

Interstitial lung disease in systemic sclerosis

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ABSTRACT: *Interstitial lung disease in systemic sclerosis. E.A. Renzoni.*

Systemic sclerosis (SSc) is a connective tissue disease characterised by fibrosis of the skin and internal organs, autoimmune abnormalities and widespread vasculopathy. A degree of interstitial lung involvement is present in the majority of patients, although clinically significant lung fibrosis is present in approximately a third. Autoantibodies are significant clinical markers; anti-topoisomerase is tightly linked to lung fibrosis, whereas anti-centromere antibodies are protective. Further evaluation of markers of progression of lung fibrosis, such as markers of epithelial permeability, will be crucial in clinical management. The clinical course of

SSc-associated interstitial lung disease is highly variable, with stability observed in a significant proportion of patients. Therefore, the decision of whether to treat is a challenging one, and should be based on evaluation of disease severity (on the basis of CT extent and lung function) and longitudinal disease behaviour. Two recently published placebo controlled randomized trials have shown a significant, if small, effect of cyclophosphamide on preventing FVC decline. However, because of the significant toxicity of cyclophosphamide, the assessment of alternative, less toxic, immunosuppressive agents for the long term management of SSc-associated interstitial lung disease is needed.

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Introduction

Systemic sclerosis (SSc) is a connective tissue disease characterised by autoimmune features, widespread vasculopathy and fibrosis of the skin and internal organs. The pattern of internal organ involvement and the natural history of the disease are highly variable. Although the etiology is unknown, most studies point to links between genetic predisposition and environmental factors. SSc has been reported to have a point prevalence between 71 per million and 158 per million in Europe, although prevalence varies widely according to ethnicity, method of case assessment and geographic area of study [1]. It predominantly affects women with a peak age of incidence between 30 and 50 [2]. The hallmark of SSc is skin involvement, which is usually accompanied by Raynaud's phenomenon. According to the extent of skin involvement, SSc patients are commonly classified into two distinct subsets, with significant prognostic indications: limited cutaneous SSc (lSSc), in which the skin lesions do not extend beyond the elbows and knees but may involve the face, and diffuse cutaneous disease (dcSSc), which also affects the thighs, arms, and torso. Patients with diffuse disease tend to have more rapid progression of their skin disease and are at higher risk of severe heart and kidney in-

volvement than lSSc [3]. However, lSSc patients are only at a slightly lower risk of severe interstitial lung disease than dcSSc [4].

Pulmonary disease is currently the major cause of death in SSc, having surpassed renal causes [5]. The two major pulmonary manifestations are interstitial lung disease (SSc-ILD) and pulmonary arterial hypertension (PAH). PAH can occur as an isolated form, in the absence of significant interstitial lung involvement, in approximately 12-16% of patients, and is associated with a long history of Raynaud's phenomenon, limited cutaneous involvement and anticentromere antibody positivity [6,7]. Compared to the idiopathic form of pulmonary hypertension (IPAH), where an actively proliferating plexiform lesion is found in the pulmonary arteriolar walls, a more fibrotic, concentric-obliterative lesion predominates in SSc-associated PAH [8]. Prior to the development of new therapies, SSc-associated symptomatic PAH had an extremely poor prognosis, with a 40% survival at 2 years [9]. Unfortunately, the few published studies seem to show that it responds less favourably than IPAH to the recently developed disease modifying therapies [10,11], although outcomes comparable to IPAH were reported in treated SSc-PAH prospectively defined by right heart catheterization [12], underlining the importance of early intervention.

The other major cause of morbidity and mortality in SSc is interstitial lung disease (SSc-ILD), which will be the focus of this review. Its prevalence varies according to a variety of parameters, including population studied, case definition and sensitivity of methods of ascertainment. While some degree of interstitial lung involvement is present in most cases in autopsy series [13], clinically significant ILD is found only in approximately one third of patients. The spectrum of disease severity varies greatly from mild and self-limited interstitial involvement to a rapidly progressive course in some patients. It is thus crucial to identify and accurately stage lung involvement in the context of systemic sclerosis.

Pathogenesis

The pathogenesis of SSc-ILD involves a variety of abnormalities, including immunological/inflammatory activation and vascular injury. The interplay between these processes is believed to lead to dysregulated fibroblast activation and unchecked remodelling of the extracellular matrix.

Although the precise sequence of events is unclear, chronic inflammation, possibly in response to an unknown injury, is believed to play a significant role in the fibrotic process. This is in contrast with idiopathic pulmonary fibrosis, where the hypothesis of inflammation preceding fibrosis has been largely abandoned, not least because anti-inflammatory and immunosuppressive agents are largely ineffective [14]. Humoral and immune cell abnormalities are found in SSc, including the presence of autoantibodies specific to the disease, chronic mononuclear cell infiltration of affected tissues, and dysregulation of lymphokine and growth factor production [15-19]. In SSc-ILD, studies on bronchoalveolar lavage have shown a gene expression signature consistent with increased expression of chemokine and chemokine receptor genes involved in the recruitment of T cells and chronic macrophage activation, with CD8+ T cells mainly expressing pro-fibrotic Th2 cytokines IL-4 and IL-5 [20]. Proteomic analysis confirms the predominant Th2 cytokine profile in SSc bronchoalveolar lavage fluid [21]. Cyclophosphamide, a potent immunosuppressant, has recently been found to be partially effective in two randomised placebo-controlled trials in SSc-ILD [22,23].

