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

Running title: SSc-ILD Disease Awareness

Dinesh Khanna[1], Donald P. Tashkin[2], Christopher P. Denton[3], Elisabetta A. Renzoni[4], Sujal R. Desai[5] and John Varga[6]

[1]University of Michigan Scleroderma Program, Division of Rheumatology/Department of Internal Medicine, Ann Arbor, MI, USA

[2]Department of Medicine, David Geffen School of Medicine at UCLA, University of California, Los Angeles, CA, USA

[3]UCL Centre for Rheumatology and Connective Tissue Diseases, Royal Free Hospital, London, UK

[4]Interstitial Lung Disease Unit, Royal Brompton Hospital, London, UK; NIHR Clinical Research Facility, Royal Brompton Hospital, London, UK

[5]National Heart and Lung Institute, Imperial College London & Department of Radiology, Royal Brompton & Harefield NHS Foundation Trust Hospital, London

[6]Northwestern Scleroderma Program, Feinberg School of Medicine, Chicago, IL, USA

Correspondence 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, USA. E-mail: khannad@med.umich.edu. Phone: +1 734-763-7182. Fax: +1 734-936-3695

Target journal: Am J Resp Crit Care Med (AJRCCM)

Number of characters in title: 92 (with spaces)

Number of characters in running title: 25 (with spaces)

Article type: Concise Clinical Review

Word count (max. 4000): 4336, *including* in-text citations

Abstract word count: 248

Figures/tables (max.6): 6 (+4 supplementary tables)

References: 95

Author contributions: Only the named authors of this manuscript contributed to the content and writing of this manuscript. None of the authors received financial compensation from an external source in return for writing or publishing this paper. All authors have contributed in the preparation of this manuscript.

Funding sources: Boehringer Ingelheim Pharmaceuticals, Inc.

Descriptor number that best classifies manuscript subject: LUNG DISEASES (9.23 Interstitial Lung Disease)

Abstract

Systemic sclerosis (SSc) is a complex, multi-organ, autoimmune disease. Lung fibrosis occurs in ~80% of patients with SSc; 25–30% 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, reduced diffusing capacity for carbon monoxide and reduced forced vital capacity. 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 non-specific 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, while all IPF patients 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

Introduction

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 3–24 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 range of possible signs, symptoms and autoantibodies (5).

Lung fibrosis occurs in up to around 80% of patients with SSc, with varying prevalence depending on ascertainment methods and 25–30% 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. Due to 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 of 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, following chronic micro-injuries in the lung. The first step in the pathological process is thought 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, while others undergo epithelial mesenchymal transition (EMT) (7). Many of the phenotypic changes occurring in respiratory epithelial cells in the context of fibrosis remain unknown and require further study. Cells undergoing EMT 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).

Transforming growth factor beta (TGF-β) 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-β, which leads to the recruitment of immune cells, including macrophages, which in turn release more TGF-β (7). Increased expression of genes regulated by TGF-β has been confirmed in patients with progressive lung fibrosis (10). Type 2 helper T-cells that secrete interleukins (IL; e.g., IL-4, 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/β-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 both 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 human leukocyte antigen (HLA)-dependent genes and non-HLA genes (Supplementary Tables 1 and 2) (15). Following 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).

In spite of 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 Friend leukemia virus integration 1 (Fli1). Fli1 appears to play a role in protecting against ILD, by up-regulating 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 being under-expressed 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 SSc patients with versus without ILD (18). Micro-ribonucleic acid (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 gender, African-American race, and the presence of anti-Scl-70 antibodies, also known as anti-topoisomerase 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), reduced diffusing capacity of the lungs for carbon monoxide (DL_{CO}) (% predicted), and decreased forced vital capacity (FVC; % predicted) (23, 24).

Similarly, risk factors for mortality in SSc-ILD include older age, male gender, extent of disease on HRCT, lower FVC and lower DL_{CO} (23). Several models including the Composite Physiologic Index; Interstitial Lung Disease-Gender, Age, Physiology Index; du Bois index; 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, gender and FVC. HRCT is routinely performed at most centers, and the findings can be integrated with pulmonary function tests (PFT) results as per the Limited/Extensive Staging System developed by Goh *et al.* for SSc-ILD (26). This staging system, which is based on the visual estimation of disease extent of disease on HRCT and, as necessary, integrated with FVC (% predicted), appears to predict the patients' 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 based on 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 based on a 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 2–4 to demonstrate examples of ILD with limited, indeterminate and extensive disease on CT, according to the Goh *et al*. 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 are not routinely used in clinical practice.

In the Scleroderma Lung Study (SLS) I and II, higher baseline skin score, older age, and a 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 PFT changes, the strongest 1-year predictor of future mortality in patients with SSc-ILD was a composite endpoint defined either by a decline from baseline in FVC of ≥ 10% or a decline of 5–9% in FVC with a decrease in DLco of ≥ 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, 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 total lung capacity, 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 non-significant trend towards improving FVC (treatment difference 4.19%, $P = 0.08$) (34). Recently nintedanib became the first FDA-approved treatment for SSc-ILD; it is indicated for slowing the rate of decline in pulmonary function in patients with SSc-associated ILD based on the results of the phase III, randomized, double-blind, placebo-controlled Safety and Efficacy of Nintedanib in Systemic Sclerosis (SENSCIS) trial (35). Primary endpoint analysis in the SENSCIS trial showed that the adjusted annual rate of decline in FVC was 52.4 mL/year in nintedanib-treated patients versus 93.3 mL/year in placebo-treated patients (difference 41.0 mL/year; 95% confidence interval [CI] = 2.9–79.0 mL/year; $P = 0.04$) 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). While 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/year versus 55.4 mL/year). 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 Bronchoalveolar Lavage Fluid Biomarkers

Blood serum or bronchoalveolar lavage 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 (Table 1 and Supplementary Table 3) (27). Autoantibodies are the only blood markers currently available in routine clinical practice (Table 1 and Supplementary Table 3). The presence of anti-Scl-70 antibodies and the absence of anti-centromere 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 Supplementary Table 3), although they are not currently available for use in routine clinical practice, with the exception of Krebs von den Lungen-6 (KL-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 chemokine (C-C motif) ligand 18 (CCL18), 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 matrix metalloproteinase-7 (MMP7) 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 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 chemokine (C-C motif) ligand 2 (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_{CO} and FVC or death within 12 months) 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 chemokine (C-X-C motif) ligand 4 (CXCL4) 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 SSc patients, and higher levels of this biomarker are associated with more rapid decline in DL_{CO} (45). Volkmann *et al.* found that plasma CXCL4 levels were higher in patients with SSc-ILD compared with healthy controls in SLS II; however, the levels did not correlate with severity of ILD at baseline. 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 chemokine (C-X-C motif) receptor 3 and CXCL4 have been reported to be increased in patients with SSc-ILD versus healthy controls, 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 SSc patients 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, bronchoalveolar lavage (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 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, APOAI, 14-3-3ε, SPFA2 and S100A6, involved in fibrosis, innate immune responses and vascular damage (55).

Comparison with Idiopathic Pulmonary Fibrosis

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 appears it is logical to explore the similarities and differences between SSc-ILD and IPF. A comparative summary is provided in Supplementary Tables 3 and 4.

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 (Supplementary Table 3 and 4), although adaptive and innate immune mechanisms are implicated in both diseases. Most patients with SSc-ILD are positive for autoantibodies (e.g., antinuclear antibodies), while 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 lipopolysaccharide, interferon-γ and granulocyte stimulating colony factor) and M2 macrophages (inducers include IL-4, IL-10 and IL-13, and TGF-β) are both involved in the pathogenesis of the disease (59). IL-4+ T cells in the BALF are associated with the severity of disease on HRCT 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 controls, whereas mast cell density was similar in patients with SSc-ILD and healthy controls (62). With regards to adaptive immunity, numbers of CD4+CD25+ 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 than in 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-β, 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) non-specific interstitial pneumonia (NSIP; Figure 5) (67), while 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 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 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 controls in the small airways of SSc patients 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 *et al.* were the first to highlight the importance of the bronchioloalveolar junction and to report overexpression of markers of the Wnt pathway (e.g., β-catenin, 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 anti-fibrotic agents nintedanib and pirfenidone have shown benefit and are approved as treatments in IPF. In SSc-ILD, nintedanib has been granted FDA approval to slow the rate of decline in pulmonary function in patients with SSc-ILD based on the results of the phase III SENSCIS trial, similar to its affect in patients with IPF. Furthermore and in line with the known safety profile of nintedanib in patients with IPF, diarrhea was the most common 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 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 adverse events (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 since IPF is the most common fibrosing ILD. This is usually straightforward in the context of the classic extra-pulmonary 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.

Conflict of Interest

Dinesh Khanna is an employee of University of Michigan and Civi Biopharma and has ownership interests in Eicos Sciences, Inc. He has received personal fees and/or grants from Actelion Pharmaceuticals Ltd, Bayer AG, Bristol-Meyers Squibb, Boehringer Ingelheim Ltd, ChemomAb Ltd, Corbus Pharmaceutical Holdings Inc., CSL Behring LLC, Cytori Therapeutics Inc., EMD Serono Inc., Genentech Inc./F. Hoffmann-La Roche Ltd, GlaxoSmithKline plc, Horizon, NIH National Institute of Allergy and Infectious Diseases, NIH National Institute of

Arthritis and Musculoskeletal and Skin Diseases, Pfizer Inc. and Sanofi-Aventis/Genzyme Corporation, and UCB S.A. Christopher Denton has received personal fees and/or grants from Actelion Pharmaceuticals Ltd, Bayer AG, Boehringer Ingelheim Ltd, CSL Behring LLC, GlaxoSmithKline plc, Inventiva, F. Hoffmann-La Roche Ltd, Sanofi and UCB. Donald Tashkin has received personal fees from Boehringer Ingelheim Ltd, EMD Serano Inc. and Genentech Inc. Elisabetta Renzoni has received lecture and advisory board fees from Boehringer Ingelheim Ltd, and F. Hoffmann-La Roche Ltd and lecture fees from Mundipharma International. Sujal R Desai is a Course Organiser for the Boehringer Ingelheim ILD Masterclass and has received honoraria and travel expenses. John Varga has received personal fees and/or research grants from NIH, Boehringer Ingelheim Ltd, Corbus Pharmaceutical Holding Inc, Bristol-Meyers Squibb, Cytori Therapeutics and Genentech.

