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Scleroderma (Systemic Sclerosis) (S Bhattacharyya, Section Editor)

Biomarkers in Systemic Sclerosis Associated Interstitial Lung Disease (SSc-ILD)

Alice Cole[1]
Christopher P. Denton[1,*]

Address
[*,1]Centre for Rheumatology, UCL Medical School, Royal Free Campus, London NW3 2QG, UK
Email: c.denton@ucl.ac.uk

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Abstract

Purpose of Review Interstitial lung disease (ILD) is the leading cause of mortality in systemic sclerosis, a rare autoimmune disease characterised by fibrosis and vasculopathy. The variety of phenotypes in SSc-ILD have inspired multiple studies aimed at the identification of biomarkers which can provide disease-specific information but due to the complex pathogenesis of SSc-ILD, it has been challenging to validate such markers. We provide a comprehensive update on those most studied along with emerging biomarkers.

Recent Findings We review the up-to-date findings with regard to the use of well-studied molecular biomarkers in SSc-ILD along with novel biomarkers offering promise as prognostic markers such as IGFBP-2 and IGFBP-7, the adipokine CTRP9, endothelial progenitor cells, and cellular markers such as $CD21^{lo/neg}$ B cells. Expression profiling data is being used in SSc patients to determine genetic and epigenetic clusters which shed further light on mechanisms involved in the pathogenesis of SSc-ILD and are likely to uncover novel biomarkers.

Summary With the exception of autoantibodies, there are no routinely measured biomarkers in SSc-ILD and reliable validation of the many potential biomarkers is lacking. Identifying biomarkers which can offer diagnostic and prognostic certainty may help patients to receive preventative treatment as part of a personalised medicine approach.

Introduction

Systemic sclerosis (SSc) is a rare, heterogeneous autoimmune disease which affects 3 to 24 per 100,000 globally. With regard to disease-specific pathology, the hallmarks of SSc are skin fibrosis, vasculopathy, and Raynaud's phenomenon. SSc also involves organs such as the lung, heart, renal system, and gastrointestinal tract [1]. The extent of organ involvement varies widely and is influenced by the primary autoantibody signature of the disease [2••].

Interstitial lung disease (ILD) affects 80% of patients with SSc [1]. A proportion of those with ILD will maintain stable disease with little progression; however, 25–30% will go on to develop progressive ILD [1]. ILD remains the leading cause of mortality amongst the population with SSc, accounting for 35% of deaths [3]. This clearly demonstrates that there is much to be gained from examining the mechanisms by which SSc-ILD becomes progressive in order to improve patient outcome.

In this article, we will review the pathogenesis of SSc-ILD with a focus on emerging biomarkers of SSc-ILD. We will also discuss recent advances in therapies including the recently licensed antifibrotic medication, nintedanib.

A biomarker in terms of SSc-ILD could include genetic polymorphism or biochemical molecules that can be identified in either serum or BALF or skin of the individual. This could be used alongside recognised markers of disease such as forced vital capacity (FVC) or extent of disease on HRCT and ideally input into an algorithm to facilitate prompt recognition of prognosis and suitable treatment.

Multiple biomarkers exist which represents the complex pathogenesis of diseases such as SSc; however, a useful exercise would be to identify those biomarkers which can further define the disease subsets and ultimately provide prognostic information which might in turn affect treatment options.

Epidemiology

EUSTAR database studying 6004 in prospective analysis observed progression of ILD in 23–27% of the cohort [4]. A large retrospective observational study found that in terms of variation between ethnic groups, lung fibrosis was more prevalent amongst Afro-Caribbean patients with SSc (31% vs. 53%, $p = 0.007$) [5]. A study examining sex differences in SSc also found that male patients with SSc are at increased risk of ILD (52% vs. 39%, $p < 0.0001$) which is also more likely to be severe [6].

The incidence of SSc-ILD development is highest within the first 5 years following diagnosis with reducing incidence thereafter [2••]. Those with diffuse disease are also at increased risk compared to those with limited disease.

Unlike its counterpart idiopathic pulmonary fibrosis which results in inevitable progression of fibrosis, SSc-ILD can follow multiple trajectories. A proportion of patients (10–20%) will never be diagnosed with ILD or it will be described at < 5% on HRCT without any impact on the pulmonary function tests. Some patients develop limited disease which is < 20% extent on HRCT but from this starting point, a proportion remain stable with minimal progression of their ILD and the remainder of the group develop a progressive disease with high mortality.

