Combined pulmonary fibrosis and emphysema in scleroderma lung disease has a major confounding effect on lung physiology and screening for pulmonary hypertension

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ABSTRACT

Objective: In systemic sclerosis (SSc), pulmonary function tests (PFTs) have been included in screening algorithms for pulmonary hypertension (PH). However, in combined pulmonary fibrosis and emphysema (CPFE), the interpretation of PFT is confounded. We examined the prevalence of CPFE in SSc patients with interstitial lung disease (SSc-ILD) and the effect of CPFE on PFTs used to evaluate the severity of SSc-ILD and the likelihood of PH.

Methods: HRCT scans of 333 SSc-ILD patients were evaluated for the presence of emphysema, extent of interstitial lung disease. The effects of emphysema were quantified on relationships between pulmonary function variables and a) the extent of SSc-ILD on HRCT; and b) echocardiographic evidence of PH.

Results**:** Emphysema was present in 41/333 cases overall (12.3%), in 26/132 smokers (19.7%) and in 15/201 life-long non smokers (7.5%). With the extent of fibrosis taken into account, emphysema was associated with signficant additional changes from expected values in DLco (- 24.1%, $p<0.0005$), pO2, ($p=0.04$), and FVC/DLco (+ 34.8%, $p<0.0005$) but not in FVC. These effects were identical in smokers and non-smokers. On multivariate analysis, the presence of emphysema had a greater effect than echocardiographic PH on FVC/DLco, whether analysed as a continuous variable or using thresholds of 1.6 or 2.0.

Conclusion: In SSc-ILD, emphysema is sporadically present in non-smokers and is associated with a low pack-year smoking history in smokers. The confounding effect

of CPFE on measures of gas exchange has major implications for the construction of screening algorithms for PH in SSc-ILD.

INTRODUCTION

In systemic sclerosis (SSc), pulmonary involvement (including interstitial lung disease and pulmonary hypertension [PH]) is the most common cause of death (1). Interstitial lung disease in SSc [SSc-ILD] ranges from sub-clinical lung involvement to major pulmonary disease progressing to respiratory failure and death (2). The severity of SSc-ILD is most **frequently** quantified in routine practice using pulmonary function tests. Carbon monoxide diffusing capacity (DLco) levels best reflect the morphologic extent of SSc-ILD on high resolution computed tomography (HRCT) (3) but are also profoundly affected by presence of PH. More recently, an increased FVC/DLco ratio has been advocated as a determinant of which SSc patients should undergo echocardiographic screening for PH (4-7).

There is reason to suspect that concurrent emphysema may seriously confound pulmonary function tests in SSc-ILD. In idiopathic pulmonary fibrosis (IPF), concurrent emphysema has been associated with spurious preservation of forced vital capacity (FVC) levels, devastating reductions in DLco levels (8) and, by implication, major effects on FVC/DLco ratios. Parallel analyses have yet to be performed in suitably large cohorts of SSc-ILD patients, despite the fact that the combination of pulmonary fibrosis and emphysema (CPFE) occurs in SSc (9).

Therefore, the aims of our study were a) to examine the prevalence of emphysema in a large SSc-ILD cohort and b) to determine whether the presence of emphysema has a

major confounding effect on pulmonary function tests used to evaluate interstitial lung disease severity and the likelihood of PH in SSc-ILD.

PATIENTS and METHODS

Methods

The study population consisted of 333 patients with SSc-ILD, referred between January 1990 and October 2004 (age 54.4 ± 13.1 years, 262 females), fulfilling the American College of Rheumatology criteria for systemic sclerosis (10**)**, with no overlap features. During this time period, our major rheumatological collaborators referred unselected patients with known or suspected SSc-ILD. After October 2004, this policy was changed to one of referral of selected patients. The diagnosis of SSc-ILD was based on evidence of interstitial lung disease on HRCT. Exclusion criteria consisted of a) the performance of HRCT scans elsewhere, unavailable for scoring; b) the separation of baseline HRCT and PFT by more than 90 days and c) smoking history not recorded (n=3). These exclusions apart, the cohort was consecutive and investigations were performed as part of a prospective clinical protocol. **Baseline data are shown in Tables 1 and 2: analyses of serological status were confined to patients undergoing testing at presentation in the same laboratory (n=308).**

Clinical data

"Ever smokers" had smoked at least one cigarette per day for at least one year. Pack-year smoking histories were recorded. In three cases, the smoking history was considered to be unreliable: these patients were excluded from the analyses.

