity of disease on HRCT in SSc-ILD (46). Plasma levels of CXCL4 correlate with the occurrence of ILD in patients with SSc, and higher levels of this biomarker are associated with more rapid decline in DL<sub>CO</sub> (45). Volkmann and colleagues found that plasma CXCL4 levels were higher in patients with SSc-ILD compared with healthy control subjects in SLS II; however, the levels did not correlate with severity of ILD at baseline (47). Plasma CXCL4 levels reduced with immunosuppressive therapy; larger declines observed over the first 12 months of treatment were associated with greater improvements in lung function over the subsequent 12 months (47). Moreover, levels of antibodies against CXCL3 (chemokine [C-X-C motif] ligand 4) and CXCL4 have been reported to be increased in patients with SSc-ILD versus healthy control subjects but lower in patients with deteriorating versus stable lung function (48). Serum levels of chitinase-3-like protein 1, also known as YKL-40, have been shown to be higher in patients with SSc with versus those without pulmonary involvement (49). Levels of chitinase 1 have been reported to be significantly higher in patients with SSc-ILD than in patients with SSc and no lung involvement; as well as being a candidate biomarker, this enzyme could be considered as a therapeutic target (50).

Currently, BAL is not routinely performed in patients with SSc-ILD; the previously observed link between BALF neutrophilia and mortality was subsequently found to be mainly related to disease severity (51, 52). However, BAL has been shown to be useful in identifying clinically unsuspected infections in a small minority of patients with SSc-ILD. If not appropriately treated, such infections have the potential to be aggravated by immunosuppressive therapy (53). In routine clinical practice, BAL is not considered to provide additional



**Table 1.** Clinically Used Biomarkers and Biomarkers under Investigation in SSc-ILD

Mechanistic Pathway and Biomarker	References
Clinically used biomarkers	
Immune dysregulation or inflammation	
Anti-centromere	20, 22, 37
Anti-Scl-70	22, 37
Nucleolar pattern on ANA (representing anti-Th/To, U3 RNP)	83
Biomarkers supported by significant clinical data	
Epithelial cell injury or barrier dysfunction	
CCL-18	40, 61
KL-6*	23, 38, 39
SP-D	84
Immune dysfunction or inflammation	
IL-6/CRP	12, 41
Biomarkers under investigation	
Epithelial cell injury or barrier dysfunction	
APOAI	55
CC16	85
ET-1	86
Isoprostane	86
SP-A	87
sE-selectin	86
sVCAM-1	86
SPFA2	55
S100A6	55
TGF-β	86
VEGF	86
14-3-3ε	55
Immune dysfunction or inflammation	
Anti-CXCR4	48
Anti-CXCR3	48
CCL2	43
CRP	88
CXCL4	45
CXCL10	89
CX3CL1	90
C3a	55
IL-10	86
IL-15	86
IL-17 <sup>†</sup>	65
IL-22 <sup>†</sup>	65
IL-23	86
miR-155	19
Remodeling and fibrosis	
Chitinase-1	50
CTGF	86
Circulating fibrocytes	88
GDF-15	88
MMP7	27
MMP12	42
MMP13	88
miR-21	19
miR-92A	91
miR-200c	88
PMN elastase	86
TIMP-1	86
TIMP-2	88
YKL-40	49

*Definition of abbreviations:* 14-3-3ε = tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein epsilon; ANA = antinuclear antibody; anti-Th/To = Th/To ribonucleoprotein antibody; APOAI = apolipoprotein A-I; C3a = complement 3 anaphylatoxin; CC16 = club cell secretory protein 16; CCL = chemokine (C-C motif) ligand; CRP = C-reactive protein; CTGF = connective tissue growth factor; CX3CL1 = chemokine (C-X3-C motif) ligand 1; CXCL = chemokine (C-X-C motif) ligand; CXCR3 = chemokine (C-X-C motif) receptor 3; ET-1 = endothelin-1; GDF-15 = growth differentiation factor-15; KL-6 = Krebs von den Lungen-6; miR = microRNA; MMP = matrix metalloproteinase; PMN = polymorphonuclear; S100A6 = S100 calcium-binding protein A6; Scl-70 = topoisomerase 1; sE-selectin = soluble E selectin; SP-A = surfactant protein A; SP-D = surfactant protein D; SPFA2 = pulmonary surfactant-associated protein A2; SSc-ILD = systemic sclerosis-associated interstitial lung disease; sVCAM-1 = soluble vascular cell adhesion molecule 1; TGF-β = transforming growth factor-β; TIMP-1 = tissue inhibitors of metalloproteinases-1; U3 RNP = fibrillar; VEGF = vascular endothelial growth factor; YKL-40 = chitinase-3-like protein 1.