Whilst the detection of ILD has improved with the availability of HRCT and the advent of surveillance pulmonary function tests (PFTs) at regular interval, the anticipation of progressive disease remains a challenge. It has been shown that a meaningful decline in lung function is predictive of

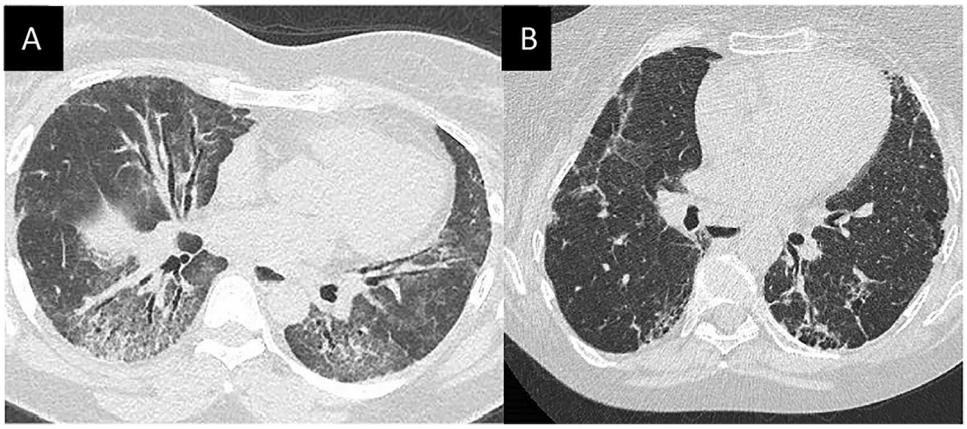

Fig. 1 **A** Extensive disease on high resolution computed tomography (HRCT), measured just above the right hemidiaphragm, the lowest of the 5 levels involved in the Goh staging. **B** Less frequently observed usual interstitial pneumonia (UIP)

mortality [7]. In practice, this is measured as FVC > 10% decline, or 5–9% decline with an associated 15% diffusing capacity for carbon monoxide (D_{LCO}) decline. By this point of detection, irreversible lung damage is likely to have occurred.

Multiple histopathological patterns of ILD are described, and these patterns are seen across other conditions such as idiopathic pulmonary fibrosis. Non-specific interstitial pneumonia is the most common pattern observed and describes temporally uniform, diffuse patchy inflammatory change which is most commonly fibrotic as opposed to cellular [8] (Fig. 1). This differs from IPF, in which the common histological pattern seen is usual interstitial pneumonia (UIP) pattern. UIP is observed in SSc-ILD, but does not confer a worse prognosis, and indeed, outcomes are more tightly linked to severity at diagnosis and serial reduction in D_{LCO} than the histopathological group [9].

Cycle of Fibrosis

Pathogenesis

The overarching process leading to SSC-ILD is believed to be altered cellular biology as a result of repeat epithelial injury leading to architectural distortion and extracellular matrix (ECM) deposition.

The first step in this process is repeat injury to alveolar epithelial cells (AEC) which activates both the innate and adaptive immune response causing an influx of inflammatory medicators which results in recruitment of fibroblasts and transformation into myofibroblasts [10]. Myofibroblast cells play an important part in SSc biology, they can arise from resident fibroblasts or epithelial mesenchymal transition and are characterised by highly expressed anti-apoptotic mediators and deposit large amounts of ECM. In response to these inflammatory changes, abhorrent healing processes occur forcing

some epithelial cells to undergo apoptosis whereas a proportion are believe to undergo epithelial-mesenchymal transition (EMT) [11].

EMT is a phenomenon also harnessed by metastatic malignant cells. In the context of AEC and fibrotic disease, it is the process by which epithelial cells gain mesenchymal function including that of increased resistance to apoptosis, increased migratory function, and increase production of ECM in response to medicators including transforming growth factor-beta (TGF-β) [11]. EMT is recognised as a response to injury in many adult tissues including lung, kidney, and eye but has been observed in SSc-ILD specifically and has also been observed in bleomycin mouse models [12]. This process may allow well differentiated AEC to lose polarity and give rise to fibroblasts and myofibroblasts, driving the fibrotic process following epithelial injury. EMT results in the loss of epithelial cell markers and gain of mesenchymal cell markers including matrix metalloproteins (MMPs) [11]. MMPs are likely to play a central role in various aspects of ILD along with EMT, including ECM remodelling and basement membrane breakdown along with playing a role in proteinase cascades [13].

Fibroblasts also play a key role in driving fibrosis in the lung. Fibrosis is initiated by activation of resident fibroblasts. Under circumstances of normal wound healing, the activated fibroblasts are at some points deactivated or undergo apoptosis; however, in SSc, there is persistent activation and production of ECM and growth factors such as fibroblast growth factor and connective tissue growth factor (CTGF:CCN2) [14]. Fibroblasts comprise a large part of the connective tissue, they deposit ECM proteins including collagen types I and III. Fibroblasts regulate the deposition of ECM by producing MMP and their inhibitors tissue inhibitors of metalloproteinases (TIMPs) [14]. Fibroblasts can be activated by multiple mechanisms including activation of tissue resident cells, EMT, endothelial-mesenchymal transition, pericyte to mesenchymal transition, and smooth muscle cell differentiation [14]. Persistent production of these myofibroblasts allows then to congregate leading to overproduction of the ECM, the hallmark feature of SSc. Along with fibroblasts, there is also influx of other immune cells including B and T cells which express inflammatory cytokines, the Th2 T-cell plays a role in driving fibrotic response [15].