Pulmonary function tests, echocardiography and HRCT were performed as previously reported (11-12).

Echocardiographic evaluation included estimation of pulmonary arterial systolic pressure from tricuspid regurgitant jet velocity, the identification of right ventricular or right atrial enlargement, and the evaluation of right ventricular function. **Echocardiographic pulmonary hypertension was defined as a pulmonary arterial systolic pressure of 40 mm Hg or greater**. Echocardiography was routinely performed at presentation: 25 of 333 patients not undergoing echocardiography at our institution were excluded from echocardiographic analyses.

HRCT sections (1.5mm - 3mm) were acquired supine, at full inspiration, at 10mm intervals (window center = -550 Hounsfield units (HU); window width = 1500 HU), using an electron beam scanner (Imatron Inc., San Francisco, CA). Scans were scored independently by two observers (AUW and SRD), blinded to clinical and lung function information. HRCT features were scored as five levels, defined using anatomical landmarks, as described previously (13-14), as follows:

- The extent of interstitial lung disease was estimated to the nearest five percent in each section, with global extent of disease on HRCT computed as the mean of the scores for section. Discrepancies of more than 20% in global scores were resolved by consensus.

- The extent of emphysema was estimated to the nearest five percent in each section, with global extent of disease on HRCT computed as the mean of the scores for section. Disagreement on the presence or absence of emphysema and discrepancies of more than 20% in global scores were resolved by

consensus. **To be scored as present, emphysema was required to be separate geographically from interstitial fibrosis. Two patients, in whom there was limited emphysematoid destruction within fibrotic lung, with no additional emphysema in other lung regions, were scored as not having emphysema.**

- **The distributions of interstitial lung disease and emphysema were quantified as upper zone extent/(upper + lower zone extent), adjusted to a percentage value. Upper zone extent was computed as the average extent of disease in the two uppermost sections. Lower zone extent was computed as the average extent of disease in the three lowermost sections.**

Data analysis

Analyses were performed using STATA software (Stata data analysis software; Computing Resource Centre, Santa Monica, CA). Data were expressed as means (SD) or medians (range), depending on distribution. Group comparisons were made using Student's *t* test, Wilcoxon rank sum, chi-squared statistics and Fisher's exact test, as appropriate. A p-value of <0.05 was considered significant. Inter-observer variation was quantified as the kappa coefficient of agreement for the presence of emphysema and the single-determination standard deviation for a) the extent of interstitial lung disease; and b) the extent of emphysema.

Correlations between the extent of interstitial lung disease and individual PFTs were examined using Pearson's product moment correlation. Univariate and multivariate analyses were performed. Logistic regression was used to identify independent associations with a) the presence of emphysema; and b) FVC/DLco threshold values of >1.6 and >2.0. Multiple linear regression models were constructed to identify the

independent determinants of levels of individual PFT. Models were formally tested for heteroscedasticity to confirm that the assumptions of parametric analysis had been satisfied.

RESULTS

Inter-observer variation for HRCT observations

Agreement was good for the presence of emphysema (kappa coefficient of agreement = 0.64). The single determination standard deviation for the extent of interstitial lung disease was 4.1%.

Sub-group differences: the presence of emphysema and smoking status

Baseline data are compared between patients with and without CPFE in Table 1. CPFE was present in 41/336 cases (12.2%) and was more prevalent in smokers (26/132, 19.7%) than in non-smokers (15/201, 7.5%), p<0.001, and in males (18/74, 24%) than in females (23/259, 9%), p<0.0005. In life-long nonsmokers, CPFE was more prevalent in males (5/26, 33%) than in females (10/175, 6%, p=0.01). Patients with CPFE were characterized by greater impairment of gas exchange and gas transfer, a higher FVC/DLco ratio and a median extent of emphysema of 5.5% (range 1-30%).