Acknowledgments

The author(s) meet criteria for authorship as defined by the International Committee of Medical Journal Editors (ICMJE). The authors received no compensation related to the development of the manuscript. Writing support and formatting assistance was provided by Leon Newman, PhD, and Ken Sutor, BSc, of GeoMed, an Ashfield company, part of UDG Healthcare plc, which was contracted and funded by Boehringer Ingelheim Pharmaceuticals, Inc. (BIPI). BIPI was given the opportunity to review the manuscript for medical and scientific accuracy as well as intellectual property considerations.

References

1. Denton CP, Hughes M, Gak N, Vila J, Buch MH, Chakravarty K, Fligelstone K, Gompels LL, Griffiths B, Herrick AL, Pang J, Parker L, Redmond A, van Laar J, Warburton L, Ong VH, Bsr, Bhpr Standards G, Audit Working G. BSR and BHPR guideline for the treatment of systemic sclerosis. *Rheumatology (Oxford)* 2016; 55: 1906-1910.
2. Denton CP, Khanna D. Systemic sclerosis. *Lancet* 2017; 390: 1685-1699.
3. Khanna D, Tashkin DP, Denton CP, Lubell MW, Vazquez-Mateo C, Wax S. Ongoing clinical trials and treatment options for patients with systemic sclerosis-associated interstitial lung disease. *Rheumatology (Oxford)* 2019; 58: 567-579.
4. Ranque B, Mouthon L. Geoepidemiology of systemic sclerosis. *Autoimmun Rev* 2010; 9: A311-318.
5. van den Hoogen F, Khanna D, Fransen J, Johnson SR, Baron M, Tyndall A, Matucci-Cerinic M, Naden RP, Medsger TA, Jr., Carreira PE, Riemekasten G, Clements PJ, Denton CP, Distler O, Allanore Y, Furst DE, Gabrielli A, Mayes MD, van Laar JM, Seibold JR, Czirjak L, Steen VD, Inanc M, Kowal-Bielecka O, Muller-Ladner U, Valentini G, Veale DJ, Vonk MC, Walker UA, Chung L, Collier DH, Csuka ME, Fessler BJ, Guiducci S, Herrick A, Hsu VM, Jimenez S, Kahaleh B, Merkel PA, Sierakowski S, Silver RM, Simms RW, Varga J, Pope JE. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. *Arthritis Rheum* 2013; 65: 2737-2747.
6. Tyndall AJ, Bannert B, Vonk M, Airo P, Cozzi F, Carreira PE, Bancel DF, Allanore Y, Muller-Ladner U, Distler O, Iannone F, Pellerito R, Pileckyte M, Miniati I, Ananieva L, Gurman AB, Damjanov N, Mueller A, Valentini G, Riemekasten G, Tikly M, Hummers L, Henriques MJ, Caramaschi P, Scheja A, Rozman B, Ton E, Kumanovics G, Coleiro B, Feierl E, Szucs G, Von Muhlen CA, Riccieri V, Novak S, Chizzolini C, Kotulska A, Denton C, Coelho PC, Kotter I, Simsek I, de la Pena Lefebvre PG, Hachulla E, Seibold JR, Rednic S, Stork J, Morovic-Vergles J, Walker UA. Causes and risk factors for death in systemic sclerosis: a study from the EULAR Scleroderma Trials and Research (EUSTAR) database. *Ann Rheum Dis* 2010; 69: 1809-1815.
7. Akter T, Silver RM, Bogatkevich GS. Recent advances in understanding the pathogenesis of scleroderma-interstitial lung disease. *Curr Rheumatol Rep* 2014; 16: 411.
8. Giacomelli R, Liakouli V, Berardicurti O, Ruscitti P, Di Benedetto P, Carubbi F, Guggino G, Di Bartolomeo S, Ciccia F, Triolo G, Cipriani P. Interstitial lung disease in systemic sclerosis: current and future treatment. *Rheumatol Int* 2017; 37: 853-863.
9. Wells AU, Denton CP. Interstitial lung disease in connective tissue disease--mechanisms and management. *Nat Rev Rheumatol* 2014; 10: 728-739.
10. Christmann RB, Sampaio-Barros P, Stifano G, Borges CL, de Carvalho CR, Kairalla R, Parra ER, Spira A, Simms R, Capellozzi VL, Lafyatis R. Association of Interferon- and transforming growth factor beta-regulated genes and macrophage activation with systemic sclerosis-related progressive lung fibrosis. *Arthritis Rheumatol* 2014; 66: 714-725.
11. Bagnato G, Harari S. Cellular interactions in the pathogenesis of interstitial lung diseases. *Eur Respir Rev* 2015; 24: 102-114.
12. De Lauretis A, Sestini P, Pantelidis P, Hoyles R, Hansell DM, Goh NS, Zappala CJ, Visca D, Maher TM, Denton CP, Ong VH, Abraham DJ, Kelleher P, Hector L, Wells AU, Renzoni EA. Serum interleukin 6 is predictive of early functional decline and mortality in

interstitial lung disease associated with systemic sclerosis. *J Rheumatol* 2013; 40: 435-446.
13. Herzog EL, Mathur A, Tager AM, Feghali-Bostwick C, Schneider F, Varga J. Review: interstitial lung disease associated with systemic sclerosis and idiopathic pulmonary fibrosis: how similar and distinct? *Arthritis Rheumatol* 2014; 66: 1967-1978.
14. Khanna D, Denton CP, Jahreis A, van Laar JM, Frech TM, Anderson ME, Baron M, Chung L, Fierlbeck G, Lakshminarayanan S, Allanore Y, Pope JE, Riemekasten G, Steen V, Muller-Ladner U, Lafyatis R, Stifano G, Spotswood H, Chen-Harris H, Dziadek S, Morimoto A, Sornasse T, Siegel J, Furst DE. Safety and efficacy of subcutaneous tocilizumab in adults with systemic sclerosis (faSScinate): a phase 2, randomised, controlled trial. *Lancet* 2016; 387: 2630-2640.
15. Stock CJW, Renzoni EA. Genetic predictors of systemic sclerosis-associated interstitial lung disease: a review of recent literature. *Eur J Hum Genet* 2018; 26: 765-777.
16. Taniguchi T, Miyagawa T, Toyama S, Yamashita T, Nakamura K, Saigusa R, Ichimura Y, Takahashi T, Toyama T, Yoshizaki A, Sato S, Asano Y. CXCL13 produced by macrophages due to Fli1 deficiency may contribute to the development of tissue fibrosis, vasculopathy and immune activation in systemic sclerosis. *Exp Dermatol* 2018; 27: 1030-1037.
17. Zhu H, Zhu C, Mi W, Chen T, Zhao H, Zuo X, Luo H, Li QZ. Integration of genome-wide DNA methylation and transcription uncovered aberrant methylation-regulated genes and pathways in the peripheral blood mononuclear cells of systemic sclerosis. *Int J Rheumatol* 2018; 2018: 7342472.
18. Mak AC, Tang PL, Cleveland C, Smith MH, Kari Connolly M, Katsumoto TR, Wolters PJ, Kwok PY, Criswell LA. Brief report: Whole-exome sequencing for identification of potential causal variants for diffuse cutaneous systemic sclerosis. *Arthritis Rheumatol* 2016; 68: 2257-2262.
19. Christmann RB, Wooten A, Sampaio-Barros P, Borges CL, Carvalho CR, Kairalla RA, Feghali-Bostwick C, Ziemek J, Mei Y, Goummih S, Tan J, Alvarez D, Kass DJ, Rojas M, de Mattos TL, Parra E, Stifano G, Capelozzi VL, Simms RW, Lafyatis R. miR-155 in the progression of lung fibrosis in systemic sclerosis. *Arthritis Res Ther* 2016; 18: 155.
20. Ashmore P, Tikly M, Wong M, Ickinger C. Interstitial lung disease in South Africans with systemic sclerosis. *Rheumatol Int* 2018; 38: 657-662.
21. Steen V. Predictors of end stage lung disease in systemic sclerosis. *Ann Rheum Dis* 2003; 62: 97-99.
22. Wangkaew S, Euathrongchit J, Wattanawittawas P, Kasitanon N, Louthrenoo W. Incidence and predictors of interstitial lung disease (ILD) in Thai patients with early systemic sclerosis: Inception cohort study. *Mod Rheumatol* 2016; 26: 588-593.
23. Winstone TA, Assayag D, Wilcox PG, Dunne JV, Hague CJ, Leipsic J, Collard HR, Ryerson CJ. Predictors of mortality and progression in scleroderma-associated interstitial lung disease: a systematic review. *Chest* 2014; 146: 422-436.
24. Wu W, Jordan S, Becker MO, Dobrota R, Maurer B, Fretheim H, Ye S, Siegert E, Allanore Y, Hoffmann-Vold AM, Distler O. Prediction of progression of interstitial lung disease in patients with systemic sclerosis: the SPAR model. *Ann Rheum Dis* 2018; 77: 1326-1332.
25. Ryerson CJ, O'Connor D, Dunne JV, Schooley F, Hague CJ, Murphy D, Leipsic J, Wilcox PG. Predicting mortality in systemic sclerosis-associated interstitial lung disease using

