Usual interstitial pneumonia is the predominant histopathology in patients with systemic sclerosis receiving a lung transplant

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Abstract

Objective

Studies identifying nonspecific interstitial pneumonia (NSIP) as the predominant histopathology in systemic sclerosis-associated interstitial lung disease (SSc-ILD) have primarily utilised surgical lung biopsies in early disease. These case series may only reflect the histopathology of early disease and differ from the histopathology of advanced disease in those with respiratory failure.

Methods

Patients receiving a lung transplant for a diagnosis of SSc at a single centre from 2000-2021 were included for retrospective analysis. All explanted lungs underwent histopathology review as part of routine care.

Results

127 patients with SSc received a native lung transplant during the study period. Usual interstitial pneumonia (UIP) was identified in 111 explants (87.4%), NSIP in 45 (35.4%) explants, organising pneumonia in 11 explants (8.7%), and lymphocytic bronchitis in 2 explants (1.6%). Areas of both UIP and NSIP were identified in 37 explants (29.1%), with only 9 explants (7.1%) showing neither UIP nor NSIP. Aspiration was identified on histology in 49 (38.6%) explants. Pathology results were available from a prior surgical lung biopsy for 19 patients, with 11 patients maintaining the same primary pathology on biopsy and explant (2 NSIP, 9 UIP) and 8 patients showing different pathology at the timepoints, all of whom had UIP on explant. Most patients (101, 79.5%) had evidence of pulmonary hypertension and vasculopathy on explant.

Conclusion

UIP is the predominant histopathology in patients with SSc receiving a lung transplant, with many patients concurrently having both NSIP and UIP or showing progression from NSIP to UIP over time before transplant.

Key words

histopathology, interstitial lung disease, lung transplantation, systemic sclerosis, usual interstitial pneumonia

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Introduction

Systemic sclerosis (SSc) is a rare autoimmune disease with the highest case-mortality amongst the rheumatic diseases. Pulmonary complications, including interstitial lung disease (ILD), are the leading cause of disease-related mortality in SSc, due to a lack of effective treatments and prevention (1, 2). Though ILD is typically detected within five years of the first non-Raynaud's symptom (3), its course is heterogenous ranging from minimal progression over decades to rapid progression to respiratory failure within several years, with over 50% of patients with SSc developing SSc-ILD (4). Male sex, anti-topoisomerase antibody (anti-Scl-70), Black race, and diffuse cutaneous disease are risk factors for SSc-ILD, however we currently lack biomarkers to identify patients at highest risk for progression to respiratory failure (5). Improving scientific knowledge regarding SSc-ILD progression to respiratory failure may improve our identification of risk factors for progression and better inform treatment approaches.

Previous studies identifying non-specific interstitial pneumonia (NSIP) as the predominant histopathology in SSc-ILD have primarily included patients with early disease. A 2002 study of 80 SSc-ILD patients, utilised open surgical lung biopsies from patients with early disease (mean disease duration 13 months), identifying NSIP in 77.5% of samples and usual interstitial pneumonia (UIP) or end-stage lung disease in 15% of samples (6). Similarly, a study of surgical lung biopsy in 22 patients with limited cutaneous SSc and SSc-ILD identified NSIP in 14 (63.64%) samples and UIP in 8 (36.36%) samples, with secondary organising pneumonia and lymphoid hyperplasia noted in several patients. While importantly defining the pathology in early disease, such studies do not sufficiently inform the pathology of patients with respiratory failure from SSc-ILD. In translational studies utilising explanted tissue, we and others have observed that the majority of explanted SSc-ILD lungs show UIP (7, 8), akin to the preponderance of UIP at autopsy (60% of samples) (9). However, the histopathology of advanced SSc-ILD has not been examined in a large series to date. Efforts to characterise ILD pattern by computed tomography (CT), have also estimated UIP to be present in a greater percentage of patients (10-12). For instance, a 2008 study examining 162 participants in the Scleroderma Lung Study found honeycomb cysts, a CT feature of UIP, in over 35% of patients (10). The repeated characterisation of SSc-ILD as an "NSIP disease" in the literature may be limiting our understanding of disease evolution in those with advanced SSc-ILD. It is critical that we understand the histopathology and mechanisms of disease in those with respiratory failure, as these patients have the highest mortality and greatest unmet clinical need.