Baseline data are compared between smokers and non-smokers in Table 2. When compared with life-long non-smokers, ever–smokers were older and more frequently male. The extent of emphysema (when present) did not differ between smokers (5.75%, range 1-30%) and non-smokers (median 5.5%, range 1 to 28.5%), p=0.74. The median smoking dose associated with the presence of emphysema in smokers (in 23 of 26 patients with exact quantification of the smoking dose) was 25 pack years (range 2 to 40 pack years). Logistic regression showed that after adjustment for age and gender, the presence of emphysema was positively linked to the pack year smoking dose, both in the whole

population (odds ratio 1.05, 95% CI 1.02, 1.08, p<0.0005) and in ever-smokers (odds ratio 1.04, 95% CI 1.00, 1.07, p=0.04).

Upper zone emphysema was more extensive on average than lower zone emphysema on paired t-testing (13.9 ± 11.3 versus 4.7 ± 6.5; p<0.00005) and was more extensive than lower zone emphysema in all but one case. The extent of interstitial lung disease was not linked either to the extent of emphysema (Rs = -0.06, p=0.72) or the proportion of emphysema in the upper zone (Rs = -0.02, p=0.88). The distribution of emphysema did not differ between smokers and non-smokers.

The effect of emphysema and smoking status on pulmonary function variables

The extent of interstitial lung disease on HRCT was negatively related to % predicted DLco (R = -0.56), % predicted FVC (R = -0.35), % predicted FEV1 (R = -0.28), pO2 (R = -0.45) and positively related to the calculated alveolar arterial oxygen gradient (R = 0.40), all p<0.0005.

As shown in **Table 3**, **the extent of interstitial lung disease and the presence of emphysema were independent determinants of % predicted DLco levels.** Smoking status provided no **independent linkage** to DLco levels **whether quantified as "ever/never smoking" or as the pack-year smoking history**. **These findings did not change with the exclusion of current smokers: the determinants of DLco were ILD extent (RC-0.59; 95%CI -0.69, -0.49; p<0.0005) and the presence of emphysema (RC-12.2; 95%CI -17.7, -6.7; p<0.0005) with no independent link to smoking status (RC-2.1; 95%CI-5.5, 1.3; p=0.22).**

The effect of emphysema, a reduction in DLco of 12.4%, represented, on average, a 24.1% reduction from the mean **observed** value of 51.4%. The negative effect of emphysema on DLco levels, after adjustment for the extent of interstitial lung disease, is shown in Figure 1 and was seen both in ever-smokers (RC = -11.2; 95% CI-17.6, -4.8; p<0.001) and non-smokers (RC = -13.9; 95% CI = -21.4, -6.3; p<0.0005) in separate models. **The presence of emphysema had a lesser impact on DLco levels when emphysema was limited (CT extent<5%; RC-8.6; 95%CI -16.2, -1.1; p=0.03) than when it more extensive (CT extent>5%; RC-14.0; 95%CI -20.7, -9.0; p<0.0005).**

In 311 patients undergoing concurrent echocardiography, the extent of interstitial lung disease, the presence of emphysema and the presence of pulmonary hypertension were independent negative determinants of % predicted DLco levels.

The independent effects of the extent of interstitial lung disease, the presence of emphysema and smoking status (current/former smokers versus lifelong non-smokers) upon other pulmonary function variables are shown in Table 2 (with adjustment for age and gender). Indices of gas exchange (pO2 levels, the alveolar-arterial oxygen gradient) were more strongly influenced by the presence of emphysema than by the smoking status. By contrast, the presence of emphysema had no independent effect on FEV1 or FVC levels (Figure 2), whereas current or former smokers exhibited relative preservation of spirometric volumes.

There was no association between the presence of emphysema and auto-antibody status (ATA positivity versus non-ATA-positivity). The inclusion of

ATA status (ATA-positivity versus ATA negativity) in the multivariate model established that this serological distinction had no independent effect (p=0.51) on the linkage between emphysema and pulmonary function variables, with no evidence of an interaction.

The determinants of the FVC/DLco ratio

FVC/DLco ratios were positively correlated with the extent of disease on HRCT (R = 0.36, p<0.0005). FVC/DLco ratios were higher in patients with PH on echocardiography (median 1.86, range 0.99 to 3.55) than in those without PH (median 1.42, range 0.73 to 3.40), p<0.0001. FVC/DLco ratios were higher in patients with emphysema (median 2.07, range 1.14 to 3.42) than in patients without emphysema (median 1.42, range 0.73 to 3.55), p<0.0001.