\*Approved by Japan's Health Insurance Program as a diagnostic marker for interstitial lung diseases in 1999.

<sup>†</sup>Circulating IL-producing T cells.

meaningful prognostic information; however, this could change if biomarkers independent of disease severity and without an equivalent correlate in the peripheral blood are identified. BALF inflammatory cytokines have been described as potential predictive biomarkers of SSc-ILD deterioration; this, however, has so far only been reported in small patient cohorts (54). Furthermore, proteomic and gene expression analysis of BALF is likely to provide insights that are specific to SSc-ILD pathogenesis that may not be possible in the peripheral blood. Proteomic analysis of BALF has also identified the differential expression of a number of potential biomarkers including C3a (complement 3 anaphylatoxin), APOAI (apolipoprotein A-I), 14-3-3ε, SPFA2 (pulmonary surfactant-associated protein A2), and S100A6 (S100 calcium-binding protein A6), involved in fibrosis, innate immune responses, and vascular damage (55).

### Comparison with IPF

Respiratory clinicians are often more familiar with IPF than SSc-ILD, IPF being the prototypic ILD; IPF affects a greater number of patients and has been researched more extensively than SSc-ILD. Not surprisingly, there is a larger literature and clinical experience in IPF compared with SSc-ILD; therefore, it is logical to explore the similarities and differences between SSc-ILD and IPF. A comparative summary is provided in Tables E3 and E4.

Although ILD occurs in a large proportion of patients with SSc, only some will experience disease that worsens over time (2). Spontaneous regression can occur, albeit rarely, in SSc-ILD, and the disease course is likely to be stabilized by treatment with immunosuppressants or as part of natural history of the disease—changing from a declining trend to stability or, in a small percentage of cases, improving over time (13, 56). In contrast, all patients with IPF have progressive fibrosis, albeit at different rates (57), which never undergoes spontaneous regression.

Immunological involvement appears to differ between SSc-ILD and IPF (Tables E3 and E4), although adaptive and innate immune mechanisms are implicated in both diseases. Most patients with SSc-ILD are positive for autoantibodies (e.g., antinuclear antibodies), whereas clinically relevant levels of

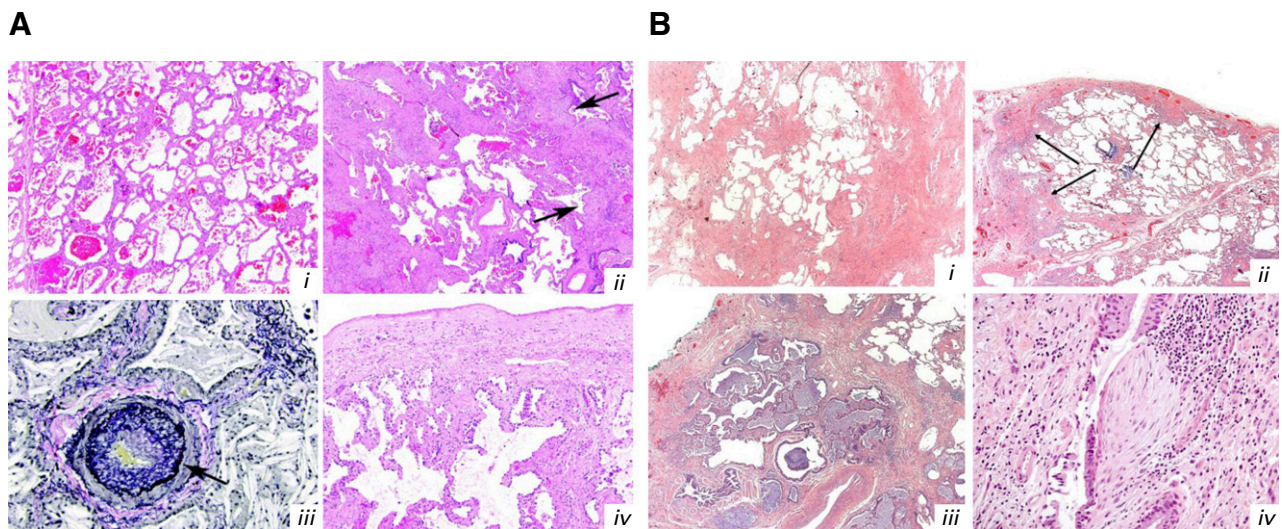
autoantibodies are believed to be absent from patients with IPF (13). A single study has reported a link between anti-HSP70 antibodies and poor survival in IPF, although currently this is not considered in routine clinical practice (58). The existence of specific activation mechanisms for different macrophage subpopulations has been described in IPF, whereby M1 macrophages (inducers include LPS, IFN- $\gamma$ , and granulocyte-stimulating colony-stimulating factor) and M2 macrophages (inducers include IL-4, IL-10, IL-13, and TGF- $\beta$ ) are both involved in the pathogenesis of the disease (59). IL-4<sup>+</sup> T cells in the BALF are associated with the severity of disease on HRCT imaging in SSc-ILD (60). Levels of CCL18 are increased in BALF and serum of patients with either IPF or SSc-ILD. In both diseases, serum CCL18 has been linked to worse prognosis independent of disease severity (40, 61), and levels of serum CCL18 appear to decrease in response to anti-IL6 therapy (14), with stabilization in lung function.