Fibroblasts are a heterogeneous group and can display various phenotypes of which some are more pro-fibrotic. The response displayed by a fibroblast varies depending on the stimulus the cell receives. Alongside this, SSc-ILD fibroblasts have been shown to behave differently to healthy populations by expressing anti-apoptotic protein B-cell lymphoma-2 (Bcl-2) in response to interleukin-6 (IL-6) [16], whereas the healthy fibroblasts displayed pro-apoptotic Bcl-2-associated X protein (BAX) (Fig. 2).

TGF-β is a well-studied participant in the pathogenesis of lung fibrosis. It exerts its effects by binding to its cell surface receptor and produce downstream activation of the transcription activator SMAD4 which leads to increase production of ECM such as collagen, plasminogen activator inhibitor-1, and CTGF: CCN2 [11]. In addition to cell surface binding, TGF-β also influences lymphocyte proliferation and supresses anti-inflammatory cytokines [10]. Forming a pro-fibrotic cycle, the injured epithelial cells recruit immune cells including macrophages which in turn release more TGF-β [17]. In vivo,

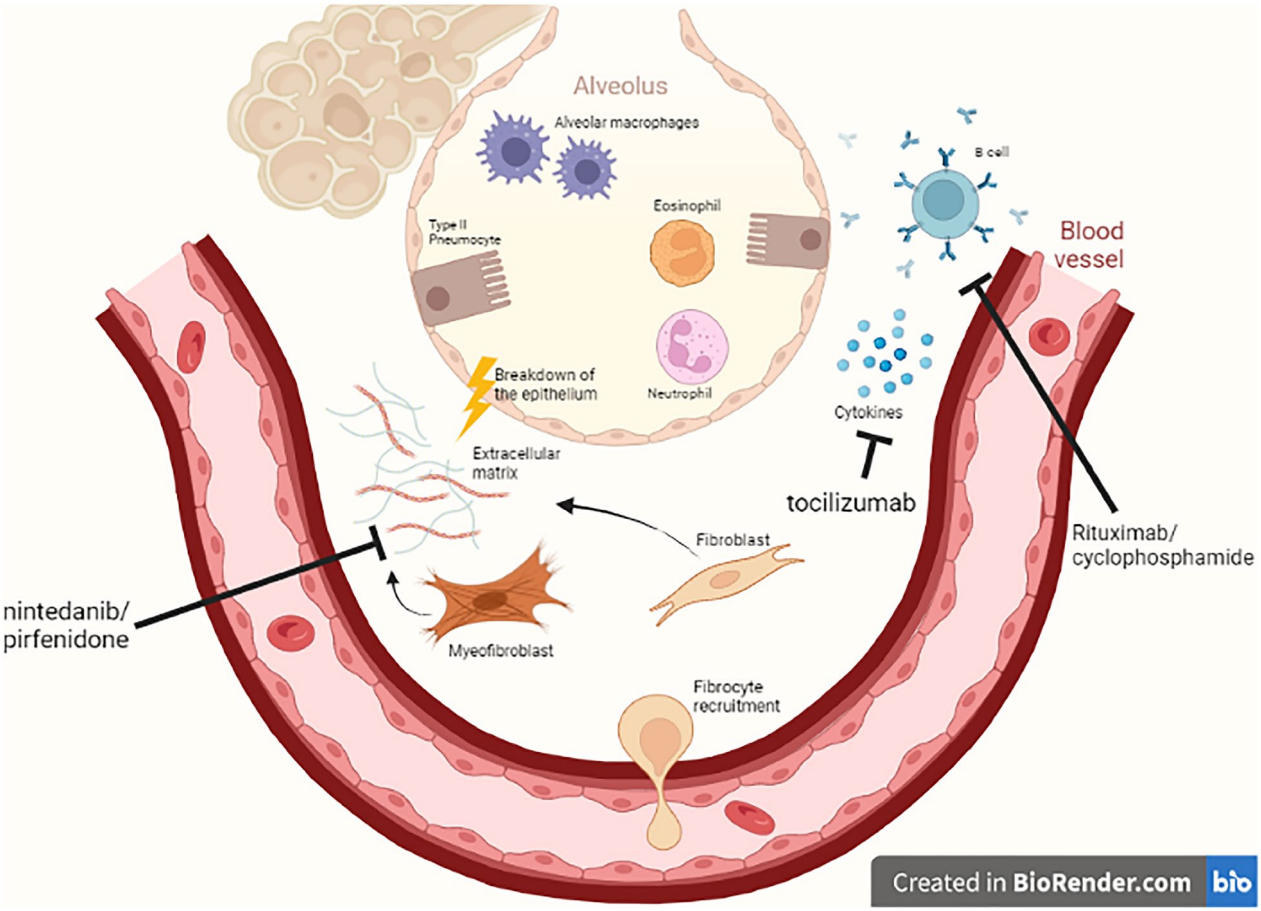

Fig. 2 Pathogenesis of SSc-ILD and the various therapeutic targets

deletion of TGF-β was found to be protective against bleomycin-induced pulmonary fibrosis [18].

Epithelial injury is also recognised in the pathogenesis of SSc-ILD. Increased 99mTc-DTPA clearance, a marker of epithelial injury, has been shown within the SSc-ILD cohort to predict a shorter time to FVC decline [19].