On multiple linear regression (after adjustment for age and gender), the independent determinants of the FVC/DLco ratio were HRCT disease extent (RC = 0.009; 95% CI 0.006, 0.011; p<0.0005), the presence of emphysema (RC = 0.55, 95% CI 0.41, 0.69; p<0.0005), the presence of PH on echocardiography (RC = 0.25; 95% 0.11, 0.38, p<0.0005) and smoking status (RC = 0.13; 95% CI 0.03, 0.22; p<0.01) **(Table 4). These findings were unchanged when analysis was confined to 211 patients with DLco levels<60% of predicted.**

The effect of emphysema, an increase of 0.55 in the FVC/DLco ratio, represented an average rise of 34.8% from the **observed** mean value of 1.58. On correction of equation heteroscedasticity (with logarithmic transformation of the FVC/DLco ratio), these findings were robust at the same level of significance, apart from the presence of PH (p<0.01). When the model was examined separately according to smoking

status, the impact of emphysema on the FVC/DLco ratio was very similar in smokers (RC = 0.56, 95% CI = 0.36, 0.77; p<0.0005) and in non-smokers (RC = 0.49; 95% CI 0.28, 0.70; p<0..0005). The confounding effect of the presence of emphysema on the FVC/DLco ratio, after adjustment for the extent of disease on HRCT, is shown in **Figure 3.**

On logistic regression, the independent determinants of an FVC/DLco ratio >2.0 were extent of disease on HRCT (odds ratio [OR] = 1.05; 95% CI 1.03, 1.07; p<0.0005), the presence of emphysema (OR = 13.8; 95% CI 5.8, 32.8; p<0.0005), increasing age (OR = 1.04; 95% CI 1.01, 10.7; p<0.01) and the presence of pulmonary hypertension (OR = 2.58; 95% CI 1.12, 5.92; p=0.02). **These findings were largely unchanged when analysis was confined to 211 patients with DLco levels<60% of predicted, although the independent linkage between PH on echocardiography and FVC/DLco>2.0 was no longer significant (p=0.06).**

DISCUSSION

The combination of emphysema and fibrosis, also known as the CPFE syndrome, has been reported in patients with CTD-ILD (9). However, the clinical significance of this proposed syndrome has yet to be established. We report a prevalence of emphysema of 12% in a large cohort of SSc-ILD patients, with emphysema present more often in current or former smokers but also ssen in 7.5% of life-long non-smokers. The presence of emphysema had a significant confounding effect on measures of gas transfer and gas exchange in both smokers and non-smokers. The FVC/DLco ratio, recently advocated as an important screening tool for pulmonary hypertension, was strikingly influenced by the presence of emphysema.

The current study of the prevalence and functional impact of emphysema in a population of over 300 SSc-ILD patients extends and refines the observations of Cottin et al in a series of 34 CTD-ILD patients with CPFE, including 10 with SSc-ILD (9). In small cohorts, emphysema had been observed in a handful of non-smoking patients with rheumatoid lung (14) and SSc-ILD (9). The size of the current cohort allows the confident conclusion to be drawn that emphysema has a significant prevalence (7.5%, 95% confidence intervals 4-12%) in non-smokers with SSc-ILD. Furthermore, the presence of emphysema in smokers with SSc-ILD was associated with a low pack-year smoking history, as also reported in rheumatoid lung (when compared with a control cohort of smokers without interstitial lung disease) (14**)**. **The size of the current cohort allows the confident conclusion to be drawn that emphysema has a significant prevalence (7.5%, 95% confidence intervals 4-12%) in non-smokers with SSc-ILD. Furthermore, in smokers with SSc-ILD with an average smoking history of only 15 pack-years, emphysema was present in 15%. Taken together, these observations raise the possibility that SSc-ILD may be a risk factor for the development of emphysema in both smokers and non-smokers. However, without a control group of smokers without interstitial lung disease, our findings are inconclusive in this regard.**