Microvascular injury is believed to be the earliest and possibly the primary event in the pathogenesis of SSc [24]. Outside of the lungs, Raynaud's phenomenon precedes the onset of skin fibrosis often by several years in most patients. Histologically, vascular damage precedes evidence of fibrosis in the skin. Nailfold capillaroscopy demonstrates capillary dropout, dilatation, and tortuosity in SSc patients. In the lungs, a study of post-mortem lung tissue identified excessive formation of irregularly shaped alveolar capillaries with an increase in the number of endothelial cells in the early stages of lung fibrosis [25]. However,

vascular regression and redistribution of vessels away from airspaces has been described in areas of established lung fibrosis [26]. Although vascular abnormalities almost certainly precede fibrosis, the sequence of events and interplay with autoimmunity is not at all clear. It has been suggested that microvascular injury induces inflammation and autoimmunity, which in turn have direct and indirect roles in inducing fibroblast activation, a key event in the development of fibrosis [27].

Fibroblasts are the main cell type responsible for the excessive extracellular matrix synthesis and deposition seen in fibrosing lung disorders. Fibroblasts can differentiate into a more metabolically active cell with features intermediate between fibroblasts and smooth muscle cells, termed myofibroblast. Myofibroblasts express high levels of α -smooth muscle actin and synthesize increased levels of collagens, TIMP and other ECM components *in vitro* [28]. Fibroblasts explanted from SSc-ILD lungs have been shown to be phenotypically different from control lung fibroblasts, although there is a degree of heterogeneity in this cell population [27,29]. SSc fibroblasts are considered to be in an activated state and the proportion of alpha-smooth muscle actin (α -SMA)-positive cells is elevated in cultures of SSc fibroblasts [30]. Although most of the studies on SSc fibroblasts have been performed on affected skin-derived cells, SSc lung fibroblasts have been shown to produce more extracellular matrix proteins, including collagen I [31], to be more contractile [32], and to respond differently to apoptotic stimuli [33], compared to control fibroblasts. It is not yet firmly established whether these differences are due to the higher proportion of myofibroblasts in this population or whether there are intrinsic, stable differences between SSc fibroblasts/myofibroblasts and their normal counterparts.

Many of the genes over-expressed by scleroderma fibroblasts mirror the gene expression profile induced by TGF β , a pleiotropic pro-fibrotic cytokine which is upregulated in several fibrotic lung diseases, including SSc-ILD [34,35]. In particular, connective tissue growth factor (CTGF) is one of the TGF β targets found to be upregulated in SSc and to have a variety of biological effects, including fibroblast proliferation, matrix production, adhesion and granulation tissue formation [34,36,37].

An intriguing question regards the origin of the stimulated fibroblasts; traditionally, they were believed to derive from the activation of resident fibroblasts induced by *in situ* cytokines and growth factors. However, accumulating evidence suggests that in fibrotic lung disease, interstitial lung fibroblasts can derive from at least two additional sources, the transdifferentiation of epithelial cells into myofibroblasts [38,39], and from a circulating fibroblast-like cell, the fibrocyte, derived from bone marrow stem cells, in response to cytokines and chemokines produced at the site of lung injury/inflammation [40,41]. It is possible that all three mechanisms are operating in SSc-ILD, although the relative contribution of each cell type has yet to be delineated.

Autoantibodies and their relation to lung involvement in SSc

Patients with systemic sclerosis express a variety of disease-specific autoantibodies, mostly mutually exclusive and associated with different subsets of the disease. Whether these autoantibodies play a direct pathogenetic role or are simply epiphenomena is unknown. However, they have clear clinical utility as markers of different subsets of disease with characteristic patterns of organ involvement, and, as mentioned later, may well identify genetic subsets of the disease.

The autoantibodies classically associated with SSc and most frequently found are anti-topoisomerase (ATA, also known as Scl-70), anti-centromere (ACA) and anti RNA polymerase I/III (ARA). Other disease-specific auto antibodies which occur less commonly include anti-Th/To, anti U3-RNP and anti-PM/Scl autoantibodies, associated with polymyositis/scleroderma overlap [42]. Non SSc-specific autoantibodies include anti-Ro (SS-A) and anti-La (SS-B), most often found in systemic lupus erythematosus and Sjogren's syndrome, anti-RNA polymerase II, also found in SLE and overlap syndromes, anti-Sm antibodies usually found in SLE, and anti U1-RNP associated with what was previously termed "mixed connective tissue disease".

The two main types of lung involvement, ILD or isolated pulmonary hypertension, are very tightly associated with specific auto-antibodies. Anti-topoisomerase antibodies (ATA), present in approximately 20% of patients, are strongly linked to the development of lung fibrosis. Roughly half of SSc patients in whom pulmonary fibrosis develops have ATA antibodies; conversely, most patients (>85%) with ATA positivity have pulmonary fibrosis [43,44]. Some studies, but not all, suggest that ATA is also predictive of higher rates of progression of lung fibrosis [45,46,4].

The less frequent anti-nucleolar antibodies, anti-U3 RNP antibody and anti TH/To, are also associated with an increased risk of pulmonary disease; the latter also seem to be associated with development of pulmonary hypertension disproportionate to the degree of interstitial involvement [47]. Interestingly, anti TH/To have also been described in a subgroup of patients with clinical idiopathic pulmonary fibrosis and a UIP pattern on histology (13/285); the prognosis of these pts did not differ from those with IPF but no autoantibodies, lending support to the suggestion that Th/To autoantibodies may be markers of aggressive lung disease [48]. Although infrequent in SSc, anti-Ro and anti La (both with and without Scl-70 antibodies) are also associated with high frequencies of lung fibrosis [49]. Interestingly, a recent study measuring rheumatoid factor isotypes in SSc, found that IgA-RF antibodies were significantly higher in those with than those without ILD (56% vs 26%) [50].

