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Role of Gastro-oesophageal reflux in systemic sclerosis and other advancements in interstitial lung diseases: preliminary data

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ABSTRACT

Background

The management of interstitial lung disease in the context of systemic sclerosis (ILD-SSc) and pleuroparenchymal fibro-elasotis PPFE associated to other ILD has important unmet clinical needs. Symptomatic gastro-oesophageal reflux [GORD] is reported in up to 90% of systemic sclerosis [SSc]. PPFE is a rare interstitial lung disease (ILD) entity characterized by pleural and parenchymal fibrosis with a striking upper-lobe predominance. PPFE can occasionally coexist with other ILDs.

Material and methods

We present preliminary baseline results of the first 44 enrolled patients (median age 52, female 70%, median FVC=74%, median DLCO= 37%, diffuse SSc 33%) of a prospective study (NCT02136394), with regards to oesophageal manometry/24hr impedance (carried out off PPIs), respiratory(K-BILD and Leicester cough questionnaires) and GORD symptoms (*UCLA SCTC GIT 2.0 Questionnaire, Reflux Disease Questionnaire RDQ)* and lung function: time to decline for FVC drop of 10% or DLCO 15% drop from Visit 1 was calculated using cox proportional hazard models.
Furthermore, a retrospective analysis of 284 IPF patients (mean age 66±1.1, males 77%, ever smokers 66%, mean FVC% 68.4±2.5, mean DLco% 36.3±1.6, average CT extent % 38.4±1.6) regarding the prevalence of PPFE is presented: average disease extent on HRCT was used as a measure of disease severity; mortality, time to irreversible decline in FVC levels of > 10% and irreversible decline in DLco levels of > 15% from baseline, were quantified from the date of the HRCT using proportional hazards analysis.

Results

Foe the ILD-SSc study: proximal reflux was detected in 50% of patients, median DeMeester score was 5. FVC% showed significant correlations ($p<0.01$) with pH recumbent clearance ($r=0.46$) and K-BILD total score ($r=0.45$). DLCO% showed significant correlations ($p<0.01$) with hiatal hernia ($r=0365$), pH recumbent clearance ($r=0.45$) and K-BILD total score ($r=0.65$).. RDQ score was significantly correlated with DeMeester($r=59$,$p<0.01$) and Time of acid pH in upright position ($r=0.48$, $p<0.01$); among respiratory questionnaires, Leicester total score showed significant correlations ($p<0.01$) with yime of acid impedance in upright position ($r=0.62$), total time of acid pH ($r=0.68$). At the univariate analysis, demeester score, % time of acid pH on recumbent position were the strongest predictor of lung function decline (respectively, $p=0.005$ and $p=0.004$).
For the PPFE study: 94 (33%) IPF patients met the HRCT criteria for PPFE. Compared to patients without PPFE, subjects with PPFE were less likely to be ever smokers ($p=0.02$), had lower FVC measurements ($p=0.0005$) and more extensive ILD on HRCT ($p=0.03$). Freestanding bronchiectasis were found in 34% of PPFE versus < 2% of patients without PPFE ($p<0.0001$). PPFE was significantly associated with increased mortality (HR: 1.54 CI: 95% 1.16-2.05, $p=0.003$), decreased time to decline in FVC (HR: 1.82 CI: 95% 1.28-2.6, $p=0.001$) and DLco (HR: 2.29 CI: 95% 1.6-3.3, $p<0.0001$). After adjusting for age, gender, smoking status, ILD severity, the association of PPFE with survival, FVC and DLco decline was confirmed.

Conclusions

We confirm an association between oesophageal dysmotility and respiratory symptoms in Ssc-ILD. Firthermore, oesophageal measures are independent predictors of ILD-SSc decline.
PPFE is an independent predictor of survival and functional worsening in IPF. Further studies in different ethnic groups and other diffuse fibrosing lung diseases are needed.

Introduction

Intertistial lung diseases (ILD) are a relatively rare heterogenous group of conditions. Idiopathic pulmunary fibrosis (IPF) is the most common and deadly form[1,2]. Despite two anti-fibrotic medications have been approved worldwide in the past few years and have been shown to slow down IPF progression[3], the only availble treatment to stop the progression of the disease is lung transplant. The clinical management of other ILD forms can be challenging as well, due to overlap of presentation/symptoms, lack of evidence for the treatment, poor understanding of the etiopathogenesis. Among the many intriguiging questions on ILD, the following will be considered on this manuscript: (a) The last update of the ATS/ERS classification of idiopatic interstitial pneumonias included the Idiopathic Pleuroparenchimal fibroelastosis (PPFE) as a distinct entity[4]. Association of PPFE to other ILD patterns have been reported, however its role on the natural history of the backgroud ILD is not known[5]. (b) Systemic sclerosis (SSc) is the connective tissue disease (CTD) with the highest frequency of ILD, ranging from 40% to 80% depending on method of ascertainment [6]. Although the majority of patients with ILD in the context of SSc (ILD-SSc) have a relatively limited disease, a proportion of them will have significant and/or progressive ILD which can further progress if left untreated[6]. The availability of reliable predictors of disease progression to avoid unncessary treatment with immunuppressive drugs in an unmet clinical need, and more research is needed to shed light on a safe prevention strategy. Microaspiration of gastric progression is believed to lead to ILD progression and can be a possible terapeutic target[7].

The role of gastro-esophageal reflux (GER) in systemic sclerosis and lung fibrosis

Systemic sclerosis (SSc) is an autoimmune condition characterized by tissue fibrosis of the skin and internal organs. Progressive interstitial lung disease (ILD) is now the main cause of death in SSc[6]. Pathogenetic pathways believed to be involved in Ssc-ILD are complex and include endothelial cell injury, inflammatory/immune activation and dysregulated fibroblast homeostasis[8]. Inhaled technetium-99m-labelled diethylene triamine pentacetate (DTPA) clearance, a marker of epithelial cell permeability, is a strong predictor of lung function decline in SSc-ILD, even when lung disease severity is taken into account[9]. Serum KL-6 (Krebs von den Lungen 6), a mucin-like glycoprotein expressed by type II pneumocytes3, is also a marker of epithelial cell damage, upregulated in SSc-ILD and in other ILDs. In SSc-ILD, serum KL-6 is correlated with ILD presence, severity and activity[10–12]. The finding of a tight link between epithelial cells markers and lung fibrosis suggests that damage to alveolar epithelial cells is likely to play a fundamental role, at least in a subset of patients (Figure 1). This highlights the need to focus on potential noxious factors for the respiratory epithelium as potential drivers of the progression of lung fibrosis. Gastro-oesophageal reflux (GER) has been suggested as a driving factor in the pathogenesis of both SSc-ILD and idiopathic pulmonary fibrosis (IPF), the most frequent idiopathic form of lung fibrosis, with many similarities to Ssc-ILD[13]. The esophagus is affected in 50-82% of patients with SSc[14]. Gastric reflux may be liquid, gaseous, or particulate; acid or nonacid; distal (localized to the distal oesophagus) or proximal (reaching the proximal oesophagus and pharynx)[15]. Reflux to the proximal oesophagus, which is intuitively linked to microaspiration into the lungs, appears to be quite common in patients with ILD-SSc and IPF[16,17]. Importantly, a significant proportion of GER reflux is asymptomatic[18].
Repeated episodes of microaspiration of gastric contents secondary to GER could lead to alveolar epithelial injury and subsequent fibrosis. The histopathological characteristics of microaspiration-related changes have been poorly studied. Bronchiolocentric organizing pneumonia, foreign bodies, intraluminal basophilic content have been described in surgical lung biopsies of ILD-SSc with possible microaspiration[19,20]. However, those histological features probably represent a small

subgroup of ILD-SSc, while the vast majority of these patients show “pure” non specific interstitial pneumonia (NSIP) histopathologic pattern and GER is virtually present in all.