risk prediction models derived from idiopathic pulmonary fibrosis. *Chest* 2015; 148: 1268-1275.
26. Goh NS, Desai SR, Veeraraghavan S, Hansell DM, Copley SJ, Maher TM, Corte TJ, Sander CR, Ratoff J, Devaraj A, Bozovic G, Denton CP, Black CM, du Bois RM, Wells AU. Interstitial lung disease in systemic sclerosis: a simple staging system. *Am J Respir Crit Care Med* 2008; 177: 1248-1254.
27. Kennedy B, Branagan P, Moloney F, Haroon M, O'Connell OJ, O'Connor TM, O'Regan K, Harney S, Henry MT. Biomarkers to identify ILD and predict lung function decline in scleroderma lung disease or idiopathic pulmonary fibrosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2015; 32: 228-236.
28. Khanna D, Nagaraja V, Tseng CH, Abtin F, Suh R, Kim G, Wells A, Furst DE, Clements PJ, Roth MD, Tashkin DP, Goldin J. Predictors of lung function decline in scleroderma-related interstitial lung disease based on high-resolution computed tomography: implications for cohort enrichment in systemic sclerosis-associated interstitial lung disease trials. *Arthritis Res Ther* 2015; 17: 372.
29. Volkmann ER, Tashkin DP, Sim M, Li N, Goldmutz E, Keyes-Alstein L, Pinckney A, Furst DE, Clements PJ, Khanna D, Steen V, Schraufnagel DE, Arami S, Hsu V, Roth MD, Elashoff RM, Sullivan KM. Short-term progression of interstitial lung disease in systemic sclerosis predicts long-term survival in two independent clinical trial cohorts. *Ann Rheum Dis* 2019; 78: 122-130.
30. Goh NS, Hoyles RK, Denton CP, Hansell DM, Renzoni EA, Maher TM, Nicholson AG, Wells AU. Short-term pulmonary function trends are predictive of mortality in interstitial lung disease associated with systemic sclerosis. *Arthritis Rheumatol* 2017; 69: 1670-1678.
31. Tashkin DP, Elashoff R, Clements PJ, Goldin J, Roth MD, Furst DE, Arriola E, Silver R, Strange C, Bolster M, Seibold JR, Riley DJ, Hsu VM, Varga J, Schraufnagel DE, Theodore A, Simms R, Wise R, Wigley F, White B, Steen V, Read C, Mayes M, Parsley E, Mubarak K, Connolly MK, Golden J, Olman M, Fessler B, Rothfield N, Metersky M. Cyclophosphamide versus placebo in scleroderma lung disease. *N Engl J Med* 2006; 354: 2655-2666.
32. Khanna D, Yan X, Tashkin DP, Furst DE, Elashoff R, Roth MD, Silver R, Strange C, Bolster M, Seibold JR, Riley DJ, Hsu VM, Varga J, Schraufnagel DE, Theodore A, Simms R, Wise R, Wigley F, White B, Steen V, Read C, Mayes M, Parsley E, Mubarak K, Connolly MK, Golden J, Olman M, Fessler B, Rothfield N, Metersky M, Clements PJ, Scleroderma Lung Study G. Impact of oral cyclophosphamide on health-related quality of life in patients with active scleroderma lung disease: results from the scleroderma lung study. *Arthritis Rheum* 2007; 56: 1676-1684.
33. Tashkin DP, Roth MD, Clements PJ, Furst DE, Khanna D, Kleerup EC, Goldin J, Arriola E, Volkmann ER, Kafaja S, Silver R, Steen V, Strange C, Wise R, Wigley F, Mayes M, Riley DJ, Hussain S, Assassi S, Hsu VM, Patel B, Phillips K, Martinez F, Golden J, Connolly MK, Varga J, Dematte J, Hinchcliff ME, Fischer A, Swigris J, Meehan R, Theodore A, Simms R, Volkov S, Schraufnagel DE, Scholand MB, Frech T, Molitor JA, Highland K, Read CA, Fritzler MJ, Kim GHJ, Tseng CH, Elashoff RM, Sclerodema Lung Study III. Mycophenolate mofetil versus oral cyclophosphamide in scleroderma-related interstitial lung disease (SLS II): a randomised controlled, double-blind, parallel group trial. *Lancet Respir Med* 2016; 4: 708-719.

34. Hoyles RK, Ellis RW, Wellsbury J, Lees B, Newlands P, Goh NS, Roberts C, Desai S, Herrick AL, McHugh NJ, Foley NM, Pearson SB, Emery P, Veale DJ, Denton CP, Wells AU, Black CM, du Bois RM. A multicenter, prospective, randomized, double-blind, placebo-controlled trial of corticosteroids and intravenous cyclophosphamide followed by oral azathioprine for the treatment of pulmonary fibrosis in scleroderma. *Arthritis Rheum* 2006; 54: 3962-3970.
35. U.S. Food & Drug Administration. FDA approves first treatment for patients with rare type of lung disease. 2019. Accessed 10 September 2019. Available from: https://www.fda.gov/news-events/press-announcements/fda-approves-first-treatment-patients-rare-type-lung-disease.
36. Distler O, Highland KB, Gahlemann M, Azuma A, Fischer A, Mayes MD, Raghu G, Sauter W, Girard M, Alves M, Clerisme-Beaty E, Stowasser S, Tetzlaff K, Kuwana M, Maher TM, Investigators ST. Nintedanib for systemic sclerosis-associated interstitial lung disease. *N Engl J Med* 2019; 380: 2518-2528.
37. Ho KT, Reveille JD. The clinical relevance of autoantibodies in scleroderma. *Arthritis Res Ther* 2003; 5: 80-93.
38. Kuwana M, Shirai Y, Takeuchi T. Elevated serum Krebs von den Lungen-6 in early disease predicts subsequent deterioration of pulmonary function in patients with systemic sclerosis and interstitial lung disease. *J Rheumatol* 2016; 43: 1825-1831.
39. Volkmann ER, Tashkin DP, Kuwana M, Li N, Roth MD, Charles J, Hant FN, Bogatkevich GS, Akter T, Kim G, Goldin J, Khanna D, Clements PJ, Furst DE, Elashoff RM, Silver RM, Assassi S. Pneumoproteins KL-6 and CCL-18 Predict Progression of Interstitial Lung Disease in Systemic Sclerosis. *Arthritis Rheumatol* 2019: [ePub ahead of print].
40. Prasse A, Pechkovsky DV, Toews GB, Schafer M, Eggeling S, Ludwig C, Germann M, Kollert F, Zissel G, Muller-Quernheim J. CCL18 as an indicator of pulmonary fibrotic activity in idiopathic interstitial pneumonias and systemic sclerosis. *Arthritis Rheum* 2007; 56: 1685-1693.
41. Elhai M, Hoffmann-Vold AM, Avouac J, Pezet S, Cauvet A, Leblond A, Fretheim H, Garen T, Kuwana M, Molberg O, Allanore Y. Performance of candidate serum biomarkers for systemic sclerosis-associated interstitial lung disease. *Arthritis Rheumatol* 2019; 1: 972-982.
42. Manetti M, Guiducci S, Romano E, Bellando-Randone S, Conforti ML, Ibba-Manneschi L, Matucci-Cerinic M. Increased serum levels and tissue expression of matrix metalloproteinase-12 in patients with systemic sclerosis: correlation with severity of skin and pulmonary fibrosis and vascular damage. *Ann Rheum Dis* 2012; 71: 1064-1072.
43. Wu M, Baron M, Pedroza C, Salazar GA, Ying J, Charles J, Agarwal SK, Hudson M, Pope J, Zhou X, Reveille JD, Fritzler MJ, Mayes MD, Assassi S. CCL2 in the circulation predicts long-term progression of interstitial lung disease in patients with early systemic sclerosis: Data From two independent cohorts. *Arthritis Rheumatol* 2017; 69: 1871-1878.
44. Chowaniec M, Skoczynska M, Sokolik R, Wiland P. Interstitial lung disease in systemic sclerosis: challenges in early diagnosis and management. *Reumatologia* 2018; 56: 249-254.
45. van Bon L, Affandi AJ, Broen J, Christmann RB, Marijnissen RJ, Stawski L, Farina GA, Stifano G, Mathes AL, Cossu M, York M, Collins C, Wenink M, Huijbens R, Hesselstrand R, Saxne T, DiMarzio M, Wuttge D, Agarwal SK, Reveille JD, Assassi S,