Pulmonary hypertension (PH) can occur in patients with SSc due to multiple mechanisms (13). Diagnosing patients with a discrete PH form guides therapeutics yet is challenging in practice due to overlapping features and the potential for coexisting mechanisms. The extent of ILD on chest CT is not predictive of the haemodynamic severity of PH (14), further complicating attempts to dichotomise patients with SSc and PH into SSc-PAH and SSc-PH-ILD subgroups. Patients with SSc-PH-ILD have increased mortality compared to those with SSc-PAH, and less improvement in functional class (15). By current United Network for Organ Sharing diagnosis classification, patients with SSc are classified as either scleroderma-restrictive or scleroderma-pulmonary hypertension at the time of transplant listing, however such dichotomisation does not reflect the fact that most patients have both ILD and PH to varying degrees.

Over 80% of patients with SSc have gastrointestinal tract involvement, with collagen accumulation in the myenteric plexus contributing to dysmotility across the gastrointestinal system (16). Oesophageal disease is intimately linked to pulmonary disease in SSc, as oesophageal aperistalsis, dysmotility, and gastroesophageal reflux (GER) all increase the risk of recurrent aspiration. Restrictive lung disease can also increase GER by elevating the thora-

coabdominal pressure gradient and decreasing the oesophageal length (17). Clinicians commonly struggle with assessing the contribution of aspiration-related injury to SSc-ILD when determining whether to start and/or escalate immunosuppression. Oesophageal disease can also influence eligibility for lung transplantation, as many centres will not transplant patients with moderate to severe dysmotility due to concern for aspiration causing allograft compromise (18).

Surgical lung biopsies provide a snapshot in time of histopathology pattern, but do not reflect the evolution of disease in any individual patient. While serial CTs are often performed in SSc-ILD and can indicate a change in tissue disease pattern, their correspondence with tissue patterns is imprecise (19-21). Patients rarely undergo multiple lung biopsies given procedural risk, making human tissue from multiple timepoints infrequently available for research purposes. To gain insight into the evolution of disease, we also reviewed pathologic data from multiple timepoints in all patients who underwent both a surgical biopsy and a subsequent lung transplant. In this largest series of lung explant pathology in SSc, we sought to define the histopathology of advanced SSc-ILD, progression of disease patterns over time, as well as the frequency of pulmonary vascular disease and histologic evidence of aspiration.

Materials and methods

Study procedures and design were approved by the University of Pittsburgh's Institutional Review Board (STUDY22020013). Patients undergoing native lung transplant at a single centre for a diagnosis of SSc between January 2000 and December 2021 were included. Eligibility criteria and clinical characterisation of the SSc lung transplant cohort at University of Pittsburgh has been previously described (22). 128 lung transplants met these initial criteria. All explanted lungs underwent histopathology review as part of routine clinical care. Histopathology reports, demographics, heart catheterisations, CT chests, prior lung biopsy pathology, autoantibody status, and preoperative medications were reviewed for all patients as available in the medical record.

Results

Study population

Of 1,790 lung transplants performed during the study period from January 2000 through December 2021, 127 native lung transplants occurred for a diagnosis of SSc (Fig. 1). 110 patients received a double lung transplant, 15 patients a single lung transplant, and 2 patients concurrently received a heart and double lung transplant. One patient underwent a native lung transplant and later a retransplant for chronic lung allograft dysfunction during the study period, with only the native lung transplant included in the data analysis. We included all patients with a diagnosis of SSc receiving a lung transplant in the defined period, including those with primarily SSc-PAH and minimal to no ILD. As many patients exhibit features of both SSc-ILD and SSc-PAH, we chose not to artificially dichotomise the study population into these two groups but rather to evaluate the burden of ILD and vascular disease in all patients. As not all patients received their rheumatology care and workup at our institution, there was significant missing autoantibody data, and we did not have autoantibody data on sufficient patients to draw conclusions regarding the association of autoantibody status with a particular histopathology pattern or features.