A study of lung tissue showed increased mast cell density in patients with IPF compared with healthy control subjects, whereas mast cell density was similar in patients with SSc-ILD and healthy control subjects (62). Regarding adaptive immunity, numbers of CD4<sup>+</sup> CD25<sup>+</sup> regulatory T cells in the lungs appear to be increased in SSc-ILD but not in IPF (63, 64). Also, increased numbers of IL-22-producing T-helper cells have been observed in SSc-ILD but not in IPF (65, 66). Consistent with these findings, individuals with SSc-ILD, but not those with IPF, benefit from CYC treatment (13). There is, therefore, good evidence to suggest that adaptive immune mechanisms play a reduced role in IPF compared with SSc-ILD. In fact, few patients with IPF are likely to respond to any immunosuppressant therapy, whereas most patients with SSc-ILD respond to such treatment. Further understanding of the phenotypes, activation mechanisms,

and roles of macrophages in lung fibrosis, both in IPF and SSc-ILD, may help in the development of therapeutic targets.

Some of the pathological pathways involved in fibrogenesis in IPF are similar to those in SSc-ILD. The initial trigger of fibrosis in both diseases appears to be epithelial and/or endothelial cell injury (13). The associated cell death has several effects, including the activation of TGF- $\beta$ , which then triggers immune responses and causes fibroblast activation, proliferation, and differentiation into myofibroblasts. These processes culminate in the excess deposition of ECM (11).

On histopathologic analysis, patients with SSc-ILD usually exhibit fibrotic (rarely cellular) nonspecific interstitial pneumonia (NSIP; Figure 5) (67), whereas usual interstitial pneumonia (UIP) may be observed only in a minority of patients with SSc-ILD. In contrast, UIP is the defining morphological pattern in patients with IPF (68). Patients with SSc-ILD and a UIP



**Figure 5.** Histopathology of systemic sclerosis-associated interstitial lung disease (SSc-ILD) and idiopathic pulmonary fibrosis (13, 68). (A) SSc-ILD. (Ai) Nonspecific interstitial pneumonia; note the diffuse alveolar septal thickening throughout the lobule with lack of peripheral accentuation in the area of an interlobular septum on the left. (Aii) Usual interstitial pneumonia; note the peripheral involvement of a pulmonary lobule sparing the centrilobular area containing the bronchovascular bundle. Arrows indicate fibroblastic foci. (Aiii) Pulmonary arterial hypertension; note the hypertensive arterial changes with prominent intimal fibrosis. Arrow indicates separation of the media and intima by the internal elastic lamina. (Aiv) Pleural fibrosis; its presence supports the diagnosis of SSc-ILD in the appropriate clinical setting. Hematoxylin and eosin (H&E)-stained sections are shown in Ai, Aii, and Aiv; Verhoeff-van Gieson-stained sections in Aiii. Original magnification  $\times 40$  in i and ii;  $\times 200$  in iii;  $\times 100$  in iv. (B) Usual interstitial pneumonia. (Bi) At low magnification, the diagnostic key is the abrupt alternating of scarred and normal lung (patchwork pattern: scar-normal-scar-normal). In the scarred areas, the alveolar architecture is obliterated. (Bii) The fibrosis frequently prevails at the periphery of the lobule in the subpleural paraseptal regions (arrows), with relative sparing of the centrilobule. This is a useful diagnostic clue, particularly in early cases like this (H&E 20). (Biii) Honeycomb consists of enlarged airspaces lined by bronchiolar epithelium, frequently filled by mucus and surrounded by dense scars. Note the architectural distortion and the abrupt transition with residual normal lung seen in the right upper corner. (Biv) A fibroblastic focus consisting of a dome-shaped proliferation of myofibroblasts immersed in a myxoid matrix. Fibroblastic foci can be covered by bronchiolar epithelium, as here, or by hyperplastic pneumocytes. H&E-stained sections are shown in Bi–Biv. (A) Reprinted from *Arthritis & Rheumatology*, Vol. 66, Herzog EL, et al., Review: Interstitial Lung Disease Associated With Systemic Sclerosis and Idiopathic Pulmonary Fibrosis: How Similar and Distinct? 1967–1978, Copyright (2014), by permission from John Wiley & Sons. (B) Reprinted from *Respiratory Medicine*, Vol. 104, Cavazza A, et al., The role of histology in idiopathic pulmonary fibrosis: An update, S11–S22, Copyright (2010), by permission from Elsevier.