Mayes M, Deng Y, Drenth JP, de Graaf J, den Heijer M, Kallenberg CG, Bijl M, Loof A, van den Berg WB, Joosten LA, Smith V, de Keyser F, Scorza R, Lunardi C, van Riel PL, Vonk M, van Heerde W, Meller S, Homey B, Beretta L, Roest M, Trojanowska M, Lafyatis R, Radstake TR. Proteome-wide analysis and CXCL4 as a biomarker in systemic sclerosis. *N Engl J Med* 2014; 370: 433-443.
46. Kafaja S, Valera I, Divekar AA, Saggar R, Abtin F, Furst DE, Khanna D, Singh RR. pDCs in lung and skin fibrosis in a bleomycin-induced model and patients with systemic sclerosis. *JCI Insight* 2018; 3.
47. Volkmann ER, Tashkin DP, Roth MD, Clements PJ, Khanna D, Furst DE, Mayes M, Charles J, Tseng CH, Elashoff RM, Assassi S. Changes in plasma CXCL4 levels are associated with improvements in lung function in patients receiving immunosuppressive therapy for systemic sclerosis-related interstitial lung disease. *Arthritis Res Ther* 2016; 18: 305.
48. Weigold F, Gunther J, Pfeiffenberger M, Cabral-Marques O, Siegert E, Dragun D, Philippe A, Regensburger AK, Recke A, Yu X, Petersen F, Catar R, Biesen R, Hiepe F, Burmester GR, Heidecke H, Riemekasten G. Antibodies against chemokine receptors CXCR3 and CXCR4 predict progressive deterioration of lung function in patients with systemic sclerosis. *Arthritis Res Ther* 2018; 20: 52.
49. Nordenbaek C, Johansen JS, Halberg P, Wiik A, Garbarsch C, Ullman S, Price PA, Jacobsen S. High serum levels of YKL-40 in patients with systemic sclerosis are associated with pulmonary involvement. *Scand J Rheumatol* 2005; 34: 293-297.
50. Lee CG, Herzog EL, Ahangari F, Zhou Y, Gulati M, Lee CM, Peng X, Feghali-Bostwick C, Jimenez SA, Varga J, Elias JA. Chitinase 1 is a biomarker for and therapeutic target in scleroderma-associated interstitial lung disease that augments TGF-beta1 signaling. *J Immunol* 2012; 189: 2635-2644.
51. Goh NS, Veeraraghavan S, Desai SR, Cramer D, Hansell DM, Denton CP, Black CM, du Bois RM, Wells AU. Bronchoalveolar lavage cellular profiles in patients with systemic sclerosis-associated interstitial lung disease are not predictive of disease progression. *Arthritis Rheum* 2007; 56: 2005-2012.
52. Strange C, Bolster MB, Roth MD, Silver RM, Theodore A, Goldin J, Clements P, Chung J, Elashoff RM, Suh R, Smith EA, Furst DE, Tashkin DP. Bronchoalveolar lavage and response to cyclophosphamide in scleroderma interstitial lung disease. *Am J Respir Crit Care Med* 2008; 177: 91-98.
53. Clements PJ, Goldin JG, Kleerup EC, Furst DE, Elashoff RM, Tashkin DP, Roth MD. Regional differences in bronchoalveolar lavage and thoracic high-resolution computed tomography results in dyspneic patients with systemic sclerosis. *Arthritis Rheum* 2004; 50: 1909-1917.
54. Schmidt K, Martinez-Gamboa L, Meier S, Witt C, Meisel C, Hanitsch LG, Becker MO, Huscher D, Burmester GR, Riemekasten G. Bronchoalveoloar lavage fluid cytokines and chemokines as markers and predictors for the outcome of interstitial lung disease in systemic sclerosis patients. *Arthritis Res Ther* 2009; 11: R111.
55. Landi C, Bargagli E, Carleo A, Refini RM, Bennett D, Bianchi L, Cillis G, Prasse A, Bini L, Rottoli P. Bronchoalveolar lavage proteomic analysis in pulmonary fibrosis associated with systemic sclerosis: S100A6 and 14-3-3epsilon as potential biomarkers. *Rheumatology (Oxford)* 2019; 58: 165-178.
56. Kim HJ, Tashkin DP, Gjertson DW, Brown MS, Kleerup E, Chong S, Belperio JA, Roth MD, Abtin F, Elashoff R, Tseng CH, Khanna D, Goldin JG. Transitions to different patterns

of interstitial lung disease in scleroderma with and without treatment. *Ann Rheum Dis* 2016; 75: 1367-1371.
57. Sharif R. Overview of idiopathic pulmonary fibrosis (IPF) and evidence-based guidelines. *Am J Manag Care* 2017; 23: S176-S182.
58. Kahloon RA, Xue J, Bhargava A, Csizmadia E, Otterbein L, Kass DJ, Bon J, Soejima M, Levesque MC, Lindell KO, Gibson KF, Kaminski N, Banga G, Oddis CV, Pilewski JM, Sciurba FC, Donahoe M, Zhang Y, Duncan SR. Patients with idiopathic pulmonary fibrosis with antibodies to heat shock protein 70 have poor prognoses. *Am J Respir Crit Care Med* 2013; 187: 768-775.
59. Zhang L, Wang Y, Wu G, Xiong W, Gu W, Wang CY. Macrophages: friend or foe in idiopathic pulmonary fibrosis? *Respir Res* 2018; 19: 170.
60. Divekar AA, Khanna D, Abtin F, Maranian P, Saggar R, Saggar R, Furst DE, Singh RR. Treatment with imatinib results in reduced IL-4-producing T cells, but increased CD4(+) T cells in the broncho-alveolar lavage of patients with systemic sclerosis. *Clin Immunol* 2011; 141: 293-303.
61. Schupp JC, Binder H, Jager B, Cillis G, Zissel G, Muller-Quernheim J, Prasse A. Macrophage activation in acute exacerbation of idiopathic pulmonary fibrosis. *PLoS One* 2015; 10: e0116775.
62. Cha SI, Chang CS, Kim EK, Lee JW, Matthay MA, Golden JA, Elicker BM, Jones K, Collard HR, Wolters PJ. Lung mast cell density defines a subpopulation of patients with idiopathic pulmonary fibrosis. *Histopathology* 2012; 61: 98-106.
63. Frantz C, Auffray C, Avouac J, Allanore Y. Regulatory T cells in systemic sclerosis. *Front Immunol* 2018; 9: 2356.
64. Hou Z, Ye Q, Qiu M, Hao Y, Han J, Zeng H. Increased activated regulatory T cells proportion correlate with the severity of idiopathic pulmonary fibrosis. *Respir Res* 2017; 18: 170.
65. Truchetet ME, Brembilla NC, Montanari E, Allanore Y, Chizzolini C. Increased frequency of circulating Th22 in addition to Th17 and Th2 lymphocytes in systemic sclerosis: association with interstitial lung disease. *Arthritis Res Ther* 2011; 13: R166.
66. Wilson MS, Madala SK, Ramalingam TR, Gochuico BR, Rosas IO, Cheever AW, Wynn TA. Bleomycin and IL-1beta-mediated pulmonary fibrosis is IL-17A dependent. *J Exp Med* 2010; 207: 535-552.
67. du Bois RM. State of the art: Mechanisms of scleroderma-induced lung disease. *Proc Am Thorac Soc* 2007; 4: 434-438.
68. Cavazza A, Rossi G, Carbonelli C, Spaggiari L, Paci M, Roggeri A. The role of histology in idiopathic pulmonary fibrosis: an update. *Respir Med* 2010; 104 Suppl 1: S11-22.
69. Bouros D, Wells AU, Nicholson AG, Colby TV, Polychronopoulos V, Pantelidis P, Haslam PL, Vassilakis DA, Black CM, du Bois RM. Histopathologic subsets of fibrosing alveolitis in patients with systemic sclerosis and their relationship to outcome. *Am J Respir Crit Care Med* 2002; 165: 1581-1586.
70. Park JH, Kim DS, Park IN, Jang SJ, Kitaichi M, Nicholson AG, Colby TV. Prognosis of fibrotic interstitial pneumonia: idiopathic versus collagen vascular disease-related subtypes. *Am J Respir Crit Care Med* 2007; 175: 705-711.
71. Song JW, Do KH, Kim MY, Jang SJ, Colby TV, Kim DS. Pathologic and radiologic differences between idiopathic and collagen vascular disease-related usual interstitial pneumonia. *Chest* 2009; 136: 23-30.

72. Peljto AL, Steele MP, Fingerlin TE, Hinchcliff ME, Murphy E, Podlusky S, Carns M, Schwarz M, Varga J, Schwartz DA. The pulmonary fibrosis-associated MUC5B promoter polymorphism does not influence the development of interstitial pneumonia in systemic sclerosis. *Chest* 2012; 142: 1584-1588.
73. Stock CJ, Sato H, Fonseca C, Banya WA, Molyneaux PL, Adamali H, Russell AM, Denton CP, Abraham DJ, Hansell DM, Nicholson AG, Maher TM, Wells AU, Lindahl GE, Renzoni EA. Mucin 5B promoter polymorphism is associated with idiopathic pulmonary fibrosis but not with development of lung fibrosis in systemic sclerosis or sarcoidosis. *Thorax* 2013; 68: 436-441.
74. Conti C, Montero-Fernandez A, Borg E, Osadolor T, Viola P, De Lauretis A, Stock CJ, Bonifazi M, Bonini M, Caramori G, Lindahl G, Blasi FB, Nicholson AG, Wells AU, Sestini P, Renzoni E. Mucins MUC5B and MUC5AC in distal airways and honeycomb spaces: Comparison among idiopathic pulmonary fibrosis/usual interstitial pneumonia, fibrotic nonspecific interstitial pneumonitis, and control lungs. *Am J Respir Crit Care Med* 2016; 193: 462-464.
75. Wu M, Assassi S, Salazar GA, Pedroza C, Gorlova OY, Chen WV, Charles J, Taing ML, Liao K, Wigley FM, Hummers LK, Shah AA, Hinchcliff M, Khanna D, Schiopu E, Phillips K, Furst DE, Steen V, Baron M, Hudson M, Zhou X, Pope J, Jones N, Docherty P, Khalidi NA, Robinson D, Simms RW, Silver RM, Frech TM, Fessler BJ, Fritzler MJ, Molitor JA, Segal BM, Movahedian M, Martin J, Varga J, Mayes MD. Genetic susceptibility loci of idiopathic interstitial pneumonia do not represent risk for systemic sclerosis: a case control study in Caucasian patients. *Arthritis Res Ther* 2016; 18: 20.
76. Juge PA, Lee JS, Ebstein E, Furukawa H, Dobrinskikh E, Gazal S, Kannengiesser C, Ottaviani S, Oka S, Tohma S, Tsuchiya N, Rojas-Serrano J, Gonzalez-Perez MI, Mejia M, Buendia-Roldan I, Falfan-Valencia R, Ambrocio-Ortiz E, Manali E, Papiris SA, Karageorgas T, Boumpas D, Antoniou K, van Moorsel CHM, van der Vis J, de Man YA, Grutters JC, Wang Y, Borie R, Wemeau-Stervinou L, Wallaert B, Flipo RM, Nunes H, Valeyre D, Saidenberg-Kermanac'h N, Boissier MC, Marchand-Adam S, Frazier A, Richette P, Allanore Y, Sibilia J, Dromer C, Richez C, Schaeverbeke T, Liote H, Thabut G, Nathan N, Amselem S, Soubrier M, Cottin V, Clement A, Deane K, Walts AD, Fingerlin T, Fischer A, Ryu JH, Matteson EL, Niewold TB, Assayag D, Gross A, Wolters P, Schwarz MI, Holers M, Solomon JJ, Doyle T, Rosas IO, Blauwendraat C, Nalls MA, Debray MP, Boileau C, Crestani B, Schwartz DA, Dieude P. MUC5B promoter variant and rheumatoid arthritis with interstitial lung disease. *N Engl J Med* 2018; 379: 2209-2219.
77. Horimasu Y, Ohshimo S, Bonella F, Tanaka S, Ishikawa N, Hattori N, Kohno N, Guzman J, Costabel U. MUC5B promoter polymorphism in Japanese patients with idiopathic pulmonary fibrosis. *Respirology* 2015; 20: 439-444.
78. Fingerlin TE, Murphy E, Zhang W, Peljto AL, Brown KK, Steele MP, Loyd JE, Cosgrove GP, Lynch D, Groshong S, Collard HR, Wolters PJ, Bradford WZ, Kossen K, Seiwert SD, du Bois RM, Garcia CK, Devine MS, Gudmundsson G, Isaksson HJ, Kaminski N, Zhang Y, Gibson KF, Lancaster LH, Cogan JD, Mason WR, Maher TM, Molyneaux PL, Wells AU, Moffatt MF, Selman M, Pardo A, Kim DS, Crapo JD, Make BJ, Regan EA, Walek DS, Daniel JJ, Kamatani Y, Zelenika D, Smith K, McKean D, Pedersen BS, Talbert J, Kidd RN, Markin CR, Beckman KB, Lathrop M, Schwarz MI, Schwartz DA. Genome-wide association study identifies multiple susceptibility loci for pulmonary fibrosis. *Nat Genet* 2013; 45: 613-620.