Histopathology patterns at explant

Usual interstitial pneumonia (UIP) was identified in 111 explants (87.4%) and NSIP in 45 (35.4%) explants (Table I). Concurrent areas of both UIP and NSIP were identified in 37 explants (29.1%), while only 9 explants (7.1%) showed neither UIP nor NSIP. Less common histopathology patterns included organising pneumonia, lymphocytic bronchitis, and respiratory and/or follicular bronchiolitis (Table II). Of the patients with organising pneumonia, 1 was identified to have overlap with rheumatoid arthritis, 1 had concurrent autoimmune hepatitis

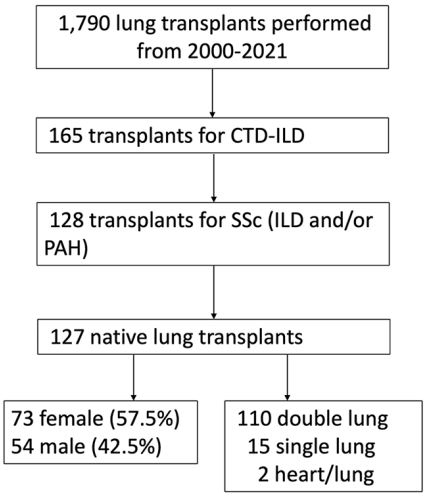

Fig. 1. Flow diagram of the cohort.
CTD-ILD: connective tissue disease-associated interstitial lung disease; SSc: systemic sclerosis; PAH: pulmonary arterial hypertension.

(with prior liver transplantation), 3 had anti-Scl70 autoantibodies, 1 had Th/To and SSA autoantibodies, and 1 had anti-centromere antibody, although autoantibody data was not available on all patients. Emphysema was noted in 16 patients (12.6%), consistent with prior descriptions of combined pulmonary fibrosis with emphysema occurring in SSc-ILD, typically in patients who are current or former smokers (23). In the subset of patients with autoantibody data available, 42 had a defined SSc antibody (Table III). Given the limited number of patients with autoantibody data, we could not make strong associations with histopathology patterns; however, the increased prevalence of predominantly vascular disease with little to no ILD in centromere patients was notable.

Diffuse alveolar damage consistent with acute lung injury, possibly stemming from an acute exacerbation of disease, was noted in 18 (14.2%) explants (Table II). While acute exacerbations of idiopathic pulmonary fibrosis are a well described event occurring in 8-14% of patients with IPF each year (24, 25), their presence in SSc-ILD has also been noted (26, 27). The presence of diffuse alveolar damage alone cannot confirm an acute exacerbation in these patients, as it can also result from drug toxicity, acute respiratory distress syndrome, toxic inhalants, and infection.

Table I. Presence of UIP and NSIP pattern in explanted SSc-ILD lungs.

Histopathology	Number of samples (%)	
UIP	111 (87.4)	
NSIP	45 (35.4)	
No UIP	16 (12.6)	6-predominant vascular disease, 2-emphysema, and vascular disease, 8-temporally homogenous NSIP
Both NSIP and UIP	37 (29.1)	
Neither UIP nor NSIP	9 (7.1)	7- predominant vascular disease, 1-emphysema and vascular disease, 1-DAD and vascular disease

SSc-ILD: systemic sclerosis-associated interstitial lung disease; UIP: usual interstitial pneumonia; NSIP: non-specific interstitial pneumonia; DAD: diffuse alveolar damage.

Table II. Less frequent histopathologic patterns in explanted SSc-ILD lungs.

Histopathology	Number of samples (%)
Diffuse alveolar damage/acute lung injury	18 (14.2)
Organizing pneumonia	11 (8.6)
Respiratory/follicular bronchiolitis	12 (9.4)
Lymphocytic bronchitis	2 (1.6)
Metaplastic ossification	20 (15.7)

SSc-ILD: systemic sclerosis-associated interstitial lung disease.

Table III. Histopathologic patterns for patients with known SSc-associated autoantibody.

Antibody positivity	Histopathology
Scl-70	UIP: n=12 NSIP: n=3 UIP and NSIP: n=8 Emphysema and vascular disease: n=1
Centromere	UIP: n=1 NSIP: n=2 UIP and NSIP: n=1 Vascular disease predominant: n=3
Th/To	UIP: n=3 UIP and NSIP: n=2
U11/U12 RNP	UIP and NSIP: n=1
U1 RNP	NSIP: n=1 UIP and NSIP: n=1
RNApol3	NSIP: n=1 UIP and NSIP: n=2

UIP: usual interstitial pneumonia; NSIP: non-specific interstitial pneumonia.