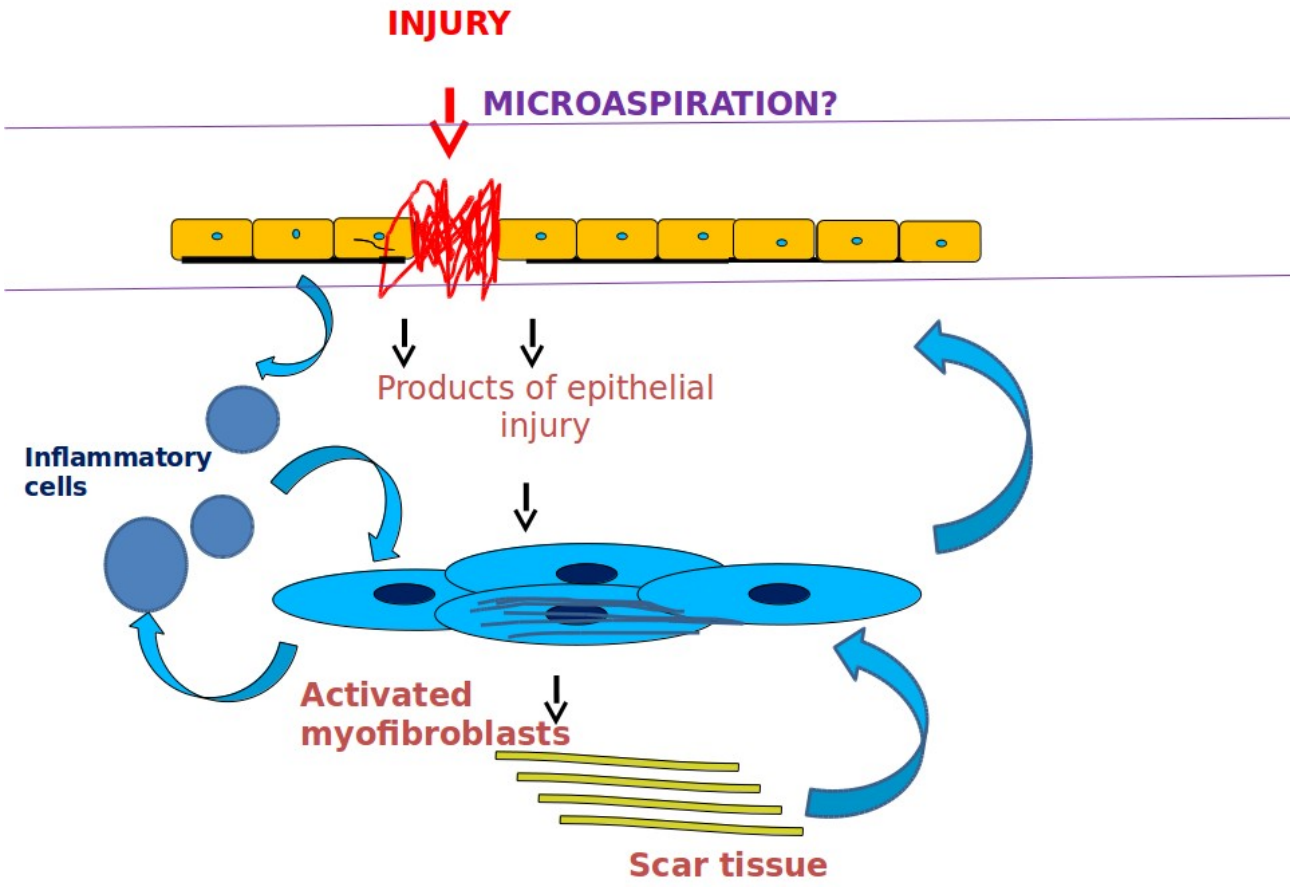


Figure 1. Cartoon showing the possible role of microaspiration in respiratory epithelial damage and subsequent lung fibrosis.

The majority of studies performed in SSc so far have used indirect methods to assess microaspiration into the lungs and have not clearly discriminated between ILD severity and progression. Of note, no study has looked for direct evidence of microaspiration into the lungs of SSc patients. Indeed GER should be considered a risk factor of microaspiration since only a proportion of reflux episodes can reach the airways.

Concentration of pepsin and bile acids in broncho-alveolar lavage (BAL) and exhaled breath condensate (EBC) have been investigated as biomarkers of microaspiration in various respiratory diseases. The first studies linking BAL pepsin and bile salts with acid reflux episodes were performed in children[21,22]. More recently, elevated BAL bile salt concentrations have been associated with early onset of bronchiolitis obliterans in 120 post lung transplant patients[23]. In the two studies performed so far on BAL pepsin levels in fibrotic lung diseases, BAL pepsin was associated with acute exacerbations[24] and severity of lung fibrosis[17] in IPF patients. Of note, the latter study by Savarino et al has shown similar results of BAL and saliva pepsin analysis in terms of correlation with lung function[17]. Although saliva measurements is mainly a surrogate marker of microaspiration, this method is non-invasive and easily repeatable. A non-invasive direct test of microaspiration is EBC pepsin. EBC, an evolving method to test pulmonary diseases[25], is easily

collected asking the patient to breath comfortably in a cold cylinder for 10 minutes. Raised levels of EBC pepsin have been associated with extra-pulmonary manifestations of GER in COPD[26], post-lung transplant[27] , IPF[28]. However, all these studies are small and some have been presented only in the form of abstract. As mentioned previously, a number of studies have suggested a link between severity of SSc-ILD and GER. Marie et al observed that patients with severe esophageal dysfunction on manometry had a significantly lower diffusing capacity of the lung for carbon monoxide (DLCO) and a higher frequency of ILD on CT, compared to those with mild or no esophageal involvement[29]. Similarly, Savarino et al reported that SSc patients with HRCT evidence of interstitial lung disease had a higher frequency of acid and non-acid reflux and a greater proportion of proximal reflux episodes, compared to those without ILD[16]. However, it remains unclear whether GER is a risk factor for ILD progression. In the prospective study of 43 SSc patients by Marie et al, at two years from baseline, average fall in DLCO was 18% in those with severe esophageal dysfunction compared to 2% in the others[29]. In a retrospective evaluation of 1043 SSc patient, GER symptoms and history of esophageal dilatation were predictive of ILD progression[30]. By contrast, in another prospective study by Gilson et al, only a trend bordering on statistical significance was observed on univariate analysis between severe esophageal dysfunction and reduction in forced expiratory volume (FVC) at follow up, which was not maintained after adjustment for disease severity, although only 7% of patients had extensive ILD involvement at baseline (FVC<70%)[31]. Troshinsky et al did not find a correlation between lung function and distal and/or proximal reflux in 39 consecutive SSc patients[32]. In IPF, recent large retrospective analyses and a number of case series have suggested a link between GER suppression and better outcome[33–35]. The larger study that retrospectively analyzed 242 IPF patients assigned to receive placebo in previous randomized clinical trials, found a significant association between reported use of anti-acid medications and slower FVC deterioration. The Authors suggested that clinical trials testing the role of anti-GER therapies in IPF are warranted[33]. Notably, though virtually all SSc patients are on anti-reflux medications, henceforth no study have investigated the role of those medications on ILD-SSc.

The confirmation of a causative link of microaspiration in the genesis and progression of lung fibrosis would have a major impact in the management of ILD-SSc patients. As discussed above, it is likely that proteases such as pepsin, and not the acidity, are the primary target for future therapies. Potent inhibitors specific for proteases, e.g. pepstatin, are available and have been tested in phase-III clinical trials[36]. Anti-reflux surgery has a variable outcome in SSc; however a carefully selected subgroup of patients could be eligible for fundoplication if this could prevent lung fibrosis. It is clear that prospective studies to assess prevalence and characteristics of microaspiration among SSc patients with ILD are required. This information could be crucial in order to highlight patients at greater risk of disease progression and to plan for appropriately designed interventional studies on the effectiveness of anti-GER treatments.

METHODS

We present preliminary data of a prospective study with an enrollment target of 100 Ssc-ILD patients. 48 consecutive patients have been enrolled so far. Inclusion criteria were (a) SSc diagnosis fulfilling published SSc diagnostic criteria[37], (b) > 5% of LD extent on high resoluton chest scan (HRCT). Exclusion criteria were poor level of English language and presence of communication problems / cognitive impairment. 40 patients will undergo broncoscopy; for this subgroup, additional exclusion criteria are FEV1 less than 1L or DLCO less than 30% of the predicted.

Patients will be asked to complete QoL, respiratory and GER-related questionnaires (see below), to provide a blood sample and undergo exhaled breath condensate collection at baseline. A subgroup of patients will be asked to undergo a bronchoscopy with BAL. Lung function tests, a high resolution chest CT, echocardiogram, full autoimmune profile, high resolution oesophageal manometry and a 24 hour impedance/pH monitoring will be carried out at baseline as part of the standard diagnostic work up. Patients will be asked to provide additional blood samples at 12 months, and undergo exhaled breath measurements at 6-12-18 months.

EBC and saliva samples will be collected from 20 healthy controls.Patients will also be asked to complete QOL, respiratory and gastro-oesophageal reflux questionnaires at six monthly intervals for 18 months after enrollment. After this time point, only clinical and lung function test data will be collected at 6-12 monthly intervals (to coincide with routine clinic visits) and up to 5 years from baseline (Figure 2).

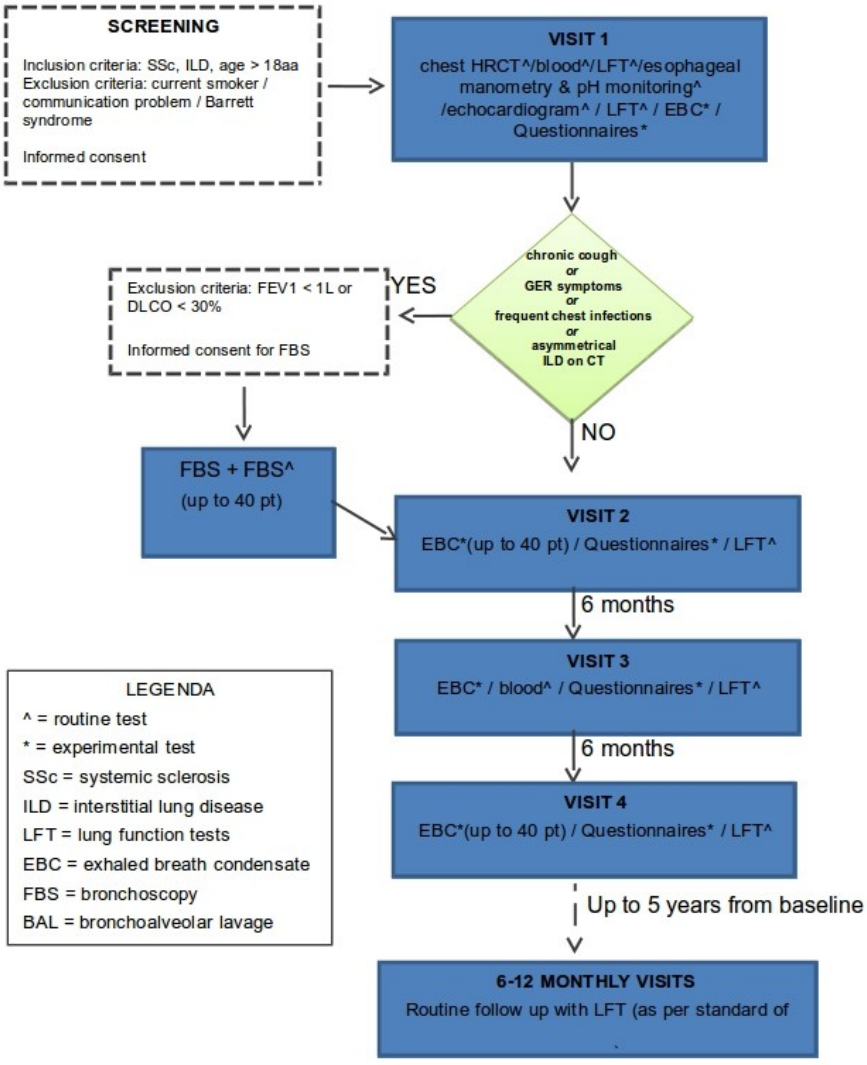


Figure 2. Study timeline

Composite physiologic index was used as a surrogate measure of ILD severity[38].