Anti-centromere antibodies (ACA) occur in approximately 20-30% of patients, although frequency varies significantly according to race, being highest in Caucasians and lowest in Hispanic, African-American and Thai patients [46]. ACA

are associated with limited cutaneous involvement, and higher risk of calcinosis and ischemic digital loss. ACA have been consistently associated with little or no lung fibrosis, and virtually no patient with this antibody has severe lung fibrosis [43]. On the other hand, ACA is associated with an increased risk of pulmonary hypertension, although not independently of the presence of limited cutaneous disease [4]. A reduced incidence of severe lung fibrosis is also found in anti-RNA polymerase I/III positive patients [45], which instead have higher frequencies of diffuse cutaneous disease and renal involvement.

Genetics

Several lines of evidence point to a genetic predisposition to SSc, including variability according to ethnic group and race, familial clustering of the disease, and the existence of genetic animal models [34,36,51-53]. An increased incidence and severity of internal organ involvement is found in several non Caucasian groups, including African-Americans [54,55]. The highest prevalence of SSc has been found in Choctaw-Native Americans living in Oklahoma, US, [56] where the frequency is more than 20 times greater than in the general population. In this population, the SSc phenotype is extremely homogeneous, with most patients displaying diffuse scleroderma, pulmonary fibrosis, and autoantibodies to topoisomerase I, suggesting that SSc subsets represent separate entities, each with its own distinct genetic risk factors [57,58].

The major histo-compatibility complex (MHC) region is the region implicated in most of the genetic studies in SSc, although associations with genes outside of the MHC region have been reported. The strongest associations described so far are between HLA class II alleles and specific autoantibodies, in turn associated with distinct clinical subsets. This suggests that different disease subtypes are associated with distinct HLA-dependent initiating factors [57]. Interestingly, the frequency of SSc-related autoantibodies varies significantly according to race and ethnicity, a finding that is possibly linked to the varying severity found in different populations. African-American patients are more likely to have diffuse cutaneous disease, anti-topoisomerase antibodies and a higher frequency of pulmonary fibrosis. On the other hand, Caucasians are more likely to have ACA antibodies and to have limited disease with less severe internal organ involvement [59-60].

Because of the very tight link between ATA and interstitial lung disease, the primary genetic association is difficult to identify. In Caucasians, ATA is associated with HLA-DRB1*1101/1104 and DPB1*1301 [43,47,57], in Japanese with DRB1*1502, and in Choctaw Indians with DRB1*1602 [61]. ACA positivity is instead weakly associated with DRB1*01 and DQB1*05 alleles, while it is negatively associated with HLA-DQB1*0201 [57]. However, a subsequent study identified a much stronger association with allelic haplotypes in the TNF-alpha gene, in linkage

disequilibrium with HLA-DRB1*01 and HLA-DQB1*0501, thus explaining the previously observed weak association [62].

Several associations with non HLA-region genes have been reported, although some have not been confirmed in separate populations and/or require further independent verification in larger cohorts. Polymorphism haplotypes of the large fibrillin gene were strongly associated with SSc in Choctaw Indians and in a Japanese population [63]. An association between SSc and a TGF β codon 10 polymorphism was found in a study from Scotland [64] but not in a Japanese study [65], although the latter reported a very weak association with SSc-associated pulmonary fibrosis. An increased frequency of two polymorphisms in the CXCR2 chemokine receptor 2 gene (CXCR2) was reported in systemic sclerosis vs controls, although this was not independently associated with pulmonary fibrosis [66]. An association between fibronectin polymorphisms and SSc lung fibrosis has been reported [67], while the finding of an association between SPARC polymorphisms and SSc-ILD in Choctaw-Indians [68] was not reproduced in a UK SSc population [69].

Overall, it is possible that clearer genetic associations will come to light if the different subsets of SSc disease are more rigorously defined and if studies are systematically repeated in geographically and ethnically different populations.

Pathology and its relationship to prognosis

The most common histological pattern in SSc-ILD is non-specific interstitial pneumonia (NSIP), as assessed by several studies performed after the recent ATS/ERS consensus classification [38, 70,71]. The hallmarks of an NSIP pattern are the homogeneity of the inflammatory/fibrotic infiltrate throughout the biopsy and scarce, if any, fibroblastic foci or areas of honeycombing. An NSIP pattern can be further subdivided into cellular and fibrotic NSIP, according to the relative predominance of interstitial inflammation versus fibrosis. According to the largest published series, out of 80 patients with SSc-ILD, 77.5% had NSIP (cellular NSIP=15; fibrotic NSIP=47), while the remainder (six in each group) were equally divided between usual interstitial pneumonia (UIP), end stage lung fibrosis and miscellaneous disorders [70]. Interestingly, although NSIP is also reported as the most frequent pattern in other connective tissue diseases, SSc-associated ILD is the most homogeneous, with patterns such as organizing pneumonia and diffuse alveolar damage described only sporadically. Furthermore, while an NSIP pattern is not infrequently associated with elements of airway involvement or organizing pneumonia in other connective tissue diseases such as rheumatoid arthritis, in SSc there tends to be a "pure" NSIP pattern; mixed patterns with overlap between NSIP, organising pneumonia and small airway involvement are almost never described.

Although historically considered similar to IPF, it is now well recognised that SSc-ILD is associat-

ed with a significantly better survival than IPF, even for similar levels of disease severity [72]. In the idiopathic interstitial pneumonias, a pattern of UIP is associated with a significantly worse prognosis compared to NSIP [73-75]. Thus, the improved survival of SSc-ILD compared to IPF, could be related to the high frequency of NSIP vs UIP. However, Bouros *et al* found no significant difference in survival between UIP and NSIP patterns of disease in the context of SSc, although the UIP group was small [70]. Furthermore, other studies have shown that in the connective tissue disease group as a whole, a UIP pattern does not imply a worse prognosis compared to NSIP [76,77]. Thus, within SSc patients, a UIP pattern does not carry the same negative prognosis as in idiopathic disease; for this reason a surgical biopsy does not seem to add significantly to the management of SSc-ILD, and does not need to be routinely performed, unless unusual features are present.