Table IV. Pathology comparison between biopsy and subsequent explant in select patients.

Surgical lung biopsy pathology	Lung explant pathology
NSIP	UIP
NSIP (N=2)	UIP and NSIP (N=2)
NSIP with DAD	UIP
Respiratory bronchiolitis	UIP
Organizing pneumonia	UIP
Organizing pneumonia with DAD	UIP
Emphysema, lung cancer	UIP and NSIP

UIP: usual interstitial pneumonia; NSIP: non-specific interstitial pneumonia; DAD: diffuse alveolar damage.

Prior surgical lung biopsies

Pathology results were available from a prior surgical lung biopsy for nineteen patients, with a median of 4 years (range 0.25–16) and mean of 5.5 years between the biopsy and transplant. Eleven patients maintained the same primary pathology on biopsy and explant (2 NSIP, 9 UIP), with 8 patients showing different primary pathology at the two timepoints (Table IV, Fig. 2). Notably, all patients with a change in histopathology between the timepoints exhibited UIP on explant, confirming that patients can evolve from other histopathologies to UIP.

Aspiration on histology

Oesophageal disease is common in patients with SSc, and can influence transplant eligibility at some centres. While a lack of findings on histopathology does not exclude prior aspiration, features including intraluminal fibroblast plugs of organising pneumonia, multinucleated giant cells, granulomas, peribronchiolar fibrosis and the identification of aspirated food particles can implicate its presence. There was strong evidence of prior aspiration (Table V, Fig. 3) in 49 (38.6%) explants. This percentage likely underestimates the true burden of aspiration-related lung injury, with sampling error, temporality of aspiration to time of transplant surgery, potential underreporting of findings, and non-specific nature of aspiration-related histology all potentially leading to underestimate.

Pulmonary vascular disease

Most patients had evidence of pulmonary hypertension or vasculopathy on explant with the most common findings including pulmonary artery atherosclerosis, occlusive fibrointimal elastosis, vessel medial hypertrophy and myointimal thickening, and plexiform lesions (Table 6, Fig. 4). Alveolar hemosiderosis was also noted in 13 (10.2%) explants, which may reflect prior repeated episodes of alveolar haemorrhage. In total 101 (79.5%) explants had pulmonary vascular disease identified on histopathology report. 116 (94.3%) of 123 patients with available data had pulmonary hypertension as defined by a