Thrombin levels have also been found to be significantly raised in SSc-ILD compared to healthy controls when measured at bronchiolar lavage (BAL) [20]. Thrombin increases fibroblast proliferation including the apoptosis-resistant myofibroblast lineage [21]. Thrombin also induced a variety of pro-fibrotic cytokines including TGF-β and ECM proteins [10]. Thrombin acts through the g-coupled receptor PAR-1 which is upregulated in SSc-ILD patients.

The Wnt pathway occurs in AEC type-2 cells which are activated during epithelial injury along with AEC type-1 apoptosis. The Wnt pathways act to upregulate WISP1 which in turn leads to increased induction of pro-fibrotic cytokines such as SPP1, MMP-7, MMP-9, and PAI-1 from AEC [22].

Risk Factors for SSc-ILD

Disease stratification is extremely important so that those with likely indolent disease can avoid chemo-toxic drugs and those with multiple risk factors for progression or high levels of established biomarkers can receive appropriate therapy at an early juncture.

The EUSTAR database which followed up 6004 European patients with SSC-ILD over a 5-year period identified male sex, high modified Rodnan Skin Score (mRSS), and presences of reflux as independent risk factors for progressive ILD [4] in patients who had 3 or more serial PFT readings over the 5-year follow up period [4].

Other risk factors for ILD also reported in large retrospective studies include older age, male sex, extent of disease on HRCT, lower FVC, and D_{LCO} [23] (Table 1).

Perhaps the most widely used determinator of risk for SSC-ILD used is the autoantibody profile of a patient. It is well established that anti topoisomerase (ATA/Scl-70) antibodies confer an increased risk of ILD, and a large retrospect cohort analyses by Niytanova et al. found that 80.3% of ATA positive patients had developed meaningful ILD after 5 years. This study elegantly demonstrated that anti-Scl-70 positivity was strongly predictive of development of ILD, independent of skin involvement (LcSSc 86.1% and DcSSc 84% at 15 years). Interestingly, the presence of ACA antibodies reduced the hazard of developing SSC-ILD significantly compared to other antibody types (hazard ratio 0.048 when compared to ATA) [2••].

Whilst it is clinically helpful to identify those patients at risk of progressive SSc-ILD, the risk factors do not offer a reliable prediction of mortality. Several models including the composite physiological index, the du Bois, and modified du Bois index have been reported to help predict mortality with the modified du Bois showing good discrimination in prediction of 1 year mortality in SSc-ILD [25].

Genome wide studies (GWAS) have identified loci associated with SSc development with specific HLA alleles conferring risk of pulmonary fibrosis in small studies (HLA-B*62 and HLA-Cw*0602) [26]. Multiple epigenetic mechanisms have been implicated which may be affected

Table 1 Clinical risk factors for ILD and progression of ILD

Clinical risk factors at ILD diagnosis and progression of ILD [24••]	Additional risk factors for progression of ILD [4]
DcSSc	Reflux
Anti topoisomerase positive	High modified Rodnan Skin Score (mRSS)
Male Afro-Caribbean	Male sex
Lower FVC	Older age at onset
Lower D_{Lco}	Raised ESR
Extent of disease on HRCT	

by environmental factors. DNA methylation analysis of SSc fibroblasts methyl-CpG-binding domain protein 2 (MBD2) mediates fibrosis via polarisation of M2 macrophages and intratracheal administration of MBD2 small interfering RNA loaded liposomes siRNA protected mice from BLM-induced lung injuries and fibrosis [27]. Histone deacetyl transferases (HDACs) have been implicated in fibrosis, interestingly either pro- or antifibrotic depending on the subgroup [28, 29]. Non-coding RNAs have also been implicated in the development SSc [30••]. Genome studies in China using the Gene Expression Omnibus (GEO) have formed a competing endogenous RNA (ceRNA) network which enable the identification of three core subnetworks (SNHG16, LIN01128, RP11-834C11.4(LINC02381)/hsa-let-7f-5p/IL6, LINC01128/has-miR-21-5p/PTX3, and LINC00665/hsa-miR-155-5p/PLS1) which are involved in immune regulation and proliferation of some cancers. The identification of these networks could lead to further novel biomarker identification [31].

Biomarkers of Disease

A useful biomarker should be easily measurable, widely applicable, and offer diagnostic and/or prognostic information about a disease. Due to the complex processes and triggers involved not only in the development of SSc but also SSc-ILD, a single and widely applicable biomarker has proved difficult to elicit, and there is currently no validated biomarker in SSc-ILD.