Radiology

As with histology, the high resolution CT pattern in SSc-ILD is relatively homogeneous, again differing from other CTD-associated interstitial lung diseases, where a greater variety is present. In SSc-ILD, the most frequent CT pattern is either predominant ground-glass opacification or an admixed ground glass/reticulation pattern, with a predominant reticular pattern present in only one third of patients (figure 1A, B, C). Interestingly, no differences were found between SSc-ILD and idiopathic NSIP; in particular, coarseness of fibrosis and proportion of ground glass opacification were virtually identical, both on univariate and multivariate analysis [78]. In contrast, SSc-ILD and idiopathic NSIP are less extensive, less coarse, and characterised by a greater proportion of ground-glass opacification than IPF, supporting the biopsy series stating that NSIP is by far the commonest histological pattern in SSc-ILD [78].

Ground glass on CT can represent either predominant inflammatory changes or fibrosis subliminal to the limits of resolution of HRCT. Although it is impossible to distinguish fibrosis from inflammation with certainty on the basis of HRCT, features suggestive of established fibrosis include admixed reticular abnormalities and the presence of traction bronchiectasis (figure 1B and 1C) [79]. However, even in the presence of these features, at least some of the ground glass may represent inflammatory changes, and the HRCT pattern can only be used as a rough guide in predicting possible reversibility with treatment.

Bronchoalveolar lavage

Although non-specific for SSc-ILD, the most common bronchoalveolar lavage (BAL) cellular profile is characterised by an increase in neutrophils, often associated with varying degrees of increase in eosinophil/lymphocyte counts. The main value of BAL in SSc is to exclude complicating infections, malignancy, and to identify un-

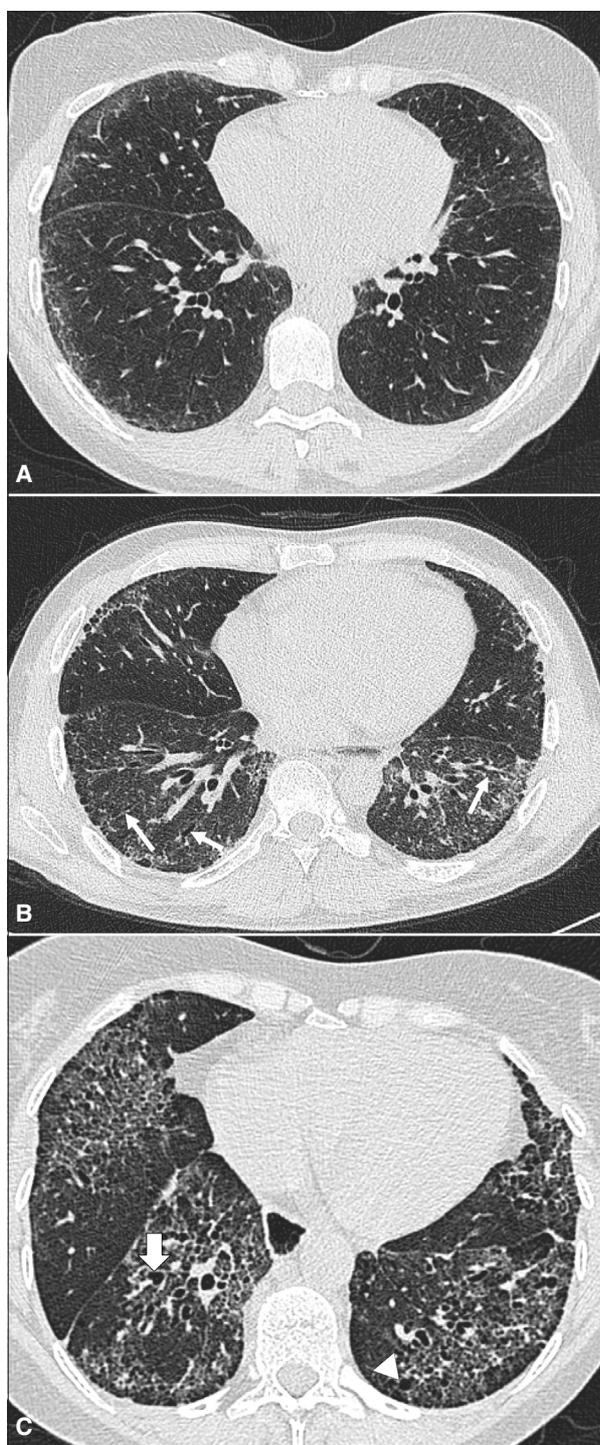


Fig. 1. - A. Peripheral ground glass opacity in a patient with SSc-ILD; traction bronchiectasis is not conspicuous, suggesting that at least part of the parenchymal disease is potentially reversible. B. Extensive ground glass and a component of reticulation with dilatation and distortion of the underlying airways (arrows) indicating established fibrosis. C. Extensive reticular pattern with minor admixed ground glass and obvious traction bronchiectasis (arrow) with some areas of honeycombing (arrowhead).

usually high counts of one (or more) cell type(s) indicating possibility of co-existing disease (eg: a striking eosinophilia could suggest a drug reaction in the appropriate clinical context).