79. Xue J, Gochuico BR, Alawad AS, Feghali-Bostwick CA, Noth I, Nathan SD, Rosen GD, Rosas IO, Dacic S, Ocak I, Fuhrman CR, Cuenco KT, Smith MA, Jacobs SS, Zeevi A, Morel PA, Pilewski JM, Valentine VG, Gibson KF, Kaminski N, Sciurba FC, Zhang Y, Duncan SR. The HLA class II Allele DRB1*1501 is over-represented in patients with idiopathic pulmonary fibrosis. *PLoS One* 2011; 6: e14715.
80. Arnett FC, Gourh P, Shete S, Ahn CW, Honey RE, Agarwal SK, Tan FK, McNearney T, Fischbach M, Fritzler MJ, Mayes MD, Reveille JD. Major histocompatibility complex (MHC) class II alleles, haplotypes and epitopes which confer susceptibility or protection in systemic sclerosis: analyses in 1300 Caucasian, African-American and Hispanic cases and 1000 controls. *Ann Rheum Dis* 2010; 69: 822-827.
81. Chilosi M, Poletti V, Murer B, Lestani M, Cancellieri A, Montagna L, Piccoli P, Cangi G, Semenzato G, Doglioni C. Abnormal re-epithelialization and lung remodeling in idiopathic pulmonary fibrosis: the role of deltaN-p63. *Lab Invest* 2002; 82: 1335-1345.
82. Khanna D, Albera C, Fischer A, Khalidi N, Raghu G, Chung L, Chen D, Schiopu E, Tagliaferri M, Seibold JR, Gorina E. An open-label, phase II study of the safety and tolerability of pirfenidone in patients with scleroderma-associated interstitial lung disease: the LOTUSS trial. *J Rheumatol* 2016; 43: 1672-1679.
83. Steen VD. Autoantibodies in systemic sclerosis. *Bull Rheum Dis* 1996; 45: 6-8.
84. Yamakawa H, Hagiwara E, Kitamura H, Yamanaka Y, Ikeda S, Sekine A, Baba T, Okudela K, Iwasawa T, Takemura T, Kuwano K, Ogura T. Serum KL-6 and surfactant protein-D as monitoring and predictive markers of interstitial lung disease in patients with systemic sclerosis and mixed connective tissue disease. *J Thorac Dis* 2017; 9: 362-371.
85. Riviere S, Hua-Huy T, Tiev KP, Cabane J, Dinh-Xuan AT. High baseline serum Clara Cell 16 kDa predicts subsequent lung disease worsening in systemic sclerosis. *J Rheumatol* 2018; 45: 242-247.
86. Lota HK, Renzoni EA. Circulating biomarkers of interstitial lung disease in systemic sclerosis. *Int J Rheumatol* 2012: 121439.
87. Ogawa F, Shimizu K, Muroi E, Hara T, Hasegawa M, Takehara K, Sato S. Serum levels of 8-isoprostane, a marker of oxidative stress, are elevated in patients with systemic sclerosis. *Rheumatology (Oxford)* 2006; 45: 815-818.
88. Bonhomme O, Andre B, Gester F, de Seny D, Moermans C, Struman I, Louis R, Malaise M, Guiot J. Biomarkers in systemic sclerosis-associated interstitial lung disease: review of the literature. *Rheumatology (Oxford)* 2019; 58: 1534-1546.
89. Antonelli A, Ferri C, Fallahi P, Ferrari SM, Giuggioli D, Colaci M, Manfredi A, Frascerra S, Franzoni F, Galetta F, Ferrannini E. CXCL10 (alpha) and CCL2 (beta) chemokines in systemic sclerosis--a longitudinal study. *Rheumatology (Oxford)* 2008; 47: 45-49.
90. Hoffmann-Vold AM, Weigt SS, Palchevskiy V, Volkmann E, Saggar R, Li N, Midtvedt Ø, Lund MB, Garen T, Fishbein MC, Ardehali A, Ross DJ, Ueland T, Aukrust P, Lynch JPr, Elashoff RM, Molberg Ø, Belperio JA. Augmented concentrations of CX3CL1 are associated with interstitial lung disease in systemic sclerosis. *PLoS One* 2018; 13: e0206545.
91. Sing T, Jinnin M, Yamane K, Honda N, Makino K, Kajihara I, Makino T, Sakai K, Masuguchi S, Fukushima S, Ihn H. microRNA-92a expression in the sera and dermal fibroblasts increases in patients with scleroderma. *Rheumatology (Oxford)* 2012; 51: 1550-1556.

808 92. Martinez FJ, Collard HR, Pardo A, Raghu G, Richeldi L, Selman M, Swigris JJ, Taniguchi H,
809 Wells AU. Idiopathic pulmonary fibrosis. *Nat Rev Dis Primers* 2017; 3: 17074.
810 93. Cappelli S, Bellando Randone S, Camiciottoli G, De Paulis A, Guiducci S, Matucci-Cerinic
811 M. Interstitial lung disease in systemic sclerosis: where do we stand? *Eur Respir Rev*
812 2015; 24: 411-419.
813 94. Wells AU, Steen V, Valentini G. Pulmonary complications: one of the most challenging
814 complications of systemic sclerosis. *Rheumatology (Oxford)* 2009; 48 Suppl 3: iii40-
815 44.
816 95. Usategui A, del Rey MJ, Pablos JL. Fibroblast abnormalities in the pathogenesis of
817 systemic sclerosis. *Expert Rev Clin Immunol* 2011; 7: 491-498.

818

819

Tables

Table 1. Clinically-used biomarkers and biomarkers under investigation in SSc-ILD

Biomarker	Mechanistic Pathway	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†	(65)
IL-22†	(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: ANA = anti-nuclear antibody; APOAI = apolipoprotein A-I; CC16 = clara cell secretory protein; CCL = chemokine (C-C motif) ligand; CTGF = connective tissue growth factor; CRP = c-reactive protein; CX3CL1 = chemokine fractalkine; CXCL = chemokine (C-X-C motif) ligand; CXCR3 = chemokine (C-X-C motif) receptor 3; C3a = complement 3 anaphylatoxin; ET-1 = endothelin-1; HP = hypersensitivity pneumonitis; a; IL = interleukin; KL-6 = Krebs von den lugen-6; MMP = matrix metalloproteinase; miR = microRNA; PMN = polymorphonuclear; Scl-70 = topoisomerase 1; SP-A = surfactant protein A; SP-D = surfactant protein D; sE-selectin = soluble E selectin; S100A6 = S100 calcium-binding protein A6; TIMP-1 = Tissue inhibitors of metalloproteinases-1; TNF-α = tumor necrosis factor; U3 RNP = fibrillarin; VCAM-1 = vascular cell adhesion molecule 1; VEGF = vascular endothelial growth factor; YKL-40 = chitinase-3-like protein 1; * = approved by Japan's Health Insurance Program as a diagnostic marker for ILDs in 1999; † = circulating interleukin-producing T cells.

Supplementary Tables

Supplementary Table 1. Statistically Significant Associations Between SSc-ILD and HLA Alleles: Studies with Ssc-ILD Cohorts ≥ 100 Patients (15). Reproduced with kind permission of Takahashi T, et al. *J Exp Med* 2017.