In order to obtain biologic sample representative of SSc-ILD, logic dictates that it might be preferable to obtain this from the lung; however, this raises the issue of multiple invasive tests. Lung biopsies are now rarely performed in patients with scleroderma so information regarding local inflammation comes from animal studies [32]. BALF again is also not routinely used to stage SSc-ILD but if found to show distinct information from serum with regard to prognostic indicators, this could change. At present, studies have shown that the neutrophilia observed is related to disease activity but not specific to lung involvement hence not routinely used in practice [24••]. Small prospective studies comparing the induced sputum and serum of SSc and SSc-ILD patients did see increased levels of markers known to be associated with inflammation and fibrosis (IGFBP-1, TGF-β, IL-8, YKL-40, and MMP-7) compared to healthy controls but there was no significant difference between the SSc groups [33], indicating this is not a sensitive method of detecting biomarkers for SSc-ILD.

ATA antibodies, whilst strongly associated with SSc-ILD, cannot serve as an independent biomarker. Multiple proteins involved in SSc pathogenesis have been studied, many showing clear associations with the presence of ILD but large-scale studies demonstrating diagnostic and prognostic power are lacking. Amongst the most studied are Krebs von Lungen-6, surfactant protein-D, and chemokine ligand-18 but other candidate markers have shown response to therapy in more recent studies as described below.

Alveolar Epithelial Injury

Krebs von den Lungen-6 (KL-6)

KL-6 is a transmembrane mucoprotein which is secreted by injured type-2 alveolar cells [19, 34]. KL-6 has been investigated as a potential biomarker in multiple studies and found to correlate with extent of fibrosis on HRCT and was negatively correlated with FVC and D_{LCO} indicating that it reflects the extent of SSc-ILD [35, 36]. A Chinese study recording KL-6 levels in CTD-associated ILD found that KL-6 levels did reduce in those patients who saw improvement in the extent of their disease after cyclophosphamide [37].

In Japan, where KL-6 levels can be routinely measured, a prospective study of 110 SSc-ILD patients found that KL-6 levels did correlate with extensive disease but there was no correlations between the trend of KL-6 in the 6 months following diagnosis and ILD progression in the first 2 years after diagnosis [38].

Patient enrolled in the Scleroderma Lung Study (SLS) II had serum KL-6 and CCL18 levels measured at baseline and 12 months, both patients receiving cyclophosphamide and MMF showed reduced levels in response the treatment but importantly, those with higher baselines levels were more likely to experience progressive ILD, indicating a role for KL-6 as a prognostic biomarker for progressive disease. Recent studies combining retrospective and prospective data and using linear mixed effect models to compare change over time have continued to offer good evidence that KL-6 is predictive of decline in D_{LCO} in both mild and severe diseases [39•].

Surfactant Protein-D (SPD)

SPD is released by alveolar type-2 pneumocytes to reduce surface tension over alveoli and prevent airway collapse. A cohort study by the Scleroderma Lung Study Research Group found that SPD levels were highly sensitive (97%) for ILD, but less specific (69%) [40] making SDP a relevant biomarker for ILD diagnosis (OR 3.15, 95% CI 1.81–5.48 [$p<0.001$]) but it does not predict disease progression [40].

Platelet Factor 4 (CXCL4)

The chemokine CXCL4 is a chemotactic agent and increases expression of profibrotic cytokines whilst also suppressing interferon-γ. CXCL4 was measured as part of the SLS II which found that despite a lack or correlation with ILD at baseline, change in CXCL4 at 12 months was predictive of progression in ILD at 12–24 months. A reduction in CXCL4 was also observed in response to immunosuppression therapy [41•].

Carbohydrate-antigen 125 (CA-125)

CA-125 is the most widely used biomarker in ovarian cancer, mainly with regard to response to chemotherapy and prognosis [42]. It also has potential as a

biomarker of epithelial injury, originally recognised in IPF [43]. In the SENSCIS trial, CA-125 demonstrated a fold decrease in the nintedanib arm, compared to placebo [44•]. Carbohydrate antigen 15.3 (CA 15.3), another marker of epithelial damage which is encoded by the same MUC1 gene which encodes KL-6, has been shown to correlate with SSc-ILD severity and when used in conjunction with HRCT had prognostic significance [45].

Immune Mediated Damage or Response to Injury

Interleukin-6

The acute phase inflammatory cytokine, IL-6, is recognised to play a role in the pathogenesis of SSc likely via its effects on both the janus kinase signal transducer of transcription factor 3 (JAK STAT3) pathway and AK-SH2 domain tyrosine phosphatase 2 (SHP2)-mitogen-activated protein (MAP) kinase pathway [46, 47]. In an exploratory cohort of SSc-ILD and IPF patients, IL-6 was found to be independently predictive of D_{Lco} decline and predictive of mortality in cases of mild ILD within the first year of diagnosis. The level of IL-6 is also closely correlated to CRP levels [48]. The Canadian Scleroderma research group found raised CRP levels were associated with early disease, DcSSc, and worse pulmonary function parameters (total lung capacity < 80% predicted) [49]. Multiple factors, including infection, can cause an elevated CRP and it is only raised in one-quarter of SSc patients. These properties reduce its strength as a candidate biomarker; however, it remains a useful tool for identifying patients with an inflammatory phenotype that may respond well to therapies such as anti-IL-6.