There is still debate on whether bronchoalveolar lavage (BAL) cellularity can be used as a basis for treatment decisions in SSc-ILD. There is no doubt

that BAL neutrophilia is associated with more progressive disease [80]. In one study, BAL fluid eosinophilia was associated with significantly increased mortality despite treatment [70]. BAL "alveolitis" (neutrophils >3% and/or eosinophils >2%) was one of the selection criteria for entry into the largest randomised clinical trial performed to date in SSc-ILD, the Scleroderma Lung Study comparing oral cyclophosphamide vs placebo [23]. However, the meaning of an increased BAL neutrophilia and/or eosinophilia is still uncertain. Increased BAL cellularity could simply be an expression of the severity of lung fibrosis and thus not independently linked to outcome. In a recently published study, BAL neutrophilia was not linked to overall mortality or rapidity of progression in SSc-ILD, once disease severity was taken into account, although early mortality (within the first two years) was weakly linked to BAL neutrophilia [81]. Even though BAL cellularity does not seem to greatly add to prognostic evaluation in SSc-ILD once extent of lung involvement has been taken into account, the association between neutrophilia and early mortality needs further exploration.

Lung function abnormalities

There are several causes of lung function impairment in systemic sclerosis, and more than one may be operating in the individual patient. The diffusion capacity for carbon monoxide (DLCO) is the most sensitive marker of functional impairment in SSc-ILD [82]. However, its evaluation always needs to be done in conjunction with lung volumes. Pure interstitial lung disease is characterised by a restrictive ventilatory pattern with reductions in FVC, TLC and increase in FEV₁/FVC ratio associated with a reduction in DLCO, which is usually more marked than the reduction in volumes. However, a disproportionate reduction in DLCO compared to lung volumes, expressed as a marked reduction in KCO (DLCO/alveolar ventilation), suggests concomitant vascular involvement, particularly if there is hypoxemia at rest or on minimal exertion [83]. An increased alveolar-arterial oxygen difference during exercise is a sensitive indicator of SSc-ILD or pulmonary vascular disease; on the other hand, a normal A-a gradient on maximal exercise reasonably excludes significant interstitial involvement [83].

In isolated pulmonary vascular involvement, DLCO and KCO are reduced while lung volumes are not affected. A similar pattern may also be the expression of concomitant interstitial lung disease and significant emphysema, which can lead to spurious preservation of lung volumes, but marked reduction in DLCO and KCO; a history of smoking and CT findings of emphysema should suggest this less frequently occurring possibility.

In patients with severe diffuse cutaneous involvement, there may be an element of extrapulmonary restriction. Rarely, it is the only abnormality present and is characterised by a restrictive ventilatory pattern, but only mildly reduced DLCO and supernormal KCO, as the total pulmonary blood volume is only minimally reduced even in the presence of severe reductions in TLC and VA.

More often it is a contributory factor leading to a more marked reduction in FVC and TLC than expected on the basis of DLCO reduction.

With the exception of patients with a history of smoking and concomitant emphysema, an obstructive pattern is rarely, if ever, seen in scleroderma.

Markers of epithelial cell permeability, damage and replication

^{99m}Tc-DTPA clearance

The clearance from the lung of inhaled ^{99m}Tc-DTPA (technetium labelled diethylene-triamine-pentacetate) is a measure of epithelial barrier integrity. An abnormally rapid clearance ($t_{1/2} < 40$ minutes), has been found in a variety of diffuse lung diseases [79,84,85]. In a group of patients with either SSc-ILD (N=53) or IPF (29), the speed of ^{99m}Tc-DTPA clearance was predictive of disease progression; a normal clearance at presentation predicted subsequent stability of lung function parameters, whereas a persistently rapid clearance was associated with higher likelihood of functional decline [85].

Serological markers of lung epithelial cells

The lung epithelium can also be assessed indirectly by studying lung epithelium specific proteins, including Krebs von den Lungen 6 (KL-6), surface protein A (SP-A) and surface protein D (SP-D).

KL-6, a mucin-like glycoprotein, is expressed on type II pneumocytes and respiratory bronchiolar epithelial cells in normal lungs [86]. In interstitial lung diseases, type II cells proliferate and replace the type I alveolar lining, possibly in response to lung injury, and are likely to participate in the pathogenesis of lung fibrosis by secreting fibrogenic cytokines, including TGF β . KL-6 is strongly expressed by proliferating/regenerating and/or damaged type II pneumocytes [87,88]. Elevated serum KL-6 is also associated with increased permeability of the alveolar capillary barrier [89].

Increased serum and BAL levels of KL-6 are found in a variety of interstitial lung diseases, including SSc-ILD [90,91] and reflect its severity, correlating inversely with DL_{CO} and FVC. Furthermore, (both baseline and) serial measurements have been observed to have predictive value [92,93]. In a longitudinal study of 39 patients with SSc, increasing levels of KL-6 were more frequently accompanied by progressive pulmonary interstitial fibrosis. In contrast, stable levels of KL-6 were more likely to be seen in patients with stable lung disease [93].

Surfactant proteins A and D (SP-A and SP-D) are also produced by epithelial type II cells and correlate with presence of interstitial involvement, KL-6 levels and inversely with pulmonary function [94,95,96]. Recently, serum levels of CCL18, a chemokine predominantly produced by alveolar macrophages, have been found to correlate with pulmonary fibrosis and, when measured serially in a group of 21 ATA+ patients with SSc-ILD, to reflect activity of interstitial lung disease [97].

Diagnosis of SSc in the context of interstitial lung disease

In most cases of SSc-ILD, the diagnosis of SSc is made on the basis of typical cutaneous findings (either limited or diffuse), a variable duration of Raynaud's phenomenon, presence of additional extracutaneous features such as oesophageal involvement, and characteristic auto-antibodies. Antinuclear antibodies are present in approximately 95% of SSc patients, but are not specific for SSc as they are found in most patients with a connective tissue disease [98], and can be found at low titre in idiopathic interstitial pneumonias. The most useful auto-antibodies in the diagnosis of SSc are ACA and ATA because they are rarely found in patients with connective tissue diseases other than SSc, family members of SSc patients, or in healthy controls [46]. Antinuclear antibodies such as RNAP I and III, as well as anti-U3-RNP and anti-Th/To are also highly specific for SSc, although less frequently found and thus of less diagnostic utility [46]. Although antinuclear antibodies are found in most patients, SSc specific antibodies are found only in a proportion of these, and it is likely that as further antigen specificities are discovered, the gap between the frequency of ANA positivity and of SSc-specific antibodies will diminish.