Tests of Oesophageal Function: Prior to testing, subjects will be asked to withhold proton pump inhibitors (PPIs) and prokinetic medication for 4 days. Following the 24hr assessment, patients will then return to their usual medications, including PPI and prokinetics, as this is currently the standard of care. After a four hour fast, patients will undergo high resolution oesophageal manometry (circumferential probe, Medtronic, Shoreview, MN) to locate the lower and upper oesophageal sphincters and undergo assessment of oesophageal motility. Immediately after, a combined impedance/pH catheter will be placed and kept for an 18-24 hour period, with digital storage of data allowing synchronised pH and impedance analysis. The use of the impedance catheter, in addition to the traditional pH measurement, will detect both gas and liquid reflux into the oesophagus, and will allow detection of reflux of non-acidic substances which would not be picked up by the pH study alone. All subjects will be asked to keep a detailed diary indicating the time of oral intake, change in position (upright and supine), and symptomatic events such as heartburn or regurgitation during the 24 hour period. The acid pH data (percent time pH<4) from the proximal electrode will be analyzed separately for the total, upright and supine positions. The definition of acid and non acid liquid reflux, as well as gas reflux will be as previously described[39].

Exhaled Breath Condensate:
Exhaled breath condensate analysis (EBC) is an evolving method to non-invasively test pulmonary disease. The patient is asked to breathe comfortably for ten minutes into a cooled cylinder, allowing the collection of the breath condensate. We plan to assess whether EBC analysis of pH and pepsin can provide a non-invasive method of testing for microaspiration into the lungs, easily repeatable and potentially applicable to the clinical setting, as a non-invasive way to monitor GER/microaspiration. We do not instead propose to study EBC bile salts, as not validated, and potentially confounded by methodological issues, related to their detergent properties. EBC collection and measurements will be performed so as to control for a number of methodological issues which can affect the results[40]. Samples will be collected as previously described[41] and aliquots will be stored at –80°C until the measurements, which will be performed within one month. Pepsin concentration will be assessed by using a commercially available detector (Peptest©). EBC pH will be quantified with a blood-gas analyser using the CO_2-loading method[40], using Kullmann's method to correct for the known low buffering capacity of EBC[42]. In the subgroup of patients undergoing BAL, EBC will be collected immediately before the BAL fluid collection on the same day, to allow direct comparison of invasive and non-invasive methods of detecting aspiration biomarkers.

Saliva analysis: Analysis of saliva samples has been investigated in a variety of systemic diseases[43]. In particular, it has been proven useful as a diagnostic marker of GER and requires no expensive equipment for collection[44]. Our aim is to measure pepsin in the saliva and assess its value as non-invasive test for detecting GER and microaspiration in SSc. Samples will be collected as previous descrided[44] and stored at –80°C until the measurements. The patient is asked to collect at least 2ml of saliva in the sample pot just before each scheduled EBC test. In order to assess variation over time and correlation with lung function parameters, we plan to collect saliva at baseline and at 12 months in all patients, and in a subgroup of 40 patients also at 6 and 18 months. Pepsin concentration will be analysed with Peptest©.

Bronchoalveolar lavage: At baseline, a subgroup of patients with SSc-ILD and characteristics as described in section 7, and agreeing to the procedure, will undergo bronchoscopy with bronchoalveolar lavage (BAL). Patients will be asked to stop any anti-reflux medications for a week (or a minimum of four days if a week not possible), to mirror the preparation for the GER assessment as outlined above. BAL fluid will be collected as previously described[45]. In addition to the tests routinely performed to assess cellularity and exclude infection, excess BAL fluid will be collected to evaluate concentration of microaspiration markers. BAL fluid will be centrifuged, and aliquots will be immediately snap-frozen at -80°. Once samples are thawed, BAL pepsin levels will be measured using a commercially available Kit (Peptes©).

Serum collection: Serum will be collected at baseline and at 12 months to measure levels of KL-6 (Krebs von den Lungen 6), a marker of alveolar epithelial damage. Raised levels of KL-6 have been associated with presence of ILD, severity and risk of progression of lung fibrosis[11,12]. At baseline, serum will be analyzed for autoimmune screen, BNP, IgG, IgA, IgM as well.

Questionnaires of GER, respiratory symptoms and quality of life: Questionnaires related to GER, respiratory, and quality of life assessment will be performed at baseline and at six monthly intervals for 18 months in all patients using a series of validated instruments. These will include the recently refined UCLA Scleroderma Clinical Trial Consortium Gastrointestinal Tract Instrument (UCLA-SCTCGIT) 2.0, including scales on reflux and bloating, developed to specifically assess the impact of gastrointestinal tract symptoms in SSc[46], with established minimally important difference estimates[47]. GER symptoms will also be assessed by a validated self-administered 12-item questionnaire

(Reflux Disease Questionnaire)[48]. In view of the relationship between GER and chronic cough, we will use the Leicester Cough Questionnaire, developed to evaluate cough severity and its impact on quality of life in patients with GER-associated cough[49]. The impact of respiratory and GER symptoms in terms of anxiety and depression will be evaluated by the 14-item self-assessment Hospital Anxiety and Depression Scale (HADS)[50]. Recently developed from a collaboration between our Unit and King's College Hospital, we will use the King's Brief Interstitial Lung Disease questionnaire (K-BILD) as a health status measure specifically designed for patients with ILD[51].

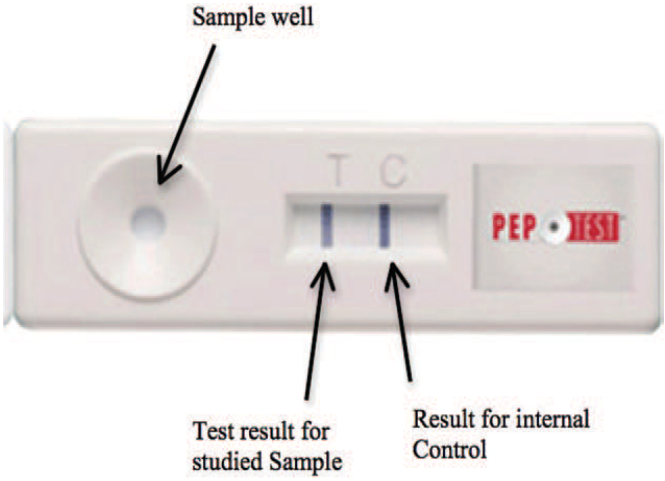


Figure 3. Peptest stick for qualitative analysis. Samples can be sent to the manufacturer for quantitative reading. Peptest is a lateral flow device.

Statistical analysis

There are a number of primary outcomes in relation to the main objectives outlined above. The first objective is to establish whether markers of microaspiration are linked to alveolar epithelial damage which will be measured by serum KL-6 (the dependent primary outcome variable). Markers of microaspiration will include EBC and/or saliva measures of pepsin, bile acids and pH in all patients and BAL measures of pepsin and bile salts in 40 patients. The variables will be transformed as appropriate so as not to violate the assumptions of multivariate linear regression.

All the variables above will be shown as median and/or mean, as appropriate according to distribution; appropriate measures of dispersion will be calculated. Primary outcomes variables (KL-6, microaspiration markers) will represent the dependent variables of multivariate models, taking into account generic (demographics, smoking status, disease duration) and specific (GER markers, autoantibody, lung disease severity) confounders.

Secondary objectives of this study include evaluating the impact on quality of life of GER related symptoms in a SSc-ILD population; for this purpose, responses to a battery of questionnaires specific for GER, SSc and ILD will be used as secondary endpoints. To assess the determinants of respiratory (cough and dyspnea) and reflux symptoms, ordered logistic regression will be used, with symptom scales as the dependent variable, and reflux patterns/markers of microaspiration as covariates, again adjusting for other covariates including ILD severity.