HLA region	Allele/Serotype	OR and *P* Value for SSc-ILD	Population	Cohort Size
DPB1	301	OR = 3.56 (1.27–10.73)*	Han Chinese	199/78[†]
		P = 0.0069		
	1301	OR = 2.25 (1.4–3.62)[‡]	Han Chinese	199/480[§]
		P = 3.3×10^{-4}		
DQB1	501	OR = 5.03[‡]	Han Chinese	134/239[§]
		P = 6×10^{-7}		
DRB1	3	OR = 2.47 (1.35–4.52)[‡]	Han Chinese	295/458[§]
		P = 0.0026		

Definition of abbreviations: HLA = human leukocyte antigen; ILD = interstitial lung disease; OR = odds ratio; SSc = systemic sclerosis; SSc-ILD = systemic sclerosis-associated interstitial lung disease.

*Versus SSc-no ILD.

[†]SSc-ILD/SSc-no ILD.

[‡]Versus control.

[§]SSc-ILD/control.

Supplementary Table 2. Statistically Significant Associations Between SSc-ILD and Non-HLA Genes: Studies with SSc-ILD Cohorts ≥ 100 Patients (15). Reproduced with kind permission of Takahashi T, et al. *J Exp Med* 2017.

Gene	Polymorphism	Function	OR and *P* Value for SSc-ILD	Population	Cohort Size
CD226	rs763361:T>A	–	OR = 1.27 (1.12–1.45)* *P* = 2.98 x 10^{-4}	French, German, Italian†	662/1642‡
	Haplotype rs763361:T>A, rs34794968:C>A, rs727088:G>A	Correlates with expression levels in T cells	OR = 1.27 (1.05–1.54)* *P* = 0.032	Spanish, German, Dutch, Italian, Swedish, British, Norwegian†	729/3,966‡
CTGF	rs918698:G>C	Alters ratio of Sp1:Sp3 binding affecting transcriptional activity	OR = 3.1 (1.9–5.0)* *P* =0.001	British	207/500‡
	rs6918698:G>C	See above	OR = 2.0 (1.5–2.6)* *P* = 0.001	Japanese	188/269‡
IRAKI	rs1059702:A>G/ rs1059703:G>A (in complete LD)	Increased NFκ-B activity	OR = 1.37 (1.16–1.62)* *P* = 1.99 x10^{-4}	French, Italian, German† (Female only)	604/2,217‡

	rs1059702:A>G/ rs1059703:G>A (in complete LD)	See above	OR = 1.30 (1.07–1.58)* *P* = 8.46 x10^{-3}	Spanish, German, Dutch, British[†] (Female only)	461/2,043[‡]
	rs1059702:A>G/ rs1059703:G>A (in complete LD)[§]	See above	OR = 1.2 (1.05–1.37)[‖] *P* = 0.007	European descent[†]	1,065/2,237[¶]
IRF5	rs2004640:G>T	Results in transcription of alternative exon 1	OR = 1.44 (1.19–1.76)*	French	280/760[‡]
	rs2004640:G>T	See above	OR = 1.38 (1.1–1.75)* *P* = 0.028	Han Chinese	502/227[‡]
	Haplotype rs3757385:G>T – rs2004640:G>T – rs10954213:G>A rs4728142:G>A	In LD with 5-bp indel which increases SP1 binding Associated with lower expression	OR = 0.64 (0.51–0.79)* Mean difference = 2.64 (0.43–4.84) *P* = 0.019	French American Caucasian	292/989[‡] 914** (Linear regression analysis with FVC % predicted)

	rs2004640:G>T[§]	See above	OR = 1.12 (1.02–1.22)[‖] *P* = 0.014	French, European Caucasian, Han Chinese[†]	1,682/2,806[¶]
NLRP1	rs8182352:T>C	–	OR = 1.19 (1.05–1.36)* *P* = 0.0065	French, German, Italian[†]	674/1,587[‡]
STAT4	rs7574865:T>G	–	OR = 1.42 (1.16–1.73)* *P* = 0.008	French	316/970[‡]
	rs7574865:T>G	–	OR = 1.86 (1.34–2.59)* *P* = 1.2 x 10^{-4}	Han Chinese	237/534[‡]
	rs7574865:T>G[§]	–	OR = 1.259 (1.07–1.47)[‖] *P* = 5.35 x 10^{-3}	French, Spanish, Han Chinese[†]	640/842[¶]
	rs10168266:C>T	–	OR = 1.73 (1.24–2.41) *P* = 7.7 x 10^{-4}	Han Chinese	237/534[‡]
	rs3821236:G>A	–	OR = 1.54 (1.07–2.22)* *P* = 0.015	Han Chinese	237/534[‡]
Unreplicated studies with small cohort sizes		–		European descent	439/399[¶]
ALOX5AP	rs10507391:A>T (NC_000013.11: g_30737959A>T)		OR = 1.45 (1.17–1.79)[‖] *P* = 0.0006		

Definition of abbreviations: ALOX5AP = arachidonate 5-lipoxygenase activating protein; bp = base pairs; CTGF = connective tissue growth factor; FVC = forced vital capacity; CD226 = cluster of differentiation 226; HLA = human leukocyte antigen; ILD = interstitial lung disease; IRAK1= Interleukin-1 receptor-associated kinase 1; IRF5 = interferon Regulatory Factor 5; LD, linkage disequilibrium; NFκβ = nuclear factor κβ; NLRP1 = NLR family pyrin domain containing 1; OR = odds ratio; SSc = systemic sclerosis; STAT4 = signal transducer and activator of transcription 4; SSc-ILD = systemic sclerosis-associated interstitial lung disease.

Corrected *P* values given where available. ORs are shown as OR (95% confidence interval), 517 where available.

*Versus control.

[†]Meta-analysis of the different populations 519 included.

[‡]SSc-ILD/control.

[§]Meta-analysis or previously published studies.

[‖]Versus SSc-no ILD.

[¶]SSc-ILD/SSc-no ILD.

**Total number of SSc patients 518, when SSc-ILD number not given.

Supplementary Table 3. Levels of Serum Biomarkers in Ssc-ILD: Comparison with Healthy Controls, Ssc Without ILD and IPF. Significant Differences Between Study Groups Were Only Seen with Respect to KL-6, SP-D and MMP7 (the Kruskal–Wallis Test was Used to Assess for Differences Across the Four Groups) (27). Data are presented as median (interquartile range). Reproduced with kind permission of Kennedy B, et al. *Diffuse Lung Dis* 2015.

	Controls	**SSc w/o ILD**	**SSc-ILD**	**IPF**	***P* Value**
KL-6 (ng/ml)	198 (52–360)	192 (0–525)	836 (431–1303)	633 (492–1,675)	0.0003*
SP-D (ng/ml)	137 (97–284)	169 (137–219)	398 (190–727)	542 (305–577)	0.0012†
MMP7 (ng/ml)	0 (0–0.06)	2.36 (1.2–5.1)	5.4 (2.6–7.25)	2.85 (1.5–3.6)	0.0009‡
TGF-β (pg/ml)	7,251 (5,654–10,034)	2,986 (2,483–4,029)	3,743 (1,855–5,500)	2,388 (1,501–7,367)	0.07
CCL18 (ng/ml)	46.85 (34.6–153.1)	49.1 (43.65–65.05)	62.05 (52.3–137.4)	48.4 (36.8–90.5)	0.58
PDGF-AA (pg/ml)	1,011 (605–2,989)	437 (314.5–649)	554 (328–935)	405 (167.5–1,222)	0.057
TNF-α (pg/ml)	2.73 (2.18–3.39)	2.53 (2.43–3.21)	3.41 (2.24–10.06)	2.78 (1.9–5.3)	0.84
VEGF (pg/ml)	60.32 (23.3–209.6)	22.9 (11.88–29.28)	24.96 (20.5–33.46)	24.14 (11.45–37.28)	0.053
Thrombomodulin (ng/ml)	3.07 (1.84–4.45)	1.36 (1.1–2.57)	1.63 (1.05–3.07)	2.57 (1.72–6.2)	0.054
PAI-1 (ng/ml)	37.2 (26.7–61.35)	21.3 (9.15–41.95)	40.55 (21.55–56.5)	32.7 (15.75–56.2)	0.35
VCAM-1 (ng/ml)	467.5 (397.1–686.6)	700.1 (567–969.5)	706.1 (583.2–801.3)	753.7 (444.5–916.3)	0.12
ICAM-1 (ng/ml)	297.7 (206.5–742.7)	259.5 (210.4–361.8)	431.4 (325.3–504.80)	416 (289.7–569.1)	0.18
P-Selectin (ng/ml)	168.5 (91.35–224.6)	131.3 (110–137.3)	133.9 (115.4–167.1)	119.1 (100.9–170.3)	0.51
L-Selectin (ng/ml)	1,397 (914.3–1,878)	1,385 (1,032–1679)	1329 (818.1–1746)	1,203 (891.4–1,784)	0.9

CCL2 (pg/ml)	84.9 (78.3–121.1)	86.7 (43.85–121.7)	145.2 (118.8–189.5)	159.4 (103.7–180.3)	0.06

Definition of abbreviations: CCL = chemokine (C-C motif) ligand; ICAM-1 = Intercellular Adhesion Molecule 1; IL = interleukin; KL-6 = Krebs von den lugen-6; MMP = matrix metalloproteinase; Pal-1 = Plasminogen activator inhibitor-1; PDGF-AA = Platelet Derived Growth Factor AA; SP-A = surfactant protein A; TGF-β = Tumor growth factor beta; TNF-α = tumor necrosis factor alpha ; VCAM-1 = vascular cell adhesion molecule 1; VEGF = vascular endothelial growth factor.