In approximately 10% of patients who are ultimately diagnosed with systemic sclerosis, skin induration is absent (*scleroderma sine scleroderma*) and patients may present with the clinical findings relative to internal organ involvement, including interstitial lung disease, which may be the sole recognized manifestation of scleroderma. Characteristic autoantibodies, symptoms of esophageal dysmotility, scattered teleangectasias, pericardial effusions and microvascular changes on capillaroscopy can all be helpful in pointing towards systemic sclerosis, particularly in this group of patients [99].

When is interstitial lung disease in SSc clinically significant?

Many patients with SSc have limited interstitial lung disease which remains stable without treatment; however, a substantial proportion of patients have clinically significant involvement. Although a clear-cut definition is lacking, most authors would agree that clinically relevant interstitial lung disease is present in at least one third of SSc patients [100]. Thus, a clinical assessment of the significance of the lung involvement is crucial, including the severity of disease and the likelihood of subsequent progression [101]. The presence of respiratory symptoms as a discriminatory factor is unreliable. Symptoms do not allow the distinction between interstitial lung disease and vascular involvement, and may be significantly confounded by concomitant muscle or joint involvement. The two most useful tools in assessing severity of disease in pulmonary fibrosis are evaluation of extent of disease on HRCT and lung function tests [102]. Lung function tests have the advantage of being easily available in all centres, whereas assessment

of average HRCT extent of disease can require specialist radiological input.

Among lung function parameters, DLCO is the best indicator of disease extent in patients with established pulmonary fibrosis [82]. However, an isolated reduction in DLCO can also indicate isolated pulmonary vascular disease, and should always be interpreted together with HRCT, clinical and autoimmune features. In the presence of a positive ATA, a reduced gas DLCO, even in the absence of significant lung volume reduction, is more likely to represent early interstitial lung disease; by contrast, the same abnormality in the context of ACA positivity, suggests pulmonary vascular disease. In the latter case, CT will confirm absent or minimal interstitial involvement. Thus, evaluation of functional indices should always be combined with an evaluation of disease extent on CT, as well as clinical and autoimmune features.

Although there is no established clear cut-off, most authors would agree that a $DLCO \leq 50\%$ and/or $FVC < 70\%$ is clinically significant and warrants consideration of treatment, whereas for lesser degrees of DLCO and/or FVC reduction, an evaluation of the inherent progressiveness of the fibrosing alveolitis becomes crucial [103]. The reasons for treating more severe disease include the fact that extensive disease has a track history of progression; furthermore, less functional reserve means that further, potentially irreversible reductions in lung function, have a significantly greater clinical impact than progression of more limited disease.

Other factors associated with disease progression include duration of systemic disease. Virginia Steen has clearly shown that the loss of volume on pulmonary function testing is greatest in the first four years of disease [4]. This may be because inflammation is greatest in early disease, and if not treated leads to subsequent progressive interstitial fibrotic disease. Particularly progressive ILD can be seen when the lung disease precedes the cutaneous manifestations of SSc.

One of the most powerful determinants of functional deterioration is observed progression on lung function. This has been observed for idiopathic fibrotic interstitial pneumonia, where a reduction in DLCO and/or FVC at six and 12 months, trumped a histological pattern of UIP vs NSIP as a predictor of mortality [104]. In a cohort of SSc-ILD, improvements in DL_{CO} (but not FVC) at 3 years, were associated with longer survival and declines in DL_{CO} were associated with increased mortality, even after adjustment for initial severity [70]. It is thus extremely important to regularly monitor pulmonary function tests particularly in those patients in whom the decision to treat is a close call. Other determinants of likelihood of progression include autoantibody status, as patients with ACA very rarely have severe ILD, and markers of epithelial damage, as discussed in the section on DTPA and epithelial markers.

Monitoring and initiating treatment

To summarise the previous sections, several features, including autoantibody subsets and clinical

parameters can help in assessing likelihood of significant interstitial lung disease. However, an adequate baseline assessment and routine monitoring is required in all SSc patients, as summarized in figure 2. Recommended investigations at baseline include PFT, chest Xray, HRCT chest and echocardiogram. Serial monitoring of lung function tests, particularly in the first 5 years after diagnosis, is crucial for detection of onset and/or progression of SSc-ILD. HRCT chest can be omitted at baseline in ACA positive patients with normal PFTs, in view of the extremely low frequency of significant interstitial lung disease in this group. Immunosuppression should be considered in patients with severe and/or progressive disease. A cut-off of $DL_{CO} < 50\%$ and/or $FVC < 70\%$ together with significant extent of abnormalities on HRCT can be used to define severe disease and initiate treatment, unless there is documented stability on sequential PFTs over several years, as summarized in figure 2. In less severe disease, an assessment of the inherent progressiveness of SSc-ILD, using serial lung function test monitoring, should be performed (fig. 2). Although not available in all centres, epithelial permeability markers such as DTPA clearance or serum KI-6, are powerful predictors of subsequent progression in SSc-ILD, and should be used in conjunction with other parameters (fig. 2). Bronchoalveolar lavage cellularity is probably not routinely useful in treatment decisions, although its role remains controversial. Although predominance of a ground glass pattern over reticulation on chest HRCT has been used as an adjunct to treatment decisions, the finding that patients with the greatest extent of fibrosis on pre-treatment chest HRCT tended to show the most significant improvement in the recently published Scleroderma Lung Study, suggests that the extent of ground glass should not be a key parameter in the decision of whether to institute immunosuppression [23].