Another aim is to establish the diagnostic value of EBC and saliva analysis, two non invasive tests of microaspiration, using established markers of GER (oesophageal manometry and pH monitoring)

as “gold standard”. Indices of lung function decline, such as fall in FVC or DLCO, will be used as endpoint to test the hypothesis that microaspiration is an independently predictive of ILD worsening.
To evaluate the association between EBC/BAL microaspiration markers and serum KL6, multivariate regression models will be constructed with KL-6 as the dependent variable (outcome), examining as covariates pepsin, pH and/or bile salts (variables of primary interest) as well as gender, duration of systemic disease, smoking status, autoantibody subsets and functional severity of lung diseases (CT extent and/or Goh stage of disease). For multivariate regression analyses, the standard regression diagnostic tests will be used (testing for heteroscedasticity and omitted variables) to ensure that the assumption of multiple linear regression are not violated, and appropriate transformation of data will be performed as required. To assess the determinants of respiratory (cough and dyspnea) and reflux symptoms, ordered logistic regression will be used, with symptom scales as the dependent variable, and reflux patterns/markers of microaspiration as covariates, again adjusting for other covariates including ILD severity, sicca symptoms, smoking and drinking habits, and anti-reflux medication.

We have calculated that the prospective study has a power of ≥80% of detecting an increase of ≥ 2 fold in EBC pepsin between progressive and stable ILD, with an alpha of 0.05, assuming a prevalence of decline in FVC>10% in 20% of patients over 18 months, as expected from the analysis of our previous cohort of SSc-ILD patients. Although this study may not have the power to assess smaller differences in microaspiration markers between patients with stable vs those with progressive ILD at 18 months, the prospective collection of lung function data and analysis with regards to baseline markers of aspiration will continue beyond the period proposed in this plan, up to five years in total, with an increasingly greater number of patients experiencing significant declines. From our previously analysed cohort of SSc-ILD patients, almost 50% of patients experience an irreversible decline in FVC>10% within four years from baseline. We expect a similar proportion of progression in this cohort, which will mean that continued collection of follow up data will continue to increase the power of the study to detect differences between patients with stable versus worsening ILD. We plan to perform a logistic regression analysis with decline of FVC>10% at 18 months as the dependent variable, and markers of microaspiration as the independent variables, again adjusting for ILD severity, age, gender, anti-reflux medication and smoking status. The correlation between BAL microaspiration markers and functional decline will be assessed using the same methods. However, as the number of patients undergoing BAL will be less than those with EBC and saliva measurements, we plan to assess the correlation between BAL and EBC/saliva markers of aspiration, using appropriate methods.
To determine whether non-invasive markers are accurate markers of BAL microaspiration markers, Spearman’s rank correlation test will be used to assess the correlation between BAL and EBC/saliva markers of microaspiration.

Results

So far, 44 patients completed Visit1, 26 Visit 2, 12 Visit 3, 5 Visit 4. Baseline characteristics are summarised in Table 1a. One patient required rescue treatment with rituximab during the study; two patients received IV cyclophosphamide and 2 other patients had their MMF dose increased. Most of the patients were already on treatment with PPI (97%), 56% on high dose PPI (>=twice a day). Moreover, half of the patients were on ranitidine as well, while 25% were on maximal anti-acid treatment (PPI+ranitidine+domperidone).
Manometry was performed in 42 patients and was typical of SSc in 59.5% of the patients. 3 patients required urgent referral to GI specialist due to severe abnormalities detected on manometry. 24H esophageal impedance was carried out in 34 patients. demeester score was abnormal (>14.1) in 50% of the subjects. Other impedance summary data are shown in Table 2a. Anti-GER treatment was modified following impedance results in 8 patients.
Average total RDQ score was lower on PPI compared to off PPI (p=0.005) (Figure 1a). Respectively, 3 and 9 persons scored total RDQ>3 on and off PPI. The other QoL questionnaires did not show significant difference between on and off PPI. Demeester score was significantly higher in patients with RDQ total above 3 compared to <3 (35 vs 4.5,p=0.003). RDQ scored at Visit 2 and 3 were lower than Visit 1 (p=0.02) (Figure 2a).

ELISA for pepsin was carried out in all patients in duplicates. The analysis of duplicated revealed important technical limitation and were not reliable, despite good standard curve (data not shown).
An alternative detection method has been used, however has been tested only on frozen samples so far, which is not recommended by the manufacturer. Pepsin was detected in none of 40 EBC samples, 8 (20%) of raw saliva samples with mean 38pg/ml (SD 103), 3 (27%) of BAL samples with mean 17.8pg/ml (SD 50).

Different correlation patterns were detected for each QoL and GER/ILD questionnaire. RDQ showed significant correlation (p<0.01) with demeester index (r=0.56), Time of acid pH in upright position (r=0.48), % of total time of acid pH (r=0.59), number of heartburns reported (r=0.6).
GIT total score showed significant correlations (p<0.01) with Time of acid pH in upright position (r=0.7), total time of acid pH (r=0.68), % of total time of acid pH (r=0.59), symptom index of cough (r=0.51). GIT heartburn subtotal correlated with demeester score (r=0.64, p<0.01).
Leicester total score showed significant correlations (p<0.01) with Time of acid impedance in upright position (r=0.62), total time of acid pH (r=0.68), % of proximal reflux (r=0.52), symptom index of cough (r=0.64).
K-BILD total score and HADS depression score showed positive correlations, respectively, with Hiatal hernia (r=0.72, p<0.01) and reported belch (r=0.64, p<0.01).
FVC% showed significant correlations (p<0.01) with pH recumbent clearance (r=0.46) and K-BILD total score (r=0.45). DLCO% showed significant correlations (p<0.01) with hiatal hernia (r=0365), pH recumbent clearance (r=0.45) and K-BILD total score (r=0.65).

Lung function time to decline data were available for 34 patients, with a mean follow-up time of 13 months. 3 and 7 patients, respectively, had a 10% FVC predicted and 15% DLCO predicted from baseline. At the univariate analysis, demeester score, % time of acid pH on recumbent position were the strongest predictor of lung function decline (combined outcome of FVC or DLCO decline), see Table 3. Other predictors of disease progression are summarized in table 3. Association to disease deterioration was maintained after adjustment for disease severity (CPI) for most of the predictors listed in table 3a.

	SSc (n=48)
Age	56.4 (29 – 78)
Female	75%
Ever smoker	36%
dSSc	29%
Scl-70	54.5%
BNP *pmol/ml*	37 (12-146)
pH (PAPs > 40mmHg on echo)	22%
On oral prednisolon	73.8%
On other immunosupp	80%
On any immunosupp	89%
Past Cyclo	57%
Past Rituximab	11%
on PPI	98%
FEV1%	70.5 (37-116)
FVC%	74 (37-128)
TLC%	69.6% (44 - 107)
KCO%	69.3 (32 - 108)
TLCO%	42 (18-72)

Table1a. Baseline characteristics. Data are presented as median (min-max) or %.

SSc=Systemic Sclerosis

	Median (min - max)
DeMeester score	5.6 (0.8 - 156)
Total acid	6 (0-56)
Total non acid	8 (1-45)
Proximal acid	2 (0-20)
Proximal non acid	3 (0-19)
Proximal reflux %	50 (0-80)
Non acid reflux %	63 (15-100)
% total time upright acid reflux	0.4 (0-0.6)
% total time recumbent acid reflux	0 (0-2.1)
Impedance recumbent clearance (sec)	0.9 (0-10.1)
Cough index association %	0 (0-88)

Table 2a. 24h esophageal impedance measures, reported as median (min-max)

	HR	p	
Demeester	1.031	0.005	$
Acid	1.06	0.01	$
Non acid	1.06	0.048	
Prox acid	1.2	0.08	
Tot reflux	1.03	0.01	$
ph_recumbent_minute	1.01	0.004	$
Ph total min	1.01	0.04	$
Impedance tot time %	2	0.039	$

Table 3a. Univariate analysis results of FVC/DLCO decline cox regression analysis.
$=p<0.005 when adding the composite physiologic index (CPI) in the multivariate model