Supplementary Table 4. Comparison of Clinical and Mechanistic Features of SSc-ILD and IPF

Feature of Comparison	SSC-ILD	IPF
Lung involvement	Lung fibrosis occurs in ~80% of patients with SSc, 25–30% of whom develop progressive ILD (2).	All patients develop characteristic progressive lung fibrosis (57, 92)
Pulmonary symptoms	Dyspnea on exertion, nonproductive cough and predominantly basal inspiratory crackles on auscultation (13, 93, 94)	Dyspnea on exertion, non-productive cough and predominantly basal inspiratory crackles on auscultation (13, 92)
Extra-pulmonary features	Multisystem characteristics of SSc (e.g., vasculopathy, Raynaud's phenomenon, immune dysfunction, skin fibrosis, gastro-esophageal reflux) (1-3)	Digital clubbing (13)
Clinical course	Variable rate of progression (some patients show rapid, early decline; disease course may be stabilized by treatment with immunosuppressants; spontaneous regression can occur [albeit infrequently]); median survival is 5–8 years (13, 56)	Progressive decline in lung function; spontaneous regression never occurs and the disease is unlikely to respond to immunosuppressant therapy; median survival is 2–3 years (13, 57)
Disease mechanisms	Repetitive endothelial/epithelial cell injury leads to activation of innate	Similar to SSc-ILD, fibroblast activation, proliferation and differentiation into

	and adaptive immune system, recruitment and activation of fibroblasts, and differentiation of fibroblasts to a myofibroblast phenotype, accumulation of ECM and development of fibrosis (7, 8, 93, 95). Increased numbers of CD4+CD25+ regulatory T-cells and IL-22-producing T-helper cells (63, 65); mast cell density similar to healthy controls (62).	myofibroblasts culminates in excess deposition of ECM (11, 95). However, unlike SSc-ILD, mast cell density is increased versus healthy controls and no increases in CD4+CD25+ regulatory T-cells or IL-22-producing T-helper cells are observed (62, 64, 66).
Autoimmune characteristics	Most patients are positive for antinuclear antibodies and other specific autoantibodies (13).	No clinically relevant levels of autoantibodies (13)
Radiographic features	NSIP pattern is typical, including ground-glass opacities with areas of subpleural sparing, reticular markings and traction bronchiectasis. UIP observed in a minority of patients, with honeycombing of lower prominence compared with IPF (13, 71).	UIP pattern with honeycombing; ground-glass opacities not seen (13, 68).

Definition of abbreviations: ECM, extracellular matrix; IL = interleukin; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis; NSIP = nonspecific interstitial pneumonia; SSc = systemic sclerosis; UIP = usual interstitial pneumonia.

Figures

Figure 1. Cellular pathogenesis of fibrotic lung injury in systemic sclerosis. ECM = extracellular matrix; EMT = epithelial-mesenchymal transition; IgG = immunoglobulin G; NK = Natural killer T cell; *including SPINT2hi, MFAP5hi and few WIF1hi fibroblasts

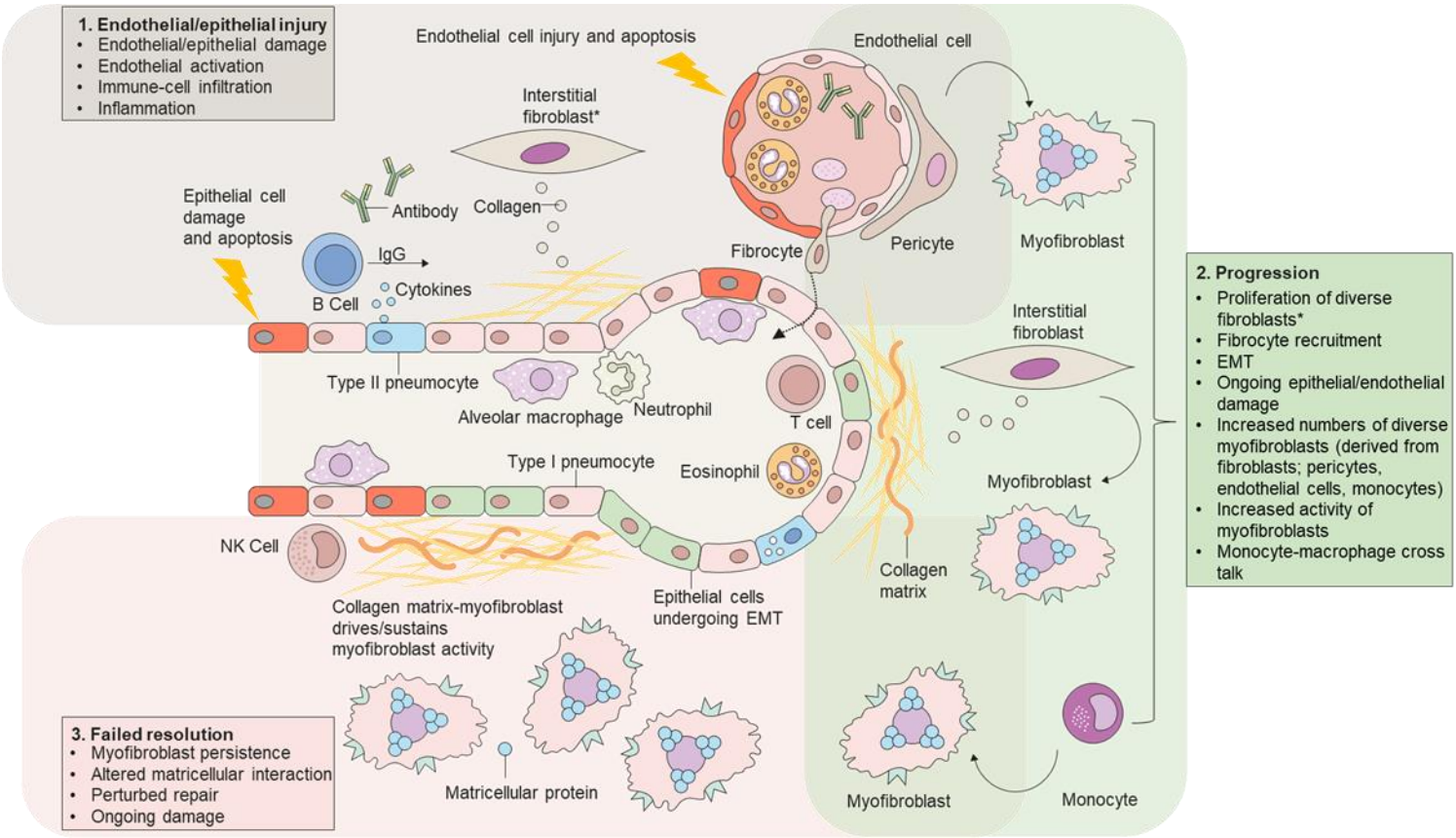


Figure 2. Limited disease (<20% extent; panels A–C) on HRCT in a 72-year-old female non-smoker. HRCT images at the level of (A) the aortic arch show no convincing ILD, and (B and C) very limited sub-pleural ground-glass opacification. ILD of 'indeterminate' extent (panels D–F) on HRCT in a 46-year-old female non-smoker with SSc. Images (A) through (D) the upper zones showing 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 NSIP pattern. Disease extent on HRCT 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. FVC = forced vital capacity; HRCT = high resolution-computed tomography; NSIP = nonspecific interstitial pneumonia

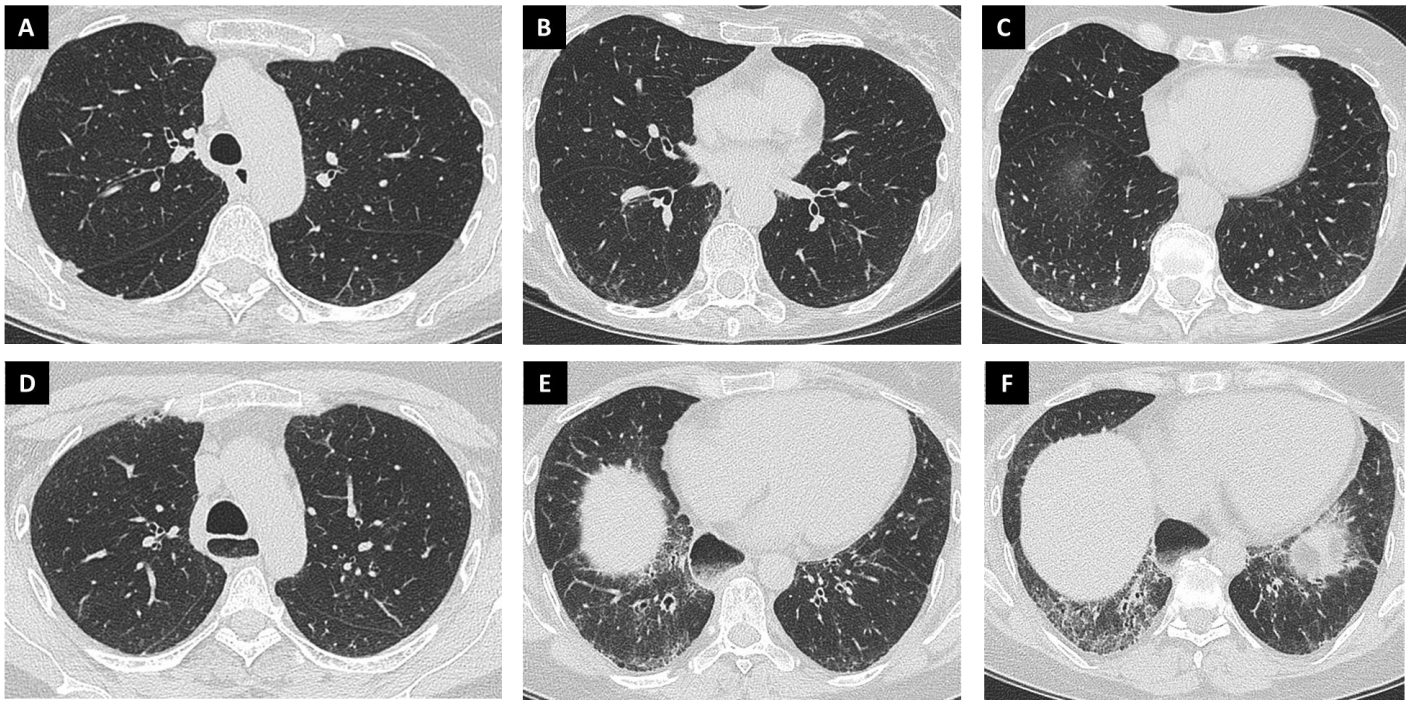


Figure 3. HRCT images in a 58-year-old female with systemic sclerosis, who never smoked; DLco 32% predicted and FVC 76% predicted. Axial images at (A) the level of the aortic arch, (B) the carina and (C) the lower lobes demonstrating extensive disease (>20% extent by visual estimation) and (D) coronal reconstruction. There is marked honeycombing, particularly in the lower lobes, indicating a UIP pattern. The coronal image shows striking lower zone preponderance of disease. FVC = forced vital capacity; HRCT = high-resolution computed tomography; DLco = diffusing capacity of the lung for carbon monoxide; UIP = usual interstitial pneumonia