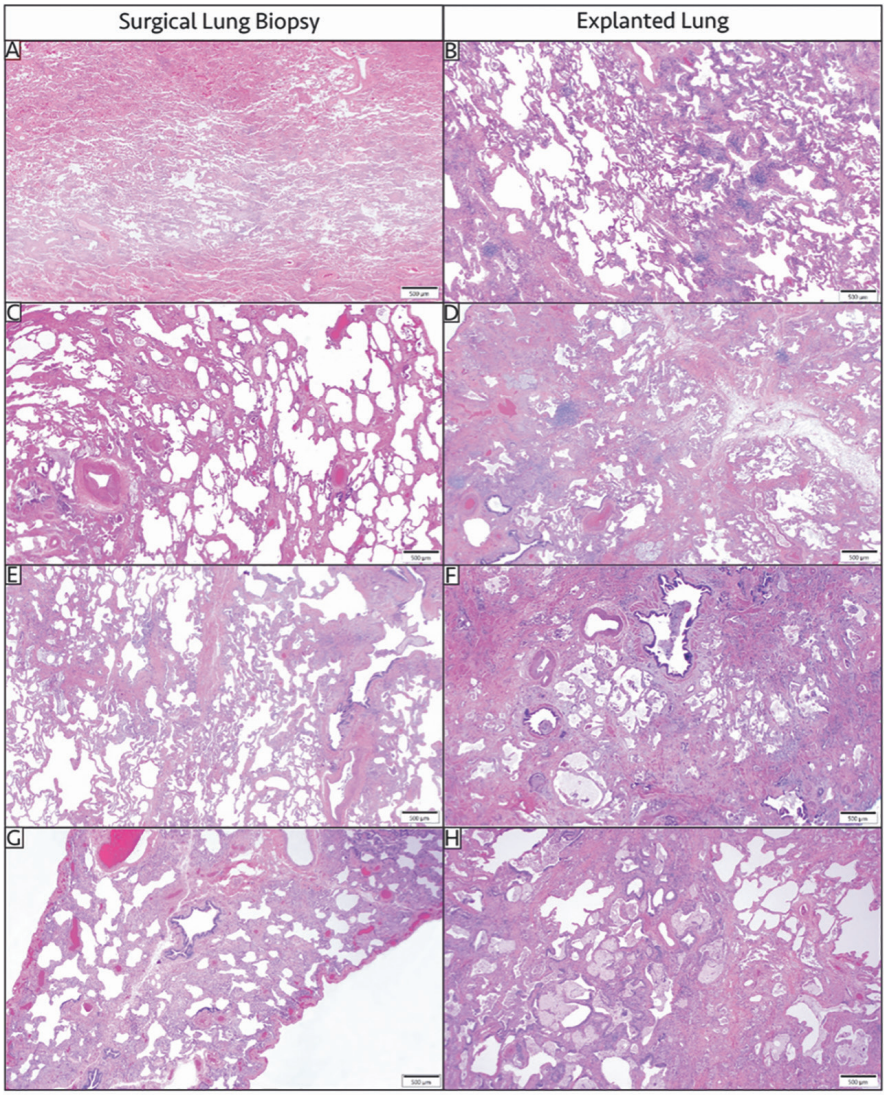

Fig. 2. Histology of surgical lung biopsy and subsequent native lung explant in four matched cases. The initial biopsy images (**A, C, E, G**) show relatively uniform interstitial expansion and fibrosis, consistent with an NSIP pattern. The explant images (**B, D, F, H**) demonstrate patchy involvement with variable degrees of fibrosis, consistent with a UIP pattern. Time between biopsy and subsequent transplant was 11 years for A/B, 4 years 5 months for C/D, 8 years for E/F, and 11 years for G/H.

Table V. Airway centric pathology findings suggestive of aspiration.

Airway-centric histopathology features	Number of samples (%)
Cholesterol clefts	19 (15)
Giant cells	26 (20.5)
Granulomas	38 (29.9)
Food particles	1 (0.8)
Any signs of aspiration	49 (38.6)

mPAP ≥20mmHg, PVR>2 Wood units and pulmonary capillary wedge pressure ≤15mmgHg and/or requiring pulmonary vasodilators (not prescribed for Raynaud's). The majority of right heart catheterisation measurements were performed within 6 months of lung transplantation. Patients with minimal to no ILD on histology were more likely to have severe occlusive fibrointimal elastosis and plexogenic pulmonary arteriopathy reported. Two patients received a concurrent heart and double lung transplant, with one demonstrating plexogenic pulmonary arteriopathy and respiratory bronchiolitis with smoker's macrophages (former smoker) on histology, and the second demonstrating severe pulmonary arteriopathy with diffuse alveolar damaging and cardiomegaly with mild subendocardial and interstitial fibrosis on histology.

Additional histology findings

Pleuritis was noted in 12 (9.5%) explants, with no vasculitis or capillaritis identified. Other significant findings included previously unidentified thromboembolism in 8 (6.3%) explants, emphysema in 16 (12.6%) explants, and tumours in 6 (4.72%) explants. The 6 tumours included 3 carcinoid tumourlets, 1 pulmonary chondroid hamartoma, 1 metastatic breast carcinoma, and 1 invasive lung adenocarcinoma. Metaplastic/dendriform ossification, the development of ossified nodules in the lung parenchyma, was identified in 20 (15.7%) explants- consistent with previous reports of its presence in 10-20% of patients with fibrotic ILD (28, 29). No patients were reported to have pleuroparenchymal fibroelastosis, although its presence has been noted radiographically in patients with systemic sclerosis (30).