pattern have a better prognosis than patients with IPF; moreover, patients with SSc and a UIP pattern do not appear to have a significantly worse survival than patients with SSc and NSIP (69, 70). Although the reasons for this are unclear, UIP in patients with a connective tissue disease is characterized by higher numbers of lymphoid follicles, smaller honeycomb cysts, and fewer fibroblastic foci compared with UIP in IPF (71).

Genetic variants associated with SSc-ILD and IPF do not appear to overlap. The association with the MUC5B (mucin 5B) promoter variant rs35705950, observed in sporadic IPF and familial idiopathic interstitial pneumonias (IIPs), is one notable example that is absent in SSc-ILD (72, 73). MUC5B expression is increased in the small airways and honeycomb cysts in UIP/IPF but similar to control subjects in the small airways of patients with SSc with an NSIP pattern (74). More generally, the genetic susceptibility loci identified in IIPs were not observed in a large North American cohort of patients with SSc-ILD (75). It is possible that the underlying genetics of ILDs are related to the different histopathological patterns. For example, rheumatoid arthritis-associated ILD with a UIP pattern is associated with the MUC5B promoter variant rs35705950 (76); however, the same variant has also been associated with idiopathic NSIP (77). Further studies are needed to characterize the link between genetic characteristics and ILD patterns. A number of HLA alleles have been associated with SSc-ILD, as discussed previously. Although associations between HLA alleles and IIP have been reported (78, 79), specific HLA allele associations do not overlap between SSc-ILD and IPF. For instance, HLA DRB1\*1501, observed to be associated with IPF (78), has been reported as protective against SSc (80).

Epigenetic changes may underpin bronchiolar remodeling and the associated formation of enlarged bronchiolized airspaces (i.e., honeycombing, which occurs to differing extents in IPF and SSc-ILD). Chilosi and colleagues were the first to highlight the importance of the bronchioalveolar junction and to report overexpression of markers of the Wnt pathway (e.g.,  $\beta$ -catenin and MMP7) in IPF but not in NSIP (81). Differences between SSc-ILD and IPF are likely in specific miRNA profiles as well as in other epigenetic parameters; further studies are needed to characterize these differences and their relevance.

Despite treatment not being the focus of this review, we briefly mention some important differences and similarities in terms of treatment of SSc-ILD and IPF as highlighted by key clinical trials. The antifibrotic agents nintedanib and pirfenidone have shown benefit and are approved as treatments in IPF. In SSc-ILD, nintedanib has been granted U.S. Food and Drug Administration approval to slow the rate of decline in pulmonary function in patients with SSc-ILD on the basis of the results of the phase III SENSICIS trial, similar to its effect in patients with IPF. Furthermore, and in line with the known safety profile of nintedanib in patients with IPF, diarrhea was the most common adverse event (AE); all reported AEs were at worst mild or moderate in severity, as reported in 49.5% and 45.0% of patients, respectively (36). The phase II LOTUSS (Safety and Tolerability of Pirfenidone in Participants with SSc-ILD) trial showed that pirfenidone administered either as monotherapy or in combination with MMF had an acceptable tolerability profile in patients with SSc-ILD. The most common AEs were nausea, headache, and fatigue, which is consistent with its tolerability profile in patients with IPF (82). SLS III (NCT03221257), for which recruitment was ongoing at the time of writing, was designed

to compare pirfenidone plus MMF with MMF alone in SSc-ILD. The results of this study, due in May 2021, may provide further data regarding the similarities and differences between treatment response in SSc-ILD and IPF.

## Conclusions

ILD is a common complication of SSc and a significant cause of morbidity and mortality. Differentiation from IPF is particularly important, because IPF is the most common fibrosing ILD. This is usually straightforward in the context of the classic extrapulmonary SSc manifestations but can be more difficult in patients with SSc sine scleroderma. Knowledge of SSc-ILD is important in our community to ensure that affected patients are managed optimally. Greater extent of lung fibrosis on HRCT, lower FVC, and early lung function decline are predictors of early mortality. Familiarity with key clinical features (including established risk factors of progressive lung disease) may prove useful in raising our alertness to the possibility of SSc-ILD in relevant patients. Perhaps most importantly, high awareness of the disease and its characteristics will be needed to realize the potential of new treatment options. ■

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