Given the high prevalence of emphysema in non-smokers, it was important to consider the possible confounding effect of inter-observer variability with regard to the presence of trivial emphysema. However, the extent of emphysema did not differ between smokers and non-smokers. More importantly, the presence of emphysema in non-smokers had the same effect on measures of gas transfer and gas exchange as in smokers. For a given extent of fibrosis, the presence of emphysema was associated

with a reduction in DLco of over 10% in both smokers and non-smokers. Strikingly, the FVC/DLco ratio rose, on average, by over 40% from the **observed** values in both smokers and non-smokers with emphysema, after adjustment for other variables. Given the major functional effects associated with emphysema in non-smokers, despite the relatively limited extent of emphysema, it appears difficult to argue that our findings have been materially affected by methodological limitations or inter-observer variability.

The reported pattern of functional impairment in patients with combined emphysema and fibrosis has consisted of **relative** preservation of lung volumes and a disproportionate reduction in DLco levels (8-9,15-17). In most reports, the relative impact of concurrent emphysema on lung volumes and measures of gas transfer has not been quantified definitively (i.e. with the extent of fibrosis also taken into account in multivariate analysis). However, in the current study, as in an early IPF study (18**)**, the presence of emphysema had a much greater effect on DLco levels than FVC levels (which were not significantly affected). These findings suggest that the presence of emphysema is unlikely to confound the accurate use of FVC in prognostic evaluation, as in an SSc-ILD staging system (19) recently validated against survival (20). **By contrast, the lack of relative preservation in FVC levels in patients with emphysema is likely to reflect the fact that spirometric volumes are influenced by smoking-related intrinsic airway inflammation, in a larger smoking patient sub-group, irrespective of the presence or absence of emphysema.**

Our observations indicate that the presence of emphysema has a major confounding effect on the contribution of pulmonary function tests to non-invasive screening for

PH in SSc-ILD. Functional abnormalities typically seen in PH include undue hypoxia (21) and reductions in DLco that are disproportionate to lung volumes, quantified historically by adjustment of DLco for alveolar volume (DLco/VA, Kco) and more recently by the FVC/DLco ratio (22). In SSc, an increased FVC/DLco ratio, alone or in combination with other variables, has provided justification for the performance of a right heart study, based on thresholds of 1.6 (4-5,7) or 2.0 (6).

However, in routine practice, pulmonary physiologic profiles suggestive of PH generally lead to the performance of echocardiography rather than immediate right heart catheterisation, a sequence reproduced in the recent prospective two-step DETECT algorithm (23). In that study, the FVC/DLco ratio was validated against echocardiographic data and was found to be one of the two non-invasive variables most strongly predictive of echocardiographic PH. However, the DETECT algorithm was developed in SSc in general and has not been tested specifically in SSc-ILD. Therefore, we report the first evaluation of the accuracy of the FVC/DLco ratio against echocardiographic data in SSc-ILD, with particular reference to the presence or absence of concurrent emphysema. Echocardiographic criteria for PH in the current study were essentially those validated in the DETECT study against the presence of PH at right heart catheterisation. Taken together, the prevalence of emphysema in SSc-ILD in the current study, the major confounding effect of emphysema on physiologic variables and the importance of the FVC/DLco ratio in the DETECT algorithm have prompted us to conclude that the DETECT algorithm may need to be adapted in patients with SSc-ILD. One important proviso is that this needs to be done in smokers and non-smokers alike. Based on our findings, it appears that major

increases in the FVC/DLco ratio are not indicative of a high likelihood of PH on echocardiography, when emphysema is present on HRCT.

Given the aims of the current study, our findings have only indirect implications with regard to pathogenetic linkages between emphysema and interstitial lung disease in SSc. However, the predilection for the development of emphysema in smoking and non-smoking patients with SSc-ILD was striking, taking into account the low pack-year history associated with emphysema in smokers. A number of candidate pathways are common to emphysema and interstitial lung disease, including oxidative stress (24-25), matrix remodeling (26-27), protein citrullination (28-30), elastin activation (31-32) and telomerase regulation (33). There is also the intriguing possibility that autoimmune pathways might be implicated both in CTD-ILD and in the pathogenesis of emphysema, accounting for the existence of emphysema in some non-smokers with SSc-ILD. Evidence of immune dysregulation has emerged in chronic obstructive pulmonary disease cohorts (34-35), including dysfunction of T regulatory cells and dendritic cells (36), autoantibody expression (32) and systemic manifestations compatible with autoimmunity **(37).**