Chemokine Ligand 1/Fractaline (CX3CL1)

CX3CL1 has shown potential as a biomarker and therapeutic target in recent studies. CX3CL is a chemokine with a unique receptor which can be expressed on multiple immune cells. A large cohort study combining two independent cohorts from Norway and California explored CX3CL in the serum and lung tissue of SSc patients with matched control. Serum levels of CX3CL correlated with D_{LCO} and lung fibrosis. Immunostaining of SSc-ILD lungs demonstrated CX3CL is expressed from epithelium and infiltrating interstitial leucocytes whereas its receptor CX3CR1 is expressed on infiltrating interstitial monocytes, particularly plasma cells. Smaller studies which preceded this work, however, had not found a clear association between CX3CL and decline in lung function [46].

Aberrant Fibrogenesis and Matrix Remodelling

Chemokine Ligand-18 (CCL18)

CCL18, formerly known as pulmonary and activated-regulation chemokine (PARC), is associated with M2-macrophage cells and is directly related to

pulmonary inflammation, and high levels have been identified in the serum and BAL of SSc-ILD patients [50•, 51, 52]. A Scleroderma Lung Study Research Group cohort study also found that CCL18 levels were independently predictive of a > 10% decline in FVC and for de novo development of extensive disease [40]. After 3 months of treatment with tocilizumab in the faSSinate trial, there was a significant reduction in CCL18 levels compare to the placebo arm, and this reduction was sustained over the 18-month trial period [53].

Matrix Metalloproteinase-9 (MMP-9)

MMP-9 (C3M) and propeptide of type VI collagen (pro-C6), both markers of ECM turnover, demonstrated fold change decreases in the arms treated with the antifibrotic nintedanib as part of the SENSCIS trial [44•]. Similar trends in biomarkers of ILD in non-IPF cases were observed in the INBUILD study [43].

Matrix Metalloproteinase-7 (MMP-7)

MMP-7 is another proteolytic enzyme which plays a role in regulating the turnover of ECM and has been shown to be significantly elevated in SSc and significantly associated with ILD [54]. A study exploring the approach of induced sputum to compare components in SSc patients vs. healthy control found that MMP-7 levels were increased but there was no difference between SSc and SSc-ILD groups [33]. MMP-12 has also been found at increased concentrations in sera, skin, and lung biopsies in SSc-ILD compared to healthy controls [55].

Emerging Biomarkers

Discussed above are some of the biomarkers which have been most scrutinised for their potential as biomarkers for SSc-ILD. There are numerous other pathways and proteins which remain under investigation.

Proteomic analysis of bronchiolar lavage fluid (BAF) in 7 patients with UIP SSc-ILD has provided fresh insight to local proteostasis. Increased protein expression in SSc-ILD BAF, particularly mannose receptor C1 (MRC1), suggests increased activity of type-2 macrophages which are involved in MMP9 mediated tissue repair and fibrosis [56•]. This analysis also identified novel potential biomarkers C3a, apolipoprotein AI (APOAI), protein S100A6, and protein 14–3-3e which affects regulation of the pulmonary surfactant-associated proteinA2 (SPFA2) gene which together may play a role in inflammatory IL-6 signalling [56•].

Altered adipose tissue metabolism has more recently been recognised as a pathogenic pathway to fibrosis in SSc and previous cross-sectional studies have demonstrated that specific adipokines are associated with SSc-ILD [57•]. Preliminary retrospective work inspecting the adipokine C1q/TNF-related protein 9 (CTRP9) found that high levels are associated with SSC-ILD whereas low levels are associated with pulmonary stability [58], whether this can offer more specific prognostic information will require further investigation.

Insulin-like growth factor binding protein-2 (IGFBP-2) has recently been shown to directly correlate with low KCO (< 70% predicted) after 2 years

in a cohort study comparing SSc both with and without ILD [59]. IGFBPs are transport proteins for IGF, and increased levels in SSc-ILD could indicate increase availability of IGF which could further contribute towards the fibrotic process. A genomic study using a weighted correlation network analysis method was able to identify key module and hub genes associated with SSc and found that IGFBP-7 is upregulated in SSc-ILD patients [60].

As discussed above, a key step in the pathogenesis of SSc-ILD is endothelial injury and dysfunctional repair. It is possible to count the number of endothelial progenitor cells which can then be used as a surrogate for endothelial regeneration or repair. In a small study, the frequency of EPCs was significantly higher in SSC-ILD patients compared to healthy controls and SSc patient with no ILD. EPC frequency was also higher in early compared to late disease [61].

Measuring dysregulated proteins as biomarkers may offer useful clinical information in SSc-ILD but it does not necessarily represent the cellular mechanisms or abnormality which is driving the process. To this end, various cell lines including monocytes and T cells have been investigated. A recently published study looking at B cells found that auto-immune-disposed subset $CD21^{lo/neg}$ B cell measured from PBMCs were significantly increased in SSc-ILD patients compared to both healthy controls and SSc no ILD patients [62•].