Pathology correlation with chest imaging

CT chest imaging reports from prior to lung transplant were available for 120 patients. The proportion of CTs containing common imaging features (pulmonary artery dilation, ground glass opacities, reticulation, traction bronchiectasis, honeycombing, and oesophageal dilation) were noted for four pathology groups (Fig. 5). Ground glass opacities were most common in the NSIP only group (57.1%), however also present in many patients with UIP only (24.3%) or concurrent UIP and NSIP (30%) on explant. Oesophageal dilation was more common in patients with UIP (57.1%) and concurrent UIP and NSIP (45.9%) compared to those with NSIP only (28.6%) or vascular predominant disease (16.7%). As these CT chests were performed over a long-time range (1998-2021), thickness of the image slice and image quality varied. As radiologic 'definitions' for UIP, NSIP, and other patterns also varied

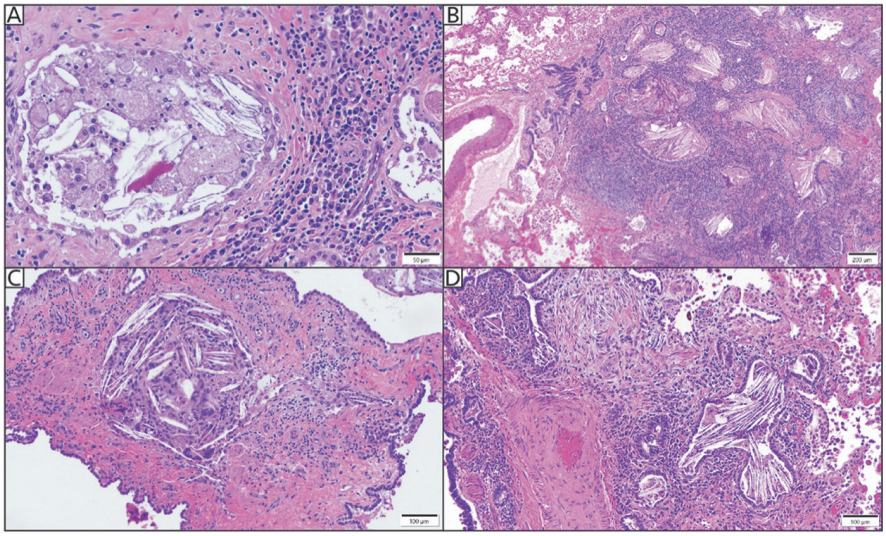

Fig. 3. Histopathology features of aspiration in SSc-ILD explants. Explanted lungs with fibrosis and airspaces filled with foamy macrophages and cholesterol clefts. (**A, B**) Foreign material and associated lymphoplasmacytic infiltrate. (**C**) Multinucleated giant cells associated with the cholesterol clefts. (**D**) Involved airspaces with adjacent organising pneumonia.

Table VI. Pulmonary vascular disease findings on histology.

Pulmonary vascular disease features	Number of samples (%)
Medial hypertrophy/myointimal thickening	79 (62.2)
Advanced pulmonary atherosclerosis	31 (24.4)
Alveolar hemosiderosis	13 (10.2)
Occlusive fibrointimal elastosis	9 (7.1)
Plexiform lesions	6 (4.7)
Any vasculopathy feature	101 (79.5)

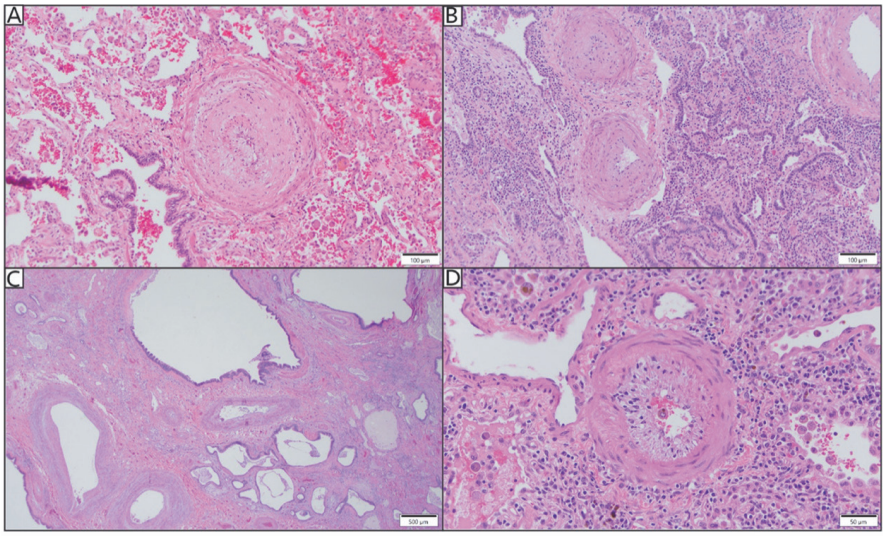

Fig. 4. Histopathology findings of pulmonary hypertension in SSc-ILD explants.
Small pulmonary arteries with features of pulmonary arterial hypertension including intimal thickening and medial hypertrophy.

over this time course, we have focused on imaging features rather than radiology pattern names from the available imaging studies.