One limitation of the current study is the fact that patients underwent right heart catheterisation only if the echocardiographic findings were suggestive of PH. The acquisition of right heart data in all cases would have allowed FVC/DLco thresholds to be evaluated for sensitivity, specificity and predictive values against proven PH. However, it is doubtful that such a protocol in a large cohort of SSc-ILD patients is achievable. It can also be argued that examination against echocardiography is **a relevant** evaluation of the true clinical utility of the FVC/DLco ratio, which may lie

in the selection of patients for echocardiography: a view cardinal to the construction of the DETECT algorithm.

In principle, the use of echocardiography to evaluate the presence of PH in patients with emphysema might be confounded by hyperinflation, causing difficulty in obtaining clear images of regurgitant flow. However, difficulties in measuring echocardiographic variables in non-extensive emphysema have not been reported

We stress that we cannot exclude the possibility that the **relatively** high prevalence of emphysema in non-smokers in SSc-ILD might apply equally to SSc patients without interstitial lung disease. The nature of our cohort, which consisted of consecutive SSc patients with known or suspected lung involvement, did not allow this possibility to be explored. Given the referral pattern to our institution, it was considered highly unlikely that the relatively small subset of patients without interstitial lung disease were representative of the larger non-referred SSc population. We suggest that further evaluations based on our findings might usefully be undertaken in SSc cohorts not selected for the presence of lung involvement. However, a powerful argument against a major excess of emphysema in the absence of interstitial lung disease is the finding that the FVC/DLco ratio was, in fact, strongly linked to echocardiographic evidence of PH in the whole SSc cohort evaluated in the DETECT study.

In conclusion, we report a predilection to emphysema in SSc-ILD, as shown by its sporadic presence in non-smokers and a low associated pack-year smoking history in smokers. The significance prevalence of emphysema in non-smokers with SSc-ILD provides indirect support for a pathogenetic basis for a syndrome of CPFE. The CPFE syndrome is associated with disproportionate impairment of measures of gas

transfer and gas exchange, including a major confounding effect on the FVC/DLco ratio. These findings have major implications for the construction of accurate screening algorithms for PH in SSc-ILD.

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REFERENCES

1. Antoniou KM, Margaritopoulos G, Economidou F, Siafakas NM. Pivotal clinical dilemmas in collagen vascular diseases associated with interstitial lung involvement. Eur Respir J. 2009;33:882-96

2. Wells AU, Margaritopoulos GA, Antoniou KM, Denton CP. Interstitial Lung Disease in Systemic Sclerosis. Semin Respir Crit Care Med 2014;35:213–21.

3. Wells AU, Hansell DM, Rubens MB, King AD, Cramer D, Black CM, et al. Fibrosing alveolitis in systemic sclerosis: indices of lung function in relation to extent of disease on computed tomography. Arthritis Rheum. 1997;40:1229-36.

4. Steen VD, Lucas M, Fertig N, Medsger TA Jr. Pulmonary arterial hypertension and severe pulmonary fibrosis in systemic sclerosis patients with a nucleolar antibody. J Rheumatol. 2007;34:2230-5.

5. Steen VD, Chou M, Shanmugam V, Mathias M, Kuru T, Morrissey R. Exercise-induced pulmonary arterial hypertension in patients with systemic sclerosis. Chest. 2008;134:146-51.

6. Hsu VM, Moreyra AE, Wilson AC, Shinnar M, Shindler DM, Wilson JE, et al. Assessment of pulmonary arterial hypertension in patients with systemic sclerosis: comparison of noninvasive tests with results of right-heart catheterization. J Rheumatol. 2008;35:458-65.

7. Launay D, Humbert M, Berezne A, Cottin V, Allanore Y, Couderc LJ, et al. Clinical characteristics and survival in systemic sclerosis-related pulmonary hypertension associated with interstitial lung disease. Chest. 2011;140:1016-24.