The complement pathway has been implicated in the process of SSc-ILD in various studies. Through activation of the classical, lectin, and alternative pathways, C3a and C5a anaphylatoxins are produced which work to recruit inflammatory cells including neutrophils, mast cells, and monocytes. C3a and C5a are observed at elevated levels in IPF and interact with TGF-β in vitro to augment epithelial injury [63, 64]. The complement pathway has been implicated in other forms of lung damage such as emphysema related to alpha-1 anti-trypsin deficiency [65•] and smoking related lung damage [66]. Emphysema is an increasingly recognised pattern of lung damage in SSc-ILD patients so there may be mechanistic parallels to be drawn from these similar patterns of lung destruction.

Current Treatment Options

Patients may first present with ILD reporting exertional or non-exertional dyspnoea or cough but in many cases, the patient may be asymptomatic. Up-to-date consensus recommends baseline PFTs and HRCT to screen for ILD at the earliest opportunity. Pulmonary manifestations of SSc can affect the parenchyma, vasculature, or musculature so it is important to incorporate these diagnoses into the differential.

Patients with SSC-ILD to the extent which impacts on PFTs typically demonstrate a restrictive pattern with low FVC and a normal or increase FVC/FEV1 ratio. The D_{LCO} will typically be reduced, and the trend in FVC and D_{LCO} is a particularly useful tool when calculating the progression of ILD [24••].

As previously mentioned, there is no consensus on thresholds for treatment in SSc-ILD. The 'Brompton UK-RSA' or 'Goh' classification can be utilised by clinicians during consultations to estimate the extent of fibrosis. CT imaging is assessed at 5 levels based on extent of grounds glass,

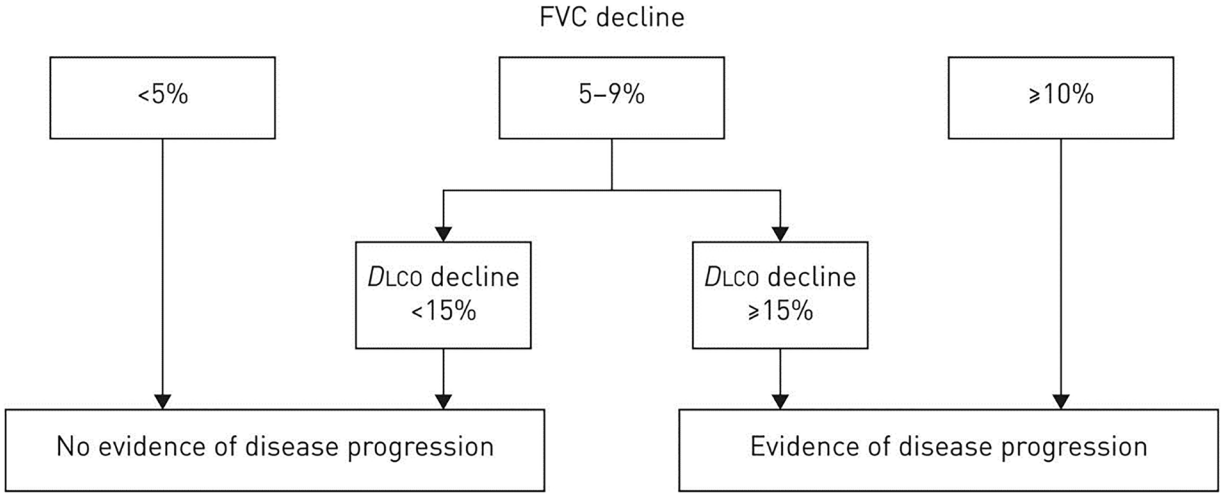

Fig. 3 Proposed algorithm to define a meaningful progression of SSc-ILD. Reproduced with kind permission from Distler et al. [24••]

reticulation, and cystic change. The resulting measure of extent defines the patient as limited (clearly < 20%), extensive (clearly > 20%), or indeterminate. Indeterminate cases are sorted into the limited or extensive category using the FVC threshold of 70% predicted [7].

An international panel of expert rheumatologists and pulmonologists collaborated to propose the following guidance to identify the change in PFT that correlates with disease progression [24••] (Fig. 3).

Once SSc-ILD has been confirmed and deemed clinically significant, several immunosuppressive therapies exist in the clinician's armamentarium.

Disease-modifying Anti-rheumatic Drugs (DMARDs)

DMARDs have been favoured as a first-line option for many years for a number of reasons including the route of delivery, cost, and side effect profile. They are effective in treating ILD, in fact the SLS II study demonstrated a significant improvement in FVC% after treatment with either mycophenolate or oral cyclophosphamide, the groups were not significantly different, but the mycophenolate was better tolerated [67]. A pooled analysis of the SLS I and II trials allowed an estimation for minimally important clinical difference (MCID) in FVC of FVC% improvement ranged from 3.0 to 5.3% and for worsening from – 3.0 to – 3.3% which will be helpful when determining treatment effects in future trials [68]. When applying the FVC MCID to results from the SENSCIS trial receiving either nintedanib or placebo, 34.5% and 43.8% had a decrease in FVC of ≥ 3.3% predicted (proposed MCID for reduction of FVC), and 23.0% and 14.9% had an increase in FVC of ≥ 3.0% predicted (proposed MCID for improvement in FVC) [69].