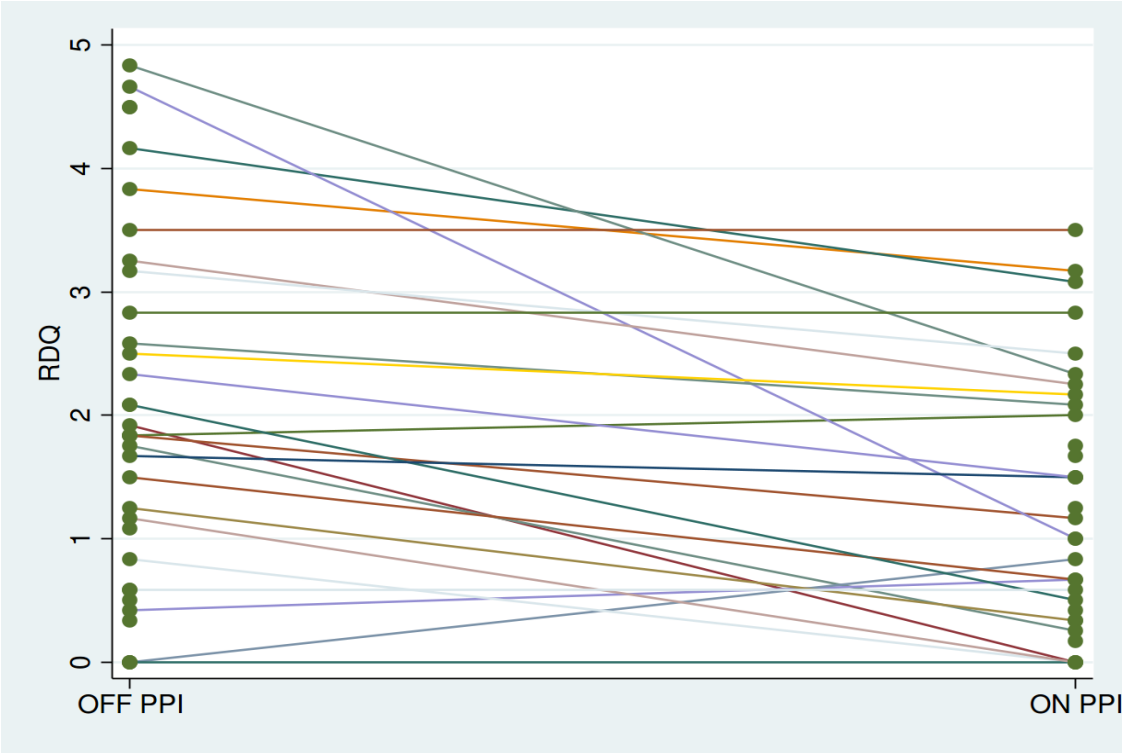


Figure 1a. RDQ scores of the same patients on and off PPI

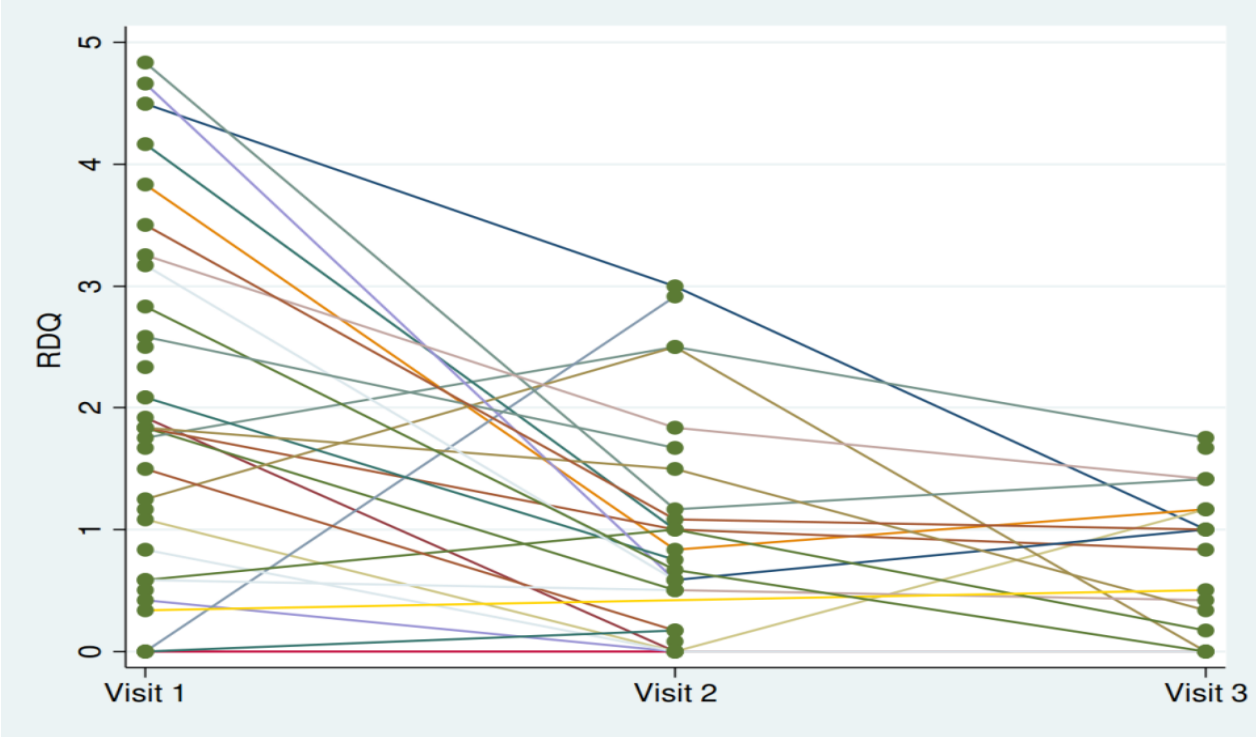


Figure 2a. RDQ scores at Visit 1, 2 and 3.

DISCUSSION

The preliminary results showed oesophageal motility measures are independent predictors of SSc-ILD progression, despite ongoing anti-acid treatment. Furthermore, there were several correlations between QoL/symptoms questionnaires/gullet motility tests and lung function. ELISA may not be a reliable detection method for pepsin on saliva and BAL, while peptest showed promising results.

Oesophageal manometry and 24h impedance monitoring are not routinely performed in SSc patients in clinical practice. The fact that impedance measures were associated to disease progression, independently of ILD severity, suggests that manometry and/or 24h impedance monitoring may guide the clinical management of SSc-ILD. A total of 10 patients required GI referral or treatment changes following those investigations. However, oesophageal manometry and impedance are semi-invasive methods and can be poorly tolerated by patients. Among the QoL and symptoms questionnaires used in this study, only RDQ showed a significant difference between on and off PPI and between follow-up visits. RDQ score was correlated with several impedance measures. RDQ may be used as a screening tool to select candidates for oesophageal motility tests.
In addition to the well reported clinical association between GER in SSc and lung involvement[30,52,53], data from animal models and "in vitro" analysis have provided a biologic rationale supporting the role of microaspiration in the genesis and/or progression of ILD. Pepsin and bile acids have a citotoxic effect on epithelial cells and can induce a pro-inflammatory response including expression of cytokines and chemokines[54]. In a rat model of chronic aspiration, interstitial pneumonitis changes followed the instillation into the airways of either whole gastric or neutralized gastric fluid, but not of hydrochloric acid[55]. Indeed the fact that the refluxate loses its acidity when it approaches the proximal esophagus, may suggest that pepsin or other proteins found in the stomach content, instead of the acidity, are causative agents of lung fibrosis. Pepsin is inactive in an alkaline environment, but it remains stable and can reactivate when exposed again to acid (other reflux episodes) or taken up within epithelial cells by endocytosis[56]. It would be tempting to suggest pepsin concentration in saliva and/or BAL as a noninvasive marker of GI and lung involvement in SSc, however we have not collected enough data yet.

It is important to bear in mind that GER is frequent in a number of chronic lung diseases and could simply be related to greater variation in the intrathoracic pressures, as the lung compliance worsens with disease progression[57]. Indeed, it has been particularly difficult to ascertain how much GER is a consequence of lung disease severity, rather than a driver of lung disease progression. In our knowledge, this study is the first to consider the confounding role of ILD severity in the impact of GER on ILD-SSc.

Surprisingly, RDQ score improved consistently from Visit 1 to Visit 2 and 3. It is possible that patient compliance to GI medications improved throughout the study for the higher frequency of follow-up and increased insight on GER problems.

As discussed in details in the 1st section of this manuscript, non-acid and/or proximal reflux are thought to be common in Ssc[58]. Our preliminary data showed acid reflux, compared to non acid and proximal reflux, has a stronger association to GI and lung disease severity in SSc. This may be explained by the putative more toxic potential of an acid content, possibly causing more significant damage to the respiratory epithelium. Clinical symptoms are felt not to be reliable as a screening tool for GI involvement SSc[18], and our data are in keeping with this.

Limitations of this study included the selection bias in terms of ILD severity, which may underestimate the results. Bearing in mind that the study is still ongoing and that not all clinical data

are available (such as CT scoring, KL6 levels, peptest results), numbers are small and results should be replicated in an independent cohort.

Pleuroparenchymal fibroelastosis pattern in IPF

Pleuroparenchymal fibroelastosis (PPFE) is a rare interstitial lung disease (ILD) characterized by pleural and sub-pleural fibrosis with striking upper lobe predominance[4] (Figure 1b). Its histopathological hallmark is elastic intra-alveolar fibrosis of subpleural regions. The etiopathogenesis of PPFE is unknown, most cases being labeled as idiopathic, and a minority can be associated with chemotherapy and bone-marrow transplant. Idiopathic PPFE has been recently included as a separate entity in the ATS/ERS classification of idiopathic interstitial pneumonias[4].

PPFE often coexists with another ILD pattern on chest CT or histopathology, and UIP has been the most common reported so far. PPFE-like histology changes can be detected in mid-lower lobes[5,59]. Thus far only a retrospective survey conducted by a japanese group has analyzed the prevalence of PPFE in IPF, and it was confirmed that PPFE is not rarely detected in IPF and shows indistinguishable histological features compared to the idiopathic form[59]. In the context of an established diagnosis of IPF or another major interstitial pneumonia, lung biopsy is not currently recommended for the only purpose of PPFE confirmation, and the diagnosis is reached on the ground of clinical and HRCT[60] characteristics.

Immune dysregulation is thought to have a major pathogenetic role in idiopathic PPFE, its treatment often consisting of a delicate balance between prevention of chest infections and careful low dose immunosuppression. This is supported by previous reports showing the occurrence of PPFE post transplantation and in association with recurrent infections[5,61–65]. Autoimmune disregulation may play an important role in IPF as well, and this may explain the reported presence of PPFE in UIP/IPF[66]. As an example, Anti–heat shock protein 70 (HSP70) antibodies were associated with poorer outcome in IPF in a recent study[67].

The main aims of this study are: to estimate the prevalence and the prognostic value of PPFE in a large unselected consecutive group of IPF patients, describe the clinical phenotype of PPFE and IPF, proposing PPFE and/or freestanding bronchiectasis as a marked of immune dysregulation.