8. Cottin V, Le Pavec J, Prévot G, Mal H, Humbert M, Simonneau G; et al. GERM"O"P. Pulmonary hypertension in patients with combined pulmonary fibrosis and emphysema syndrome. Eur Respir J. 2010;35:105-11.
9. Cottin V, Nunes H, Mouthon L, Gamondes D, Lazor R, Hachulla E, et al. Combined pulmonary fibrosis and emphysema syndrome in connective tissue disease. Arthritis Rheum 2011;63:295-304.
10. Masi AT, Rodnan GP, Medsger TA Jr. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980;23:581–590.
11. Goh NS, Veeraraghavan S, Desai SR, Cramer D, Hansell DM, Denton CP et al. Bronchoalveolar lavage cellular profiles in patients with systemic sclerosis-associated interstitial lung disease are not predictive of disease progression. Arthritis Rheum. 2007;56:2005-12.
12. Desai SR, Veeraraghavan S, Hansell DM, Nikolakopolou A, Goh NS, Nicholson AG et al. CT features of lung disease in patients with systemic sclerosis: comparison with idiopathic pulmonary fibrosis and nonspecific interstitial pneumonia. Radiology. 2004;232:560-7.
13. Copley SJ, Wells AU, Sivakumaran P, Rubens MB, Lee YC, Desai SR et al. Asbestosis and idiopathic pulmonary fibrosis: comparison of thin-section CT features. Radiology 2003; 229:731-736.
14. Antoniou KM, Walsh SL, Hansell DM, Rubens MR, Marten K, Tennant R, et al. Smoking-related emphysema is associated with idiopathic pulmonary fibrosis and rheumatoid lung. Respirology. 2013;18:1191-6

15. Wiggins J, Strickland B, Turner-Warwick M. Combined cryptogenic fibrosing alveolitis and emphysema: the value of high resolution computed tomography in assessment. Respir Med. 1990;84:365-9.

16. Wells AU, Hansell DM, Rubens MB, Cailes JB, Black CM, du Bois RM. Functional impairment in lone cryptogenic fibrosing alveolitis and fibrosing alveolitis associated with systemic sclerosis: a comparison. Am J Respir Crit Care Med. 1997;155:1657-64.

17. Akagi T, Matsumoto T, Harada T, Tanaka M, Kuraki T, Fujita M et al. Coexistent emphysema delays the decrease of vital capacity in idiopathic pulmonary fibrosis. Respir Med. 2009;103:1209-15.

18. Wells AU, King AD, Rubens MB, Cramer D, du Bois RM, Hansell DM. Lone cryptogenic fibrosing alveolitis: a functional-morphologic correlation based on extent of disease on thin-section computed tomography. Am J Respir Crit Care Med. 1997;155:1367-75.

19. Goh NS, Desai SR, Veeraraghavan S, Hansell DM, Copley SJ, Maher TM et al. Interstitial lung disease in systemic sclerosis: a simple staging system. Am J Respir Crit Care Med. 2008;177:1248-54.

20. Moore OA, Goh N, Corte T, Rouse H, Hennessy O, Thakkar V, et al. Extent of disease on high-resolution computed tomography lung is a predictor of decline and mortality in systemic sclerosis-related interstitial lung disease. Rheumatology (Oxford). 2013;52:155-60.

21. Zisman DA, Karlamangla AS, Kawut SM, Shlobin OA, Saggar R, Ross DJ, et al. Validation of a method to screen for pulmonary hypertension in advanced idiopathic pulmonary fibrosis. Chest 2008; 133: 640-645.

22. Nathan SD, Shlobin OA, Ahmad S, Urbanek S, Barnett SD. Pulmonary hypertension and pulmonary function testing in idiopathic pulmonary fibrosis. Chest 2007; 131:657-663.

23. Coghlan JG, Denton CP, Grünig E, Bonderman D, Distler O, Khanna D et al. Evidence-based detection of pulmonary arterial hypertension in systemic sclerosis: the DETECT study. Ann Rheum Dis. 2013 May 18. [Epub ahead of print]

24. Vuorinen K, Ohlmeier S, Leppäranta O, Salmenkivi K, Myllärniemi M, Kinnula VL. Peroxiredoxin II expression and its association with oxidative stress and cell proliferation in human idiopathic pulmonary fibrosis. J. Histochem. Cytochem. 2008; 56: 951–9.