Discussion

Understanding the histopathology and active mechanisms in patients with advanced SSc-ILD is imperative as these patients have the highest unmet need for strategies to improve long-term outcomes. We identified that UIP is the predominant histopathology in patients with SSc receiving a lung transplant, with many patients concurrently having both NSIP and UIP, or showing a progression from NSIP to UIP over time. Additionally, isolated pulmonary hypertension or ILD was rare in this advanced cohort, with most patients having both ILD and significant vascular disease at the time of transplant.

We identified eight patients with a change in primary histopathology pattern between lung biopsy and transplantation, all of whom had UIP on explant histology. Others have previously noted that patients with NSIP on lung biopsy may be later recategorised as UIP based on re-biopsy, lung transplant, or continued radiographic imaging- with their histology typically having "extensive NSIP-like areas in otherwise conventional UIP" (31). This occurrence could be due to sampling limitations or the surgical selection of biopsy site, as a prior analysis of multi-lobe surgical biopsies for ILD diagnosis identified different diagnoses between lobes in 26% of patients (n=28 of 109) (32). This study identified no difference in the survival of patients with UIP in all lobes *versus* those discordant for NSIP and UIP in different lobes, however, did find improved survival in patients with NSIP in all lobes, supporting the common practice that patients with a histologic pattern of UIP in any area of lung tissue are classified overall as having UIP. Katzenstein *et al*. also previously evaluated the evolution of ILD pathology in 20 patients receiving a lung transplant after previous surgical lung biopsies (33). They observed that NSIP-like areas were present in 12 of 15 biopsies and 16 of 20 explant specimens from patients with UIP, with extensive NSIP-like areas noted in many of these samples. Unlike our study, they did not identify any cases of explants showing UIP that were proceeded by biopsy findings of NSIP. Of note, only 2 patients in this study had a clinical diagnosis of autoimmune disease (1 SSc, 1 polymyositis).

Despite over twenty years of substantial

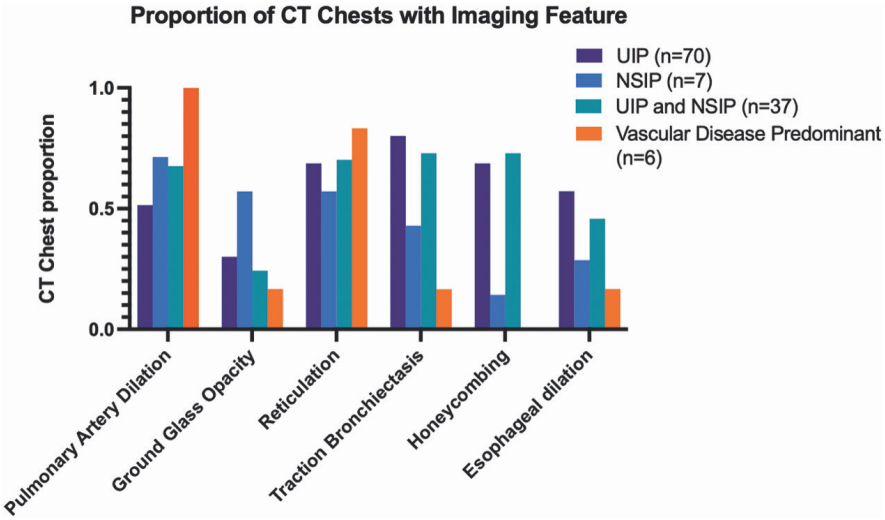

Fig. 5. 120 patients had available imaging reports from CT chest imaging prior to lung transplantation. Imaging features identified by the interpreting radiologist were noted, with graph depicting the proportion of CT chests for each group that noted the particular feature. Patients were divided based on explant pathology into the groups of: UIP, NSIP, UIP and NSIP, and vascular predominant disease.