Nintedanib

The SENSCIS trial for nintedanib met its primary endpoint, showing a reduction in the rate of progression of ILD. As of late 2021, nintedanib has been licensed in the UK for use in SSc-ILD demonstrating progression despite immunosuppression by the National Institute for Health and Care Excellence (NICE) [70] and can be used safely in combination with MMF [71].

The latest analysis of the SENSCIS data focussed on the reduction in decline in FVC (mL/year) in patients with risk factors for progressive ILD. Patients receiving nintedanib with elevated CRP (>6 mg/L) or thrombophilia (>330 × 10^9/L) saw a difference of 52.5 mL/year in rate of decline in the nintedanib arm. A reduction in rate of FVC decline was also seen in patients with DcSSc and those with high mRSS (>15) [72••]. The change in FVC for the nintedanib was also sustained, as observed in the SENSCIS-ON open arm extension trial [73]. Patients in these high-risk groups might typically receive immunosuppression but would not normally be treated with nintedanib, particularly early in their disease course. This analysis demonstrates that the addition of antifibrotic therapy can salvage FVC decline which may lead to better long-term outcomes.

Rituximab and Cyclophosphamide

Findings from the DESIRES trial suggest that rituximab is an effective therapy for reducing fibrosis particularly in the skin and in ILD, administered at 375 mg/m^2 weekly for 4 weeks [74••]. In double-blind, randomised study, FVC improved in rituximab group vs. placebo (0.09% increase versus a 2.87% decrease), inflammatory ground glass findings on HRCT also reduced in the rituximab group compared to placebo (0.32% reduction in ILD areas on CT compared with a 2.39% increase in the placebo group). D_{LCO} decreased in both groups during follow up with no statistical difference between groups (1.32% and 3.56% in rituximab and placebo) but the D_{LCO} in the rituximab group did stabilise from 4 weeks onwards [74••].

An open label randomised trial in India involving 60 patients with DcSSc saw an increase in FVC 61.30 (11.28) to 67.52 (13.59) in the rituximab arm compared to the cyclophosphamide arm 59.25 (12.96) to 58.06 (11.23) at 6 months [75]. There were also more adverse reactions in the cyclophosphamide arm including pneumonia and herpes zoster. A Russian open label prospective study involving 107 patients with SSc-ILD over a period of 13 months found an increase in FVC in each treatment arm (cyclophosphamide $p = 0.034$ and rituximab 0.000045) and again, the therapy was better tolerated in the rituximab arm [76].

Retrospective comparisons have also been conducted [77], and these, along with the studies outlined above, have shown efficacy of both cyclophosphamide and rituximab therapy, and head to head randomised control trials such as the RECITAL trial [78] are needed to help inform decision-making for treatment in SSc-ILD.

Tocilizumab (TCZ)

The FocuSSed trial, a large phase III randomised control trial comparing TCZ (anti-IL-6) to placebo in combination with its predecessor, FaSSinate, demonstrated a clear stabilisation in lung function particularly within the DcSSc subgroup who have fibrosis present at treatment initiation [79, 80].

Transplant

Autologous stem cell transplant (SCT) has been studied in three randomised controlled trials which have demonstrated that they are an effective treatment stabilising pulmonary disease, improving lung volumes, and reducing the extent of ILD observed on HRCT. There is significant morbidity associated with undergoing SCT, and extensive ILD has been associated with poor outcomes [81•]. It has been suggested that the optimal patient groups for SCT are early diffuse patients with rapidly progressive disease who have not yet developed significant visceral involvement [82].

Solid organ transplant such as heart and lung transplant is a therapeutic option for a small number of patients with SSc-ILD. Unfortunately, transplant does not exclude recurrence, and gastro-oesophageal reflux presents a significant barrier to transplant for SSc patients.

Conclusion

At present in clinical practice, immunosuppressive treatment is initiated after fall in FVC which implies a certain degree of ILD has already occurred. By combining biomarkers of disease alongside risk factors such as male age, high mRSS, and reflux, there may be scope to develop a reliable algorithm by which allows targeted therapy to be initiated earlier in these individuals with the goal to prevent rather than stabilise ILD.

The introduction of platforms such as GWAS, proteomics, and metabolomics is deepening our understanding of the pathophysiology and architecture of systemic sclerosis. With these developments, a breadth of candidate biomarkers are being studied but the challenge lies in finding readily measurable biomarker which offer specific diagnostic and prognostic value above that of PFTs and imaging. The numerous environmental triggers and epigenetic mechanisms involved in the pathogenesis of SSc make finding a single biomarker which can accurately represent SSc-ILD a further challenge. Large randomised controlled trials which have facilitated new licensed treatments in SSc-ILD have also offered valuable insight into the response of candidate biomarkers but further large-scale studies focussing on biomarkers are needed to validate these in order to incorporate them into routine disease stratification.

Compliance with Ethical Standards

Conflict of Interest
Alice Cole declares that she has no conflict of interest. Christopher P. Denton declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent
This article does not contain any studies with human or animal subjects performed by any of the authors.

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•• Of major importance

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