Treatment

Until recently, only uncontrolled or retrospective clinical trials were available in SSc-associated lung fibrosis, although most had suggested the effectiveness of cyclophosphamide in stabilizing or improving FVC and/or DLCO [105-108]. These findings have recently been confirmed by two randomized placebo controlled trials (RCT). The Scleroderma Lung Study evaluated 162 patients randomized to receive either placebo or oral cyclophosphamide (in addition to low dose prednisolone) for one year [23]. The study met the primary outcome, the observed absolute difference in FVC% at 12 months between treated and untreated patients, although the difference was small (2.53%, $p=0.03$). Interestingly, the largest effect was seen in patients with more severe lung fibrosis, as assessed by CT scoring, emphasizing the importance of patient selection [23]. Among secondary outcome measures, a significant, but again small, beneficial effect was observed in the skin thickness and dyspnoea scores.

The other recently published RCT in SSc-ILD is the FAST trial (fibrosing alveolitis in scleroder-

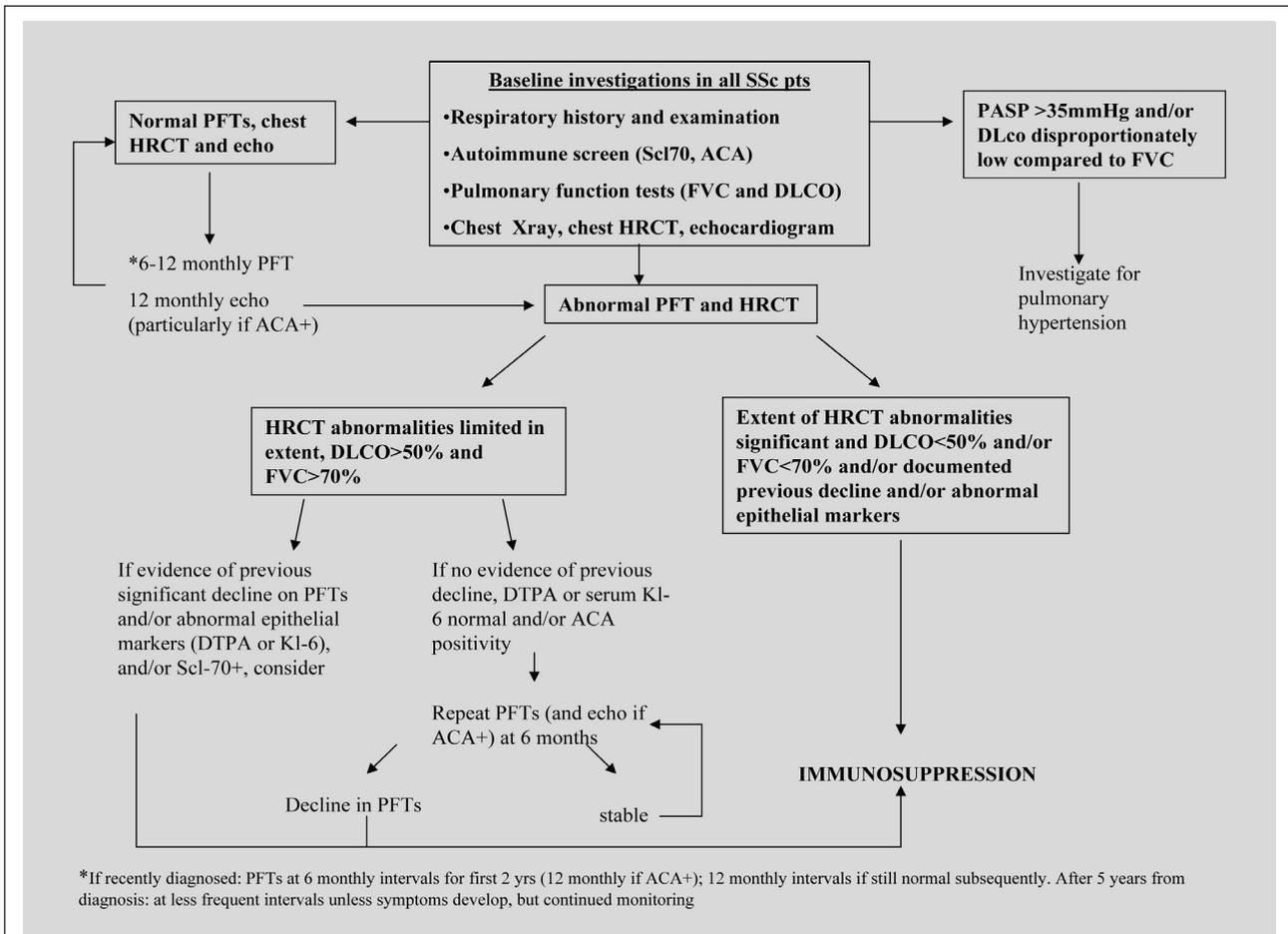


Fig. 2. - Algorithm for monitoring and initiating treatment for interstitial lung disease in patients with systemic sclerosis.

ma trial) performed in 45 patients, comparing placebo with monthly iv cyclophosphamide for 6 months, followed by oral azathioprine and low dose prednisone for a total of 24 months [22]. Although this study did not reach a significant result, there was a clear trend towards a difference in change in FVC at one year in the treatment group ($p=0.08$), with a 4.19% change in FVC at one year favouring the treatment group. Interestingly the magnitude of the effect was similar to the oral cyclophosphamide Scleroderma Lung Study. By contrast, use of intravenous cyclophosphamide was associated with less toxic side effects (haematuria, haemocytopenia) than the oral cyclophosphamide trial. Furthermore, although long term follow up data was lacking, experience gained in the treatment of other autoimmune disorders, indicates that intermittent monthly iv boluses of cyclophosphamide are associated with lower risks of cancer and gonadal failure compared to oral daily administration of the drug [109].