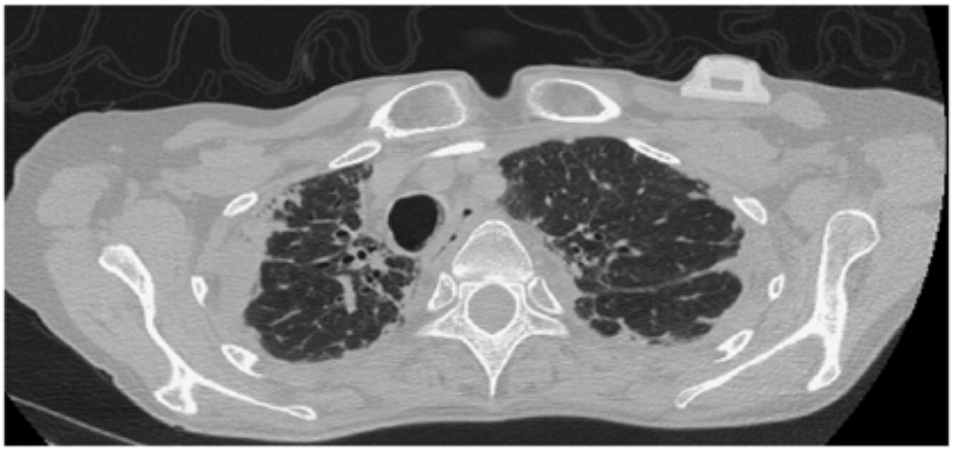


Figure 1b. Chest CT of a PPFE study-patient.

METHODS

Study population and clinical information:

A retrospective analysis of an interstitial lung disease database identified all new consecutive patients with a multidisciplinary team diagnosis of idiopathic pulmonary fibrosis (IPF) according to ATS/ERS published guideline and/or diagnostic inclusion criteria of INPULSIS trial [4,68], over a four and a half year period (January 2007 to July 2011). Patients with a non-contrast, supine, volumetric thin section CT were captured, and subsequent exclusions are shown

Approval for this retrospective study of clinically indicated CT and pulmonary function data was obtained from the Institutional Ethics Committee and informed patient consent was not required.

Pulmonary function tests:

Pulmonary function tests (lung volumes, Dlco, SpO2 at rest) were analyzed if performed within 6 months of the corresponding HRCT scan. Pulmonary function tests results were expressed as percent predicted values using the patient's age, sex, race and height. The composite physiological index (CPI) was calculated using the formula: 91.0 - (0.65 x % predicted DLco) - (0.53 x % predicted FVC) + (0.34 x % predicted FEV_1)[69].

Chest CT protocol:

The CT scans were obtained using a 64-slice multiple detector CT scanner (Somatom Sensation 64, Siemens, Erlangen, Germany) or a 4-slice multiple detector CT scanner (Siemens Volume Zoom, Siemens, Erlangen, Germany. To satisfy requirements for processing by the CALIPER algorithm, all scans were reconstructed using a high spatial frequency, B70 kernel (Siemens, Munich, Germany). All patients were scanned from lung apices to bases, supine, at full inspiration, with 1.0mm section thicknesses using a peak voltage of 120kVp with tube current modulation (range 30-140 mA). Images were viewed at window settings optimized for the assessment of the lung parenchyma (width 1500 H.U.; level -500 H.U.).

Evaluation of underlying CT fibrotic pattern

To establish the underlying pattern of fibrosis in each case, a single radiologist (DMH) with over 30 years thoracic imaging experience, who was blinded to all clinical information, evaluated every volumetric CT. The CT fibrotic pattern was categorized as representing either a definite or possible UIP pattern, or a pattern inconsistent with UIP according to the 2011 ATS/ERS/JRS/ALAT IPF diagnostic guidelines[70].

CT scoring of parenchymal features:

Each CT was also independently evaluated for features of interstitial lung disease (ILD) and PPFE by two radiologists (WH, HB) with 3 and 4 years thoracic imaging experience respectively, blinded to all clinical information. An initial training dataset of 15 non-study cases was used to help to identify pre-existing biases. The scores of the test cases were reviewed and the most widely discrepant results discussed with a third radiologist (JJ).

i) Parenchymal pattern extent scoring:

All CTs were scored at five representative axial CT levels using a continuous scale. The anatomical levels chosen were as follows: (1) the origin of the great vessels from the aorta, (2) the main carina, (3) the pulmonary venous confluence, (4) a point halfway between level 3 and 5, (5) immediately above the dome of the right hemidiaphragm.

At each level, the total extent of ILD and emphysema were initially estimated to the nearest 5%. The scores for the five sections were averaged to generate overall ILD and emphysema extents for each patient. The ILD score at each level was then sub-classified into four patterns: reticular pattern, ground glass opacification with traction bronchiectasis, ground glass opacification without traction bronchiectasis and honeycombing, using definitions from the Fleischner Society glossary of terms

for thoracic imaging with minor modifications. To derive a CT level percentage for each of the four parenchymal patterns, the total ILD extent scored on a single level was multiplied by the individual parenchymal pattern extent at the same level and divided by 100. The four parenchymal pattern scores were then averaged across the five sections to generate a mean percentage for each pattern for each patient.

ii) PPFE and airway scoring

PPFE was scored on CT as pleurally or parenchymally based, well-demarcated, angular consolidative aggregations with or without evidence of volume loss. Such aggregations were separately categorized as an apical pleural cap if they occurred within 1cm, in the z-axis, of the lung apex. CTs were scored for PPFE on a lobar basis using a categorical scale that took into account the average extent of involvement of the pleural surface. PPFE was given a lobar score of: absent =0, <10% of pleural surface =1, 10-33% of pleural surface =2, >33% of pleural surface =3. The lobar PPFE scores were then summed following consensing (described below) for analysis.

Bronchial wall thickening and dilatation were scored on a lobar basis using four-point categorical scales. Bronchial wall thickening was scored subjectively using the following scale: absent =0, trivial =1, mild =2, moderate to severe =3. Bronchial wall dilatation was scored in relation to the diameter of the accompanying pulmonary artery as follows: diameter less than adjacent pulmonary artery =0, diameter equal to adjacent pulmonary artery =1, diameter>1x but <1.5x diameter of the adjacent pulmonary artery =2, diameter>1.5x diameter of the adjacent pulmonary artery =3.

Binary presence/absence scores for each lung were derived for four separate variables: (1) As previously stated, consolidation or fibrosis occurring within 1 cm (in the z-axis) of the lung apex was categorized as an apical cap and was thereby distinguished from PPFE. (2) Concentric pleural thickening separate to areas of PPFE involving over 15% of the surface area of one hemithorax were documented as smooth pleural thickening. (3) The presence of calcified pleural plaques consistent with asbestos exposure were noted; as was fibrocalcific disease in the upper lobes compatible with old TB (4). Lastly, for each individual patient, evidence of a focal mid-line depression of the skin contour in the immediate suprasternal space was also documented.

iii) Consensus derivation for PPFE scores and final identification of PPFE cases

Given that PPFE is a relatively new radiological sign, deriving a consensus for the PPFE scores of the two radiologists, was achieved with a third experienced scorer (DMH) with over 30 years thoracic imaging experience. The main aim of consensing was to identify, on a lobar basis, those cases in which the presence of PPFE may have been over-scored. Therefore, any case in which only one of the original two scorers had identified PPFE in the lungs (presence versus absence of PPFE) was adjudicated by the third scorer. Furthermore, as an additional failsafe to identify over-estimation of PPFE, any cases in which the maximum lobar PPFE extent identified by both scorers was <15% (Grade 1/trivial PPFE), was also adjudicated by the third scorer. Once a consensus for all the lobar scores had been reached, the lobar scores were summed for each patient (PPFE extent). Presence of PPFE was defined as PPFE extent>0; PPFE moderate-severe when PPFE extent >= 2.

Statistical analysis

Data are presented as mean and SD, median and IQR, or percentage as required. Statistical analysis was performed using STATA (v. 14, StataCorp, Texas). Differences between groups were analyzed using either t-test, Mann-Whitney, Kruskal Wallis or Chi-square as appropriate. Cox regression model was used to estimate the association between mortality/lung function decline and PPFE. The assumption of proporzional hazard was assessed using Schoenfeld residuals after the estimation of the univariate and multivariate models. The following covariates were included in all multivariate cox-regression models, unless specified differently in the result section: age at the time of chest CT, gender, smoking status, average ILD extension on CT, presence of bronchiectasis. Study outcomes (survival, lung function deterioration) were analyzed for 3 groups: (1) demographics including age, gender, smoking status; (2) measures of ILD severity; (3) PPFE status. There is no agreement on the

method to estimate the C statistic of cox survival models. C-statistic is considered useful to evaluate the general adequacy of risk prediction models. We computed the C-statistic using the Harrell's C coefficient. A value of 0.5 indicates no predictive discrimination, and values of 0 or 1.0 indicate perfect separation of subjects with different outcomes. In general, a C-static >0.7 suggests substantial concordance.
The weighted Kappa statistic was used to estimate the interobserver agreement for the categorical variables PPFE and freestanding bronchiectasis.
In order to validate the results in terms of risk prediction, we used bootstrap resampling which allows estimation of the standard error and confidence intervals. A two sided p value < 0.05 was considered statistically significant for all the analysis.