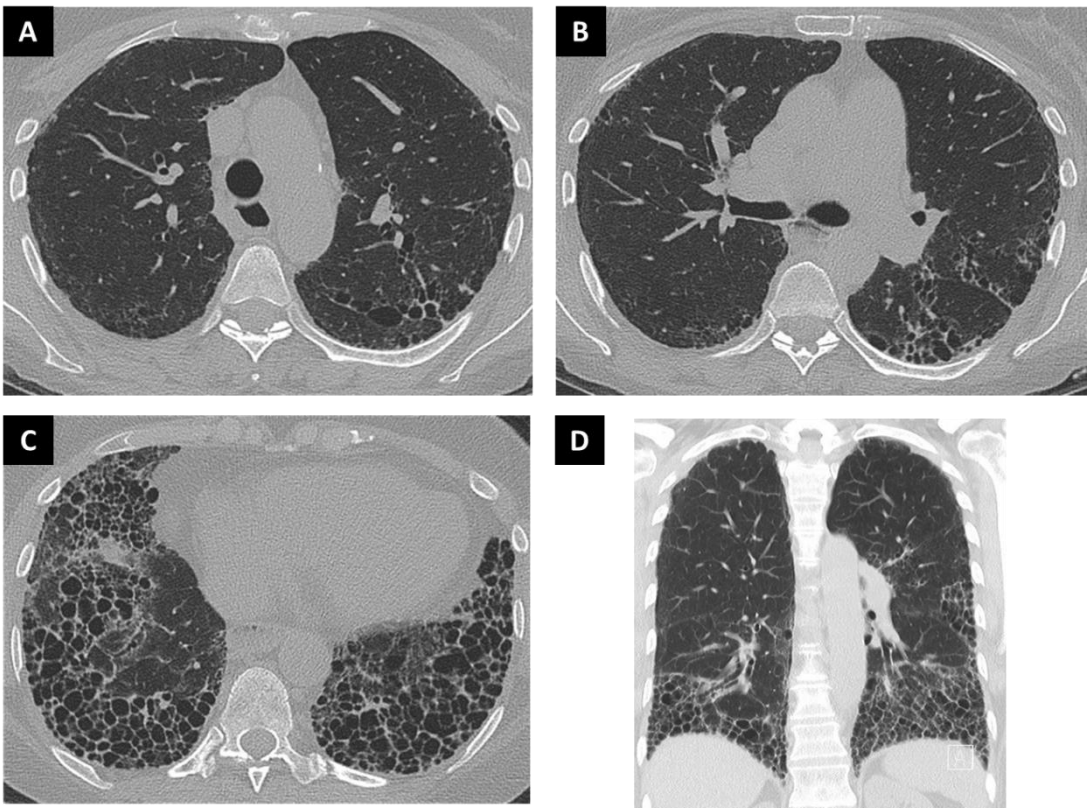


Figure 4. CT in 52-year-old male, ex-smoker with a DLco of 22% and FVC 56% predicted. Axial images at (A) the level of the arch, (B) the pulmonary venous confluence and (C) the costophrenic recesses showing extensive (>20%) disease. There is predominant ground-glass opacification with fine reticulation, no honeycombing but severe traction bronchiectasis. The CT features are consistent with a fibrotic NSIP pattern. Note also the marked esophageal dilatation. DLco = diffusing capacity for carbon monoxide.

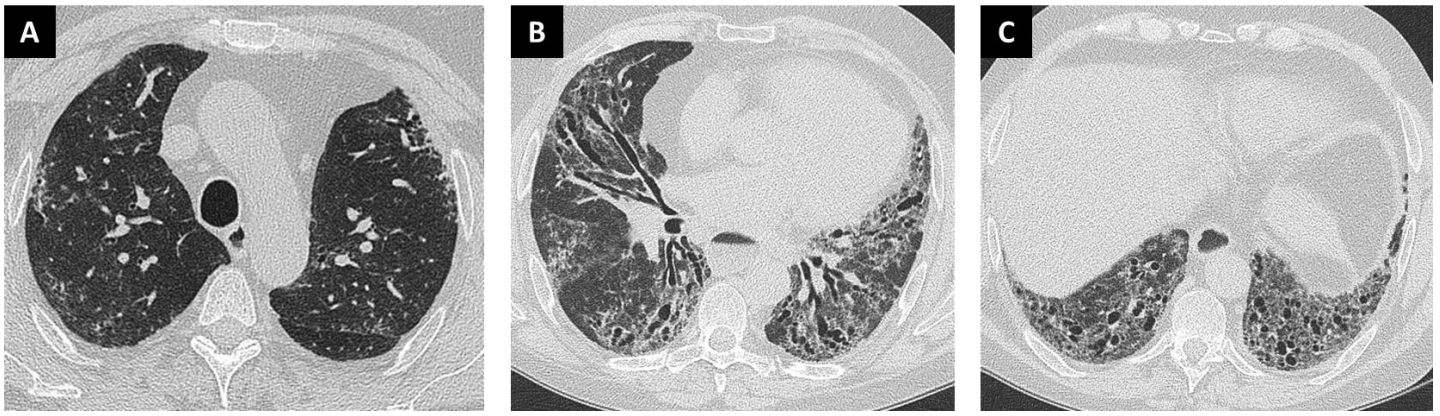
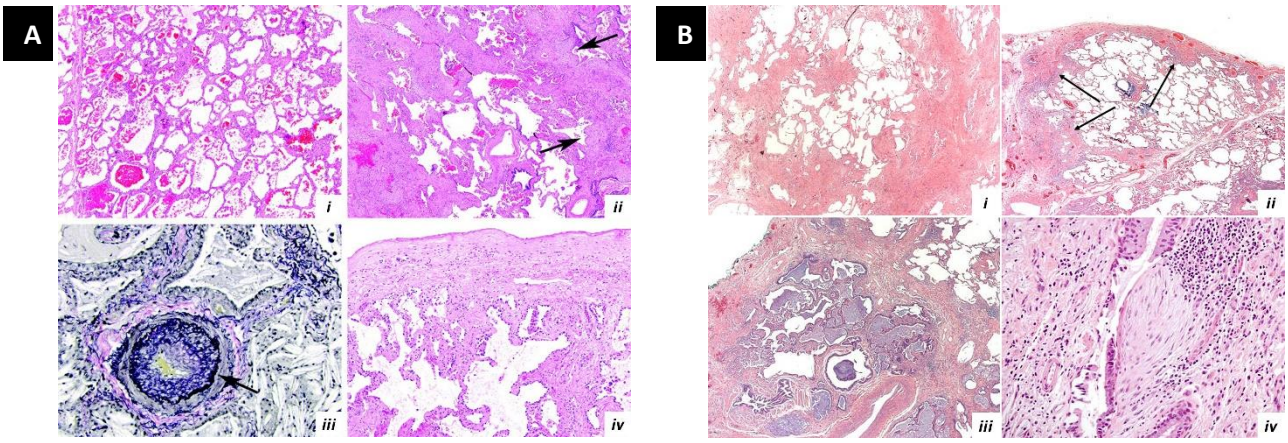


Figure 5. Histopathology of systemic sclerosis-associated interstitial lung disease (SSc-ILD) and idiopathic pulmonary fibrosis (IPF) (13, 68). Reproduced with kind permission of (*A*) Cavazza A, et al. *Respir Med* 2010, and (*B*) Herzog EL, et al. *Arthritis Rheumatol* 2014.

(*A*) SSc-ILD. *i*, 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. *ii*, UIP; note the peripheral involvement of a pulmonary lobule sparing the centrilobular area containing the broncho-vascular bundle. Arrows indicate fibroblastic foci. *iii*, 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. *iv*, Pleural fibrosis; its presence supports the diagnosis of SSc-associated ILD in the appropriate clinical setting. Hematoxylin and eosin stained sections are shown in *i*, *ii*, and *iv*; Verhoeff-van Gieson stained sections in *iii*. Original magnification × 40 in *i* and *ii*; × 200 in *iii*; × 100 in *iv*. (*B*) UIP. *i*) 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. *ii*) The fibrosis frequently prevails at the periphery of the lobule in the subpleurale-paraseptal regions (arrows), with relative sparing of the centrolobule. This is a useful diagnostic clue, particularly in early cases like here (haematoxylineeosin 20). *iii*) 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. *iv*) 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 hyperplasic pneumocytes. Hematoxylin and eosin stained sections are shown in *i*, *ii*, *iii* and *iv*. Original magnification × 20 in *i* and *ii*; × 20 in *iii*; × 100 in *iv*. UIP = usual interstitial pneumonia.


A
i
ii
iii
iv
B
i
ii
iii
iv