Several authors have questioned the clinical significance of the small, albeit significant, treatment effects observed in both trials. However, as recently outlined [110], this appears to be a common feature of all the recent RCTs performed in fibrotic lung diseases. In the presence of a placebo arm and of the trialled medication being available for open treatment for patients unwilling to participate, patients selected for entry will inevitably tend to have less severe and/or inherently progressive disease. This is

particularly true for SSc-ILD, where there was a weight of evidence in favour of cyclophosphamide's effectiveness before the two RCTs commenced. Thus, many of the patients in the placebo arm will tend to be stable, and treatment effects will inevitably tend to be diluted [111].

Unresolved issues regarding treatment include which long-term immunosuppressive agents to use after the induction period with cyclophosphamide, so as to minimise cyclophosphamide-induced morbidities. The similar results obtained in the FAST trial in which six months of cyclophosphamide were followed by azathioprine, suggest that a protocol of induction (with cyclo) / maintenance (with less toxic immunosuppressants) regimen is a viable option. Indeed, the superior efficacy of cyclophosphamide over less toxic immunosuppressants remains to be proven, and it may be that less aggressive drugs such as azathioprine are equally effective even when used as first line agents, although controlled studies are needed.

Although less toxic than cyclophosphamide, azathioprine is also burdened by relatively frequent side effects, particularly liver function abnormalities. Mycophenolate mofetil (MMF) was developed as an alternative to azathioprine with a more selective mode of action and, therefore, fewer side effects [112].

A retrospective study comparing 109 SSc pts treated with MMF with 63 SSc patients receiving other immunosuppressive drugs suggested that

oral mycophenolate may be effective in preventing progression of lung fibrosis [113]. A prospective but small and uncontrolled study found significant improvement in FVC and DLCO after 4-6 months of mycophenolate treatment used as a first line agent in recent onset SSc-ILD [114]. The role of less toxic immunosuppressants needs to be established by controlled clinical trials.

Issues regarding transplant

Despite the encouraging results obtained with cyclophosphamide on prevention of progression of SSc-ILD, in some patients lung fibrosis continues to progress despite treatment, leading to respiratory insufficiency and death. Lung transplantation represents the only hope for survival in these patients, until more effective treatments are discovered. Until recently, very little data was available on the outcome of lung transplantation in scleroderma, with only a handful of cases found in the published literature [115-117]. However, Schachna L *et al.* have recently published the results of a study comparing long term survival in 29 pts with scleroderma, 70 with IPF and 38 with idiopathic pulmonary hypertension, representing the total number of transplants for these conditions in two US centres over a 12.5 year period [118]. Among systemic sclerosis patients, indications for transplantation were interstitial lung disease in 15 pts, pulmonary arterial hypertension in 11 and both in three patients. Despite the common perception that systemic disorders might represent a contraindication to transplant, the study concluded that scleroderma patients undergoing lung transplantation have similar rates of survival to the two other lung-only disorders at two years, although there was a non significant trend towards a higher early mortality within the first six months. Interestingly, four out of the seven deaths occurring in SSc patients in the first month post-transplant were attributed to primary graft failure and all occurred in patients with severe pulmonary hypertension undergoing single lung transplantation.

Future directions

In view of the toxicities of the current immunosuppressive regimens and of the presence of a significant proportion of patients who continue to progress despite optimal current management, alternative, more effective treatment options are needed. The activation of T and B cells early in the course of the disease suggests that these cells and their cytokines are potential targets for therapeutic interventions [15]. Approaches that alter the balance between T_H1 and T_H2 cytokines by inhibiting T_H2 cytokines, have been shown to be beneficial in animal models of SSc [119]. Conversely, a syndrome similar to SSc develops in mice deficient for the T_H1 cytokine interferon- γ receptor [18]. B cell depletion was associated with reduced skin fibrosis in tight-skin mice model of SSc [27]. Targeting of costimulation molecules which modulate activation of T cells, such as CTLA4, is also being evaluated in SSc. Autologous stem cell transplan-

tation associated with very high doses of cyclophosphamide is currently being evaluated for rapidly progressive diffuse cutaneous disease. Although effective on skin disease, it is associated with significant morbidity and mortality and its effectiveness on lung involvement is unclear as none of the trials were designed to specifically investigate effects on interstitial lung disease [120,121].

Among pro-fibrotic cytokines, TGF β and CTGF are natural treatment targets, and have been evaluated in a Phase I/II trials to assess safety and tolerability. Disappointingly, a recent study showed no significant effect on skin fibrosis of anti-TGF β treatment, although the study was not designed to evaluate SSc-ILD [122].

Other anti-fibrotic strategies currently being evaluated in idiopathic pulmonary fibrosis, such as N-Acetylcysteine and pirfenidone, will also need to be tested in SSc-ILD. Clinical trials are also needed to evaluate the treatment of pulmonary hypertension associated with SSc-ILD, to assess whether the drugs that are used in isolated pulmonary hypertension are similarly effective when pulmonary hypertension occurs in association with interstitial involvement.

Over the last ten years we have seen unquestionable progress in the treatment of both interstitial lung disease and isolated pulmonary hypertension in SSc. Further understanding of the molecular mechanisms through which vascular disease, autoimmunity and fibrosis interlink will inevitably lead to improved treatment strategies in SSc. The use of the high throughput parallel methods of analysing global gene expression, protein and metabolic profiles in different disease subsets, including SSc patients with and without ILD and/or PAH, will hopefully reveal the pathways specifically involved in development and progression of interstitial lung disease, allowing for the development of more effective targeted therapies.

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