RESULTS

Prevalence of PPFE, clinical and radiological features

Patient characteristics, stratified according to the three categories of PPFE extent, are summarized in table 1. PPFE elements were detected in 92 out of 274 IPF patients (33.6%). Patients with PPFE were characterized by lower FVC levels, higher prevalence of freestanding bronchiectasis, higher count of lymphocytes in the BAL and higher prevalence of lifelong never smokers. Two thirds of the PPFE subgroup had extensive PPFE (66%). Limited and extensive PPFE showed comparable demographics, lung function parameters, smoking status and PH prevalence.

Baseline HRCT measurements are summarized in table 2. Morphological associations with PPFE (table 2) consisted of freestanding bronchiectasis ans presence of emphysema (both $p<0.0001$). BAL neutrophil count was higher in patient with freestanding bronchiectasis.

PPFE was independently associated both with presence of standalone bronchiectasis (OR 44.1, 95% CI 10.2-195.4, $p<0.0001$) and lower prevalence of emphysema (OR 44.1, 95% CI 9.14-195.4, $p<0.0001$).

Interobserver agreement and adjudication process

Both absence/presence of freestanding bronchiectasis and absence/presence of PPFE elements showed a substantial inter-observer agreement ($k=0.7$). Similar values were obtained for absence/presence of emphysema ($k=0.68$) and identification of the three PPFE categories ($k_w=0.75$).

Final discrepancies between the two scorers in the adjudications of presence and grade of PPFE occurred in 41 out of 267 patients (15%). Additional 8 patients were scored with trivial PPFE on both upper lobes by both scorers, and 1 patient had no PPFE on the leff upper lobe and moderate PPFE on right upper lobe. Overscoring could be ascribed to: (1) diffuse ILD with more prominent abnormalities on lower lobes, and, as part of the same process, similar changes on upper lobes mimicking pleuro-parenchimal abnormalities; (2) apical pleural cap; (3) extrapleural irregularities spreading into fissures; (4) presence of extrapleural fat; (5) false positive abnormalities mimicking PPFE, e.g. dendriform ossification (figure ???).

Subanalysis of extensive PPFE vs limited PPFE

No patient with limited PPFE showed presence of standalone bronchiectasis on chest CT, while it was found on half of the extensive PPFE group ($p<0.0001$). Other clinical and radiological parameters showed borderline or no significant difference between the two groups (table1).

Presence of PPFE elements: association with functional decline and survival

204 of 274 patients (74.5%) died during a median follow-up of 25 months. Two and five year survival were, respectively, 54.4% and 28.1%. Decline in FVC was seen in 132 of 209 patients (63%), with a median time to decline of 9.4 months. Decline in DLco was seen in 137 of 209 patients (65.6%), with a median time to decline of 9.2 months.
Presence of PPFE elements on HRCT was associated with increased mortality (HR: 1.71 CI: 95% 1.24 – 2.36, p=0.001), decreased time to decline in FVC (HR: 2.24 CI: 95% 1.52-3.31, p<0.0001) and DLco (HR: 2.74 CI: 95% 1.82-4.13, p<0.0001) (table 3). Other univariate predictors of survival and lung function deterioration are shown in table 3.

When adjusted for demographics and chest CT extent, presence of PPFE showed a bordeline association with survival and was an independent marker of FVC and DLCO (see tablexxxx). The C-index of this model was 0.66. Using CPI instead of CT extent as marker of severity, the survival association with PPFE reached statistical significant (see supplementary material???). The confounding effect of pulmonary hypertension on survival was analyzed on a subgroup of 145 patients. Running the multivariate model without the inclusion of PH in this subgroup showed an adjusted HR of 1.75 (CI 95%: 1.16 – 2.65, p=0.007); the inclusion of PH as a covariate gave comparable results in terms of effect size (HR_{adj}: 1.79 CI: 95% 1.18 – 2.7, p=0.006).

Extensive PPFE was associated with mortality independently of ILD extent. After adjusting for demographics, smoking status, extent on HRCT, the association of extensive PPFE elements with survival, FVC and DLCO decline was confirmed (table 4). The C-index of the survival model was 0.66 (CI 95% 0.62-0.70). Using the CPI as measure of lung severity in the cox multivariate models gave similar results (data not shown).

Sub analysis in patients with surgical lung biopsy and influence of the CT classification ?????

Definite UIP pattern on CT was found in 35% of both PPFE and no PPFE subgroups (p=0.9) (Table 2b). PPFE association with mortality was not confounded by neither the radiological classification of UIP nor applying the ATS/ERS classification of IPF. The inclusion of the CT classification in the multivariate survival model confirmed the 66% increase of mortality associated with the presence of severe PPFE (HR_{adj}: 1.67 CI: 95% 1.2 – 2.3, p=0.002).

Prevalence and extent of PPFE were not different between patients with and without biopsy. Stratifying the analysis according to the ATS/ERS diagnostic criteria [ref] this classification did not show significant differences in terms of association between presence of extensive PPFE and survival. On multivariate analysis, subjects with definite IPF and MDT diagnosis IPF without meeting ATS/ERS criteria showed, respectively, an excess of 72% (p=0.022) and 61% of mortality due the presence of extensive PPFE.

DISCUSSION

PPFE elements are prevalent in IPF and are associated with worse survival. PPFE in the context of IPF, compared to IPF alone, has a distinctive phenotype characterized by a striking prevalence of freestanding bronchiectasis, prominent BAL lymphocytosis, more restrictive disease and increased frequency of never smokers. PPFE, with or without freestanding bronchiectasis, is a marker of immune dysregulation in IPF.

Our study showed a prevalence of 33% of PPFE in IPF. A case series of 12 PPFE cases found an UIP pattern on histology in 25% of the patients[5], while a recent retrospective study in 110 IPF Japanese patients showed a lower PPFE prevalence of 10% using radiological criteria or 8% using histological criteria[71]. We found 40% of patients satisfied the HRCT criteria for PPFE in the subgroup of 50 subjects with UIP pattern on pathology. Compared with Oda's study, our patients with histology data had similar age, slightly lower FVC and far lower DLCO. It is possible that PPFE prevalence is influenced by ethnicity or genetic background.

The clinical and radiological features of PPFE in the context of IPF have many similarities with IPPFE. The pleuroparenchimal abnormalities on CT are indistinguishable between IPPFE and PPFE in the context of other ILDs[65]. PPFE has specific histological characteristics, however lung biopsy is rarely warranted due to the high risk of pneumothorax[5,66,72]. IPPFE is thought to occur more frequently in non smokers, at age below 60 years, equally in male and females[60]. Platythorax, the anterioposterior flattening of the chest, has been described in IPPFE[73]. A recent survey reported shorter anteroposterior chest diameter in PPFE and IPF compared to IPF alone[59]. Our study did not include the presence of plathythorax, however the lower mean FVC% in PPFE and IPF compared to IPF alone would fit with more frequent plathythorax in the PPFE subgroup.

The striking prevalence of freestanding bronchiectasis and BAL lymphocytosis inflammation confirms the role of immune dysregulation in the pathogenesis of PPFE even in the context of other ILD. This is indeed in keeping with data gathered from small series of IPPFE. Reddy et al reported a history of recurrent chest infections for 7 out of 12 IPPFE patients, 4 of whom had positive autoantibodies[5]. Fungal or atypical mycobacterial infections[5,74,75] and daptomycin-induced eosinophilic pneumonia[76] have been reported as well. PPFE has been described as a rare complication of some chemotherapeutic agents, lung, bone marrow or haematopoietic stem cell transplantation[74]. SP-D is implicated in the immune host defence of the lung, and is elevated in the BAL and/or serum of several ILD subtypes[77]. A recent small series of idiopathic PPFE found increased serum SP-D levels in 82% of 17 IPPFE patients[78]. Currently, although not proved and often not effective to slow down the disease progression, the mainstay of the treatment for idiopathic PPFE is a delicate combination of prophylactic antibiotics and low dose immunosuppression.

Our radiological classification of PPFE was different from previous studies. For example, ODA et al identified patient as having "definite", "consistent" or "inconsistent" with PPFE on the basis of the preferential distribution of the pleuroparenchimal lesions on upper lobes[71]. Our PPFE staging was obtained using the average PPFE extent (visually estimated according to the amount of pleural involvement) across all lobes. In our knowledge, this is the first survey providing interobserver agreement data for PPFE. The kappa score for presence of PPFE was substantial, close to the previously shown high reproducibility of other HRCT features such as freestanding bronchiectasis and UIP[79].
Recent research has shown the potential clinical role of immune dysregulation in IPF patients. A well conducted retrospective study suggested that autoantibodies to heat shock protein 70 were associated to worse survival in 121 IPF patients[67]. The discovery of the single-nucleotide polymorphism rs35705950 of the MUC5b gene in around 30% if IPF subjects in the study of

Seibold et al started a new era in the ILD research[80]. Since then, several groups have confirmed the role of MUC5b and airway mucosal host immunity in IPF[81–83]. MUC5b is more expressed in the distal airways than honeycomb cysts of IPF patients. Compared to controls, IPF subjects have a higher BAL bacterial load, which is linked to survival and absence of the minor allele rs3570950 MUC5b polymorphism[84]. A randomized controlled trial published in 2013 found an increased survival of IPF patients trated with co-trimoxazole[85]. Taken together, these findings may support the role of immune-driven damage in a subgroup of IPF patients, eventually leading to PPFE-like changes. We suggest that PPFE may be considered a marker of clinically significant immune-disregulation in IPF.