progress in the field of ILD since this study of lobar histology was published, numerous questions remain regarding the connection between NSIP and UIP. Do patients progress from NSIP in early stages to UIP in later disease? If so, then why are some found to have UIP in early disease and why do some still have only NSIP at transplant? Do NSIP and UIP develop concurrently in different areas of lung parenchyma due to unique mechanisms, either related to the severity of injury or to the nature of the injury? If so, where does the NSIP go in those patients who transition to UIP by transplant? While our data supports the hypothesis that NSIP progresses to UIP, we cannot exclude the possibility that in the explanted lungs with NSIP and UIP, these patterns occurred due to different local mechanisms-such as increased immunologic derangement and/or vasculopathy in areas of NSIP *versus* increased epithelial injury due to aspiration in areas of UIP.

Although most SSc lung pathology studies have utilised biopsies in early disease, lung explant pathology has been reported in more limited cohorts. A 2021 study examining fundoplication post lung transplant in 36 patients with SSc noted the presence of UIP on explant in 13 patients (36.1%) and NSIP on explant in 23 patients (63.9%) (34). Eleven patients were noted to have diagnoses of other autoimmune disease as well including systemic lupus erythematosus, Sjögren's syndrome, rheumatoid arthritis, dermatomyositis, and polymyositis which may have biased the pathology distribution. Nevertheless, the discordant rates of UIP in this transplant population and ours could result from different selection criteria for lung transplant candidacy between institutions, subjective interpretation differences by different pathologists, reporting difference on including all *versus* only the predominant pattern, or random variation in the patterns of disease. More consistent with our observations, another single-centre study previously reported lung transplant outcomes from 14 patients with SSc, noting that 9 (64.3%) explants had UIP, 4 explants had NSIP (28.6%), and 2 (14%) had both UIP and NSIP on pathology (35). The predominance of an SSc-ILD vs SSc-PAH phenotype in the patients was also not detailed in prior studies.

In survival analyses of patients with SSc and other ILDs, NSIP (or non-UIP pattern) is consistently associated with improved mortality compared to UIP. Patients are typically classified to such group assignments based on their initial CT (or pathology), however this may misinform the expected trajectory of patients who evolve from NSIP to UIP. Perhaps the ILD community should revisit whether the earliest or the latest timepoint CT or pathology pattern is the best way to classify such patients. Additionally, our work raises the question of how early versus late developing UIP groups differ- including possible differences in genetic polymorphisms, environmental exposures, and other clinical manifestations such as oesophageal disease that merit further study. The increased prevalence of oesophageal dilation on CT imaging in patients with UIP, compared to those with no UIP, could support an increased role of epithelial injury in this subset.

As a large volume lung transplant centre that historically has not excluded patients from transplant listing based on oesophageal disease, we routinely transplant patients with SSc and were uniquely positioned to perform this study. By utilising explanted lung tissues only, our study has a selection bias for patients with severe SSc-ILD and is intended to reflect only this advanced cohort rather than the entire SSc-ILD patient population. While our study was limited by its retrospective design, all histopathology review was performed at the time of transplant as part of routine clinical care. Biases of individual pathologists towards over- or under-calling specific patterns cannot be excluded from effecting our work or the previous histopathology case series(6). However, as the explant tissues in our study were examined by a total of 7 different thoracic pathologists, we believe this is unlikely to have a significant effect on our findings. As many patients did not receive their rheumatology care at our institution, there was significant missing data on disease duration and extent of skin involvement, which could not be included in our analyses.

As the largest series of SSc pulmonary explant pathology, our study confirms previous observations that NSIP progresses to UIP in many patients with advanced ILD and highlights the need to include tissues from SSc patients with UIP in translational research moving forward. Finding evidence of aspiration in such a large percentage of the explanted lungs again raises the concern for the contribution of aspiration

to SSc-ILD pathogenesis. Our work also demonstrates that most patients with SSc and advanced respiratory disease have both ILD and PH, with a wide range of disease severity for both pathologies, rather than a dichotomous divide of patients with SSc and pulmonary disease into SSc-PAH and SSC-ILD(± PH). More work is needed to identify those with SSc-ILD at greatest risk of progression in order to guide earlier and more aggressive therapy in these patients, as well as the enrichment of clinical trial cohorts.

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