PPFE is an independent predictor of survival and disease deterioration in IPF, even after taking into account... It is not clear why a disease apparently confined to the upper-lobes can confer such risk of mortality. Firstly, previous studies reported histological characteristics of PPFE in middle and lower lobes as well[64,76,86]. Some of our patient had radiological evidence of PPFE outside the upper lobes. Secondly, the combination of low FVC, normal FEV1/FVC ratio and high RV/TLV ratio in PPFE may suggest abnormal chest wall expansion. Thirdly, the background immune-driven dysregulation and frequent chest infections may reduce progressively the lung functional reserve and/or trigger the progression of IPF.

Our study has a number of limitations. This is a retrospective study, therefore possible unknown sources of bias may have not been taken into account. However survival data for patients lost at follow-up have been updated contacting patients' GP, and we had consistent results in terms of survival prediction when using in turn different estimations of lung disease severity. We had a relatively low number of PPFE cases, but survival outcomes have been validated using a bootstrapping method. Regarding the presence of pulmonary hypertension, only trans-thoracic echocardiogram data were available. To reduce the risk of overestimation, we used a conservative threshold of RVSP (50mmHg).

In summary, we suggest IPF-associated PPFE is a distinct IPF phenotype characterized by immune-dysregulation and worse survival compared to IPF alone. PPFE is surprisingly prevalent in IPF, and its impact on treatment response and disease management in general should prompt future research. A unified radiological classification of PPFE is needed as well to compare results between different studies.

Table 1. Patient characteristics.

	No PPFE (n=177)	Limited PPFE (n=29)	Estensive PPFE (n=61)	p
Gender: Female *n (%)*	41 (22.5)	6 (19.4)	15 (24.6)	NS
Age *Mean, SD*	66.3 (8.9)	64.7 (10.5)	67.2 (9.1)	NS
Smoking Ever smoker *n, (%)*	126 (69.2)	18 (58.1)	34 (55.7)	NS
Lung function *mean (SD)*				
FVC % of predicted	71.3 (20.5)	63.7 (16.8)	61.2 (19.6)	0.0004
FEV1 % of predicted	72.2 (19.1)	68.3 (16)	67.1 (20.3)	0.06
DLco % of predicted	36.9 (12.9)	37.7 (13.1)	34.2 (13.4)	NS
RV/TLC	37.5 (7.9)	36.6 (6.5)	39.9 (7.7)	0.046
CPI *mean (SD)*	53.9 (11.3)	55.6 (10.8)	59 (11.9)	0.007
PH	41 (51.3)	11 (45.5)	24 (55.8)	NS
BAL *mean (SD)*				
Neutrophils %	13.2 (15.4)	6.8 (5.3)	18.8 (16.3)	0.004
Lymphocytes %	6.7 (5)	8.5 (5.9)	11.3 (7.1)	0.005
Eosinophils %	5 (5.1)	4.2 (3.4)	5.4 (4.5)	NS

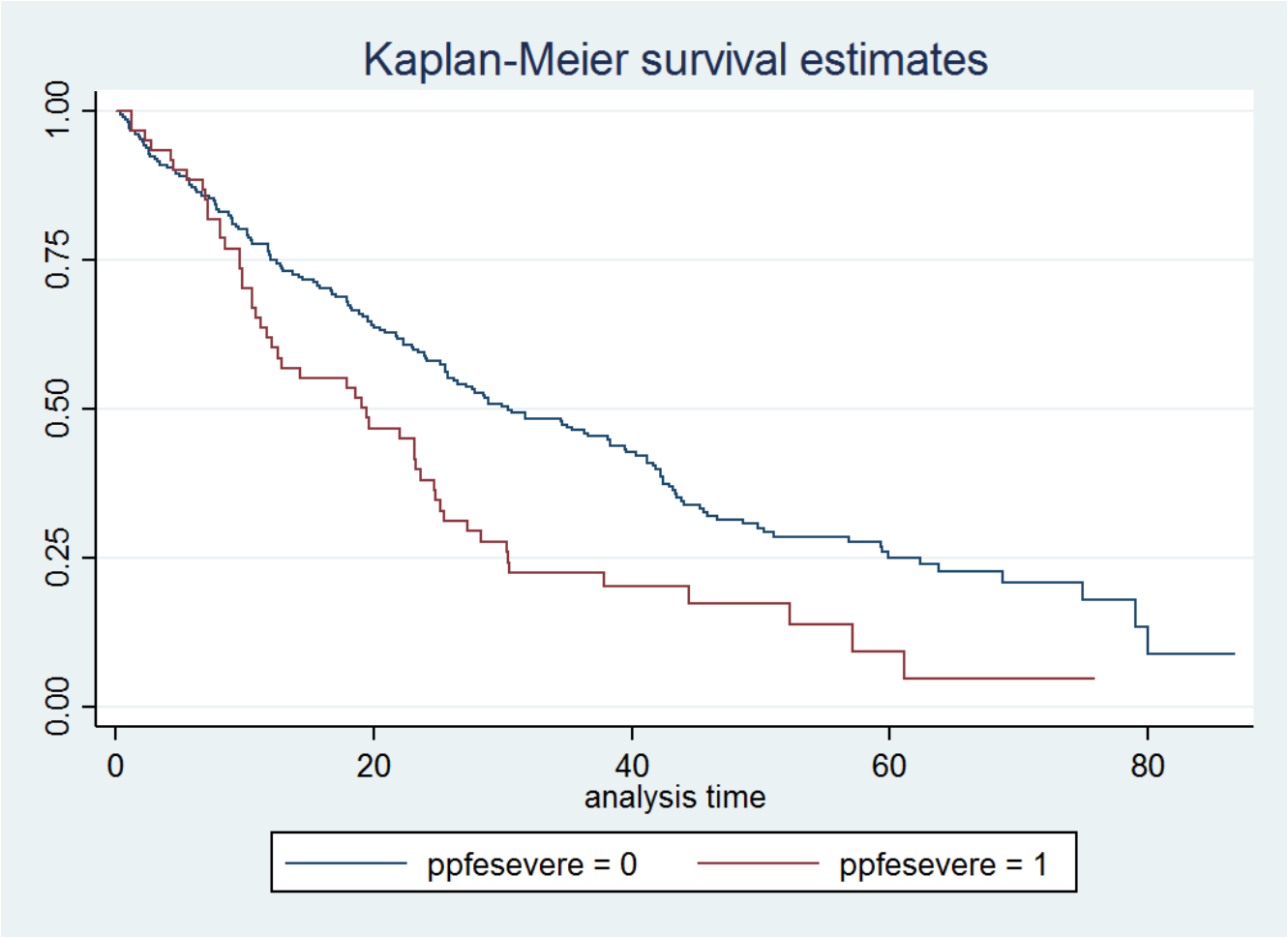
NS: $p > 0.05$

Table 2. High resolution computed tomography parameters in presence versus not presence of PPFE element

	No PPFE (n=182)	Limited PPFE (n=31)	Estensive PPFE (n=61)	p
Overall extent of fibrosis *Mean (SD)*	37.1% (13.7)	41.8% (14.4)	40.2% (11.7)	NS
Reticulation *Mean (SD)*	33.7% (11.6)	37.7% (11.8)	37.1% (9.3)	0.025
Ground glass attenuation (no tractions) *Mean (SD)*	0.1% (0.85)	0.28% (1.1)	0 (0)	NS
Ground glass attenuation (with tractions) *Mean (SD)*	1.2% (2.7)	1.6% (3.3)	1% (3.7)	NS
Honeycomb *Mean (SD)*	1.3 (4.7)	2.3% (7.2)	1.6 (7.2)	NS
Emphysema				
Total extent *mean (SD)*	7.6 (12.8)	1% (2.9)⌋	1.8 (4.9)¤	0.0001
Present *n (%)*	76 (45.5)	6% (20)⌋	11 (18.3)¤	<0.0001
Freestanding bronchiectasis *n (%)*	2 (1.1)	0 (0)	31 (50.8)¤†	<0.0001
CT classification^ *n (%)*				
definite / possible UIP	63 (34) / 119 (65)	9 (29) / 22 (71)	23 (38) / 38 (62)	NS

NS: p value > 0.05

¤: significant difference compared to no PPFE ($p < 0.05$)
†: significant difference compared to limited PPFE ($p < 0.05$)
⌋: significant difference compared to no PPFE ($p < 0.05$)



P=0.0005 (log-rank)

Figure 2b. Kaplan Meier graph of mortality according to PPFE status.

Table 3. Relationship between presence of PPFE elements and functional decline/mortality (univariate analysis)

HR (p)°	**Time to FVC 10% decline**	**Time to DLco 15% decline**	**Mortality**
extensive PPFE yes/no	2.24 (<0.001)	2.73 (<0.0001)	1.71 (0.001)
Extent	1.01 (0.07)	1.01 (0.057)	1.04 (<0.0001)
Bronchiectasis yes/no	2.13 (0.003)	1.85 (0.02)	1.47 (0.07)
Honeycomb yes/no	1.4	1.1	1.49 (0.019)
FVC%	0.99	0.99 (0.003)	0.97 (<0.0001)
DLCO%	0.98 (0.006)	0.98 (0.031)	0.94 (<0.0001)
CPI	1.02 (0.01)	1.02 (0.003)	1.07 (<0.0001)

°p reported in brackets when <0.1

Extent = Overall extent of fibrosis
PPFE = Pleuroparechymal fibroelastosis
CPI = Composite physiologic index [REF]

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