25. Volonte D, Kahkonen B, Shapiro S, Di Y, Galbiati F. Caveolin-1 expression is required for the development of pulmonary emphysema through activation of the ATM-p53-p21 pathway. J Biol Chem. 2009;284:5462-6.

26. Zandvoort A, Postma DS, Jonker MR, Noordhoek JA, Vos JT, Timens W. Smad gene expression in pulmonary fibroblasts: indications for defective ECM repair in COPD. Respir Res. 2008;9:83.

27. Gauldie J, Kolb M, Ask K, Martin G, Bonniaud P, Warburton D. Smad3 signaling involved in pulmonary fibrosis and emphysema. Proc Am Thorac Soc. 2006;3:696-702.

28. Padyukov L, Silva C, Stolt P, Alfredsson L, Klareskog L. A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis. Arthritis Rheum. 2004;50:3085-92.

29. Klareskog L, Stolt P, Lundberg K, Källberg H, Bengtsson C, Grunewald J, et al. A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. Arthritis Rheum. 2006;54:38-46.

30. Bongartz T, Cantaert T, Atkins SR, Harle P, Myers JL, Turesson C et al. Citrullination in extra-articular manifestations of rheumatoid arthritis. Rheumatology (Oxford). 2007;46:70-5.

31. Greene CM, Low TB, O'Neill SJ, McElvaney NG. Anti-proline-glycine-proline or antielastin autoantibodies are not evident in chronic inflammatory lung disease. Am J Respir Crit Care Med. 2010;181:31-5.

32. Rinaldi M, Lehouck A, Heulens N, Lavend'homme R, Carlier V, Saint-Remy JM et al. Antielastin B-cell and T-cell immunity in patients with chronic obstructive pulmonary disease. Thorax. 2012;67:694-700.

33. Faner R, Rojas M, Macnee W, Agustí A. Abnormal lung aging in chronic obstructive pulmonary disease andidiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2012;186:306-13.

34. Brusselle GG, Joos GF, Bracke KR.New insights into the immunology of chronic obstructive pulmonary disease. Lancet. 2011 Sep 10;378(9795):1015-26

35. Marsland BJ, Königshoff M, Saglani S, Eickelberg O. Immune system dysregulation in chronic lung disease. Eur Respir J. 2011 Sep;38(3):500-1.

36. Plumb J, Smyth LJ, Adams HR, Vestbo J, Bentley A, Singh SD.Increased T-regulatory cells within lymphocyte follicles in moderate COPD. Eur Respir J. 2009 Jul;34(1):89-94

37. Barnes PJ, Celli BR. Systemic manifestations and comorbidities of COPD. Eur Respir J. 2009 May;33(5):1165-85

Table 1: Baseline data compared between patients with CPFE and patients without emphysema and non-smokers. CPFE patients were more often male smokers, and were characterized, lower DLco and po2 levels and a higher FVC/DLco ratio. [Abbreviations: ILD = interstitial lung disease; PH = pulmonary hypertension {as judged by echocardiography)].

	CPFE (n=41)	**Pulmonary fibrosis without emphysema (n=292)**	**Significance**
Age (years)	53.1 ± 15.1	54.4 ± 12.8	p = 0.57
Gender (M;F ratio)	18/74 (males 24%)	23/259 (males 9%)	p<0.0005
Ever-smokers	26/41 (63%)	106/292 (36%)	p = 0.001
ATA positivity (n=308)	14/39 (36%)	127/269 (47%)	p = 0.19
Extent of ILD	Median 24.5% (1 – 70%)	Median 15.5% (1 – 84%)	p = 0.08
Prevalence of PH (n=311)	9/37 (24%)	38/236 (16.0%)	p = 0.10
FEV1 (% predicted)	73.6 ± 18.9	75.3 ± 19.6	p = 0.59
FVC (% predicted)	77.0 ± 22.3	75.7 ± 21.8	p = 0.73
DLco (% predicted)	37.8 ± 13.0	53.3 ± 17.8	p<0.0005
FVC/DLco ratio	Median 2.07 (1.14 – 3.42)	Median 1.42 (0.73 – 3.55)	p<0.0005
pO2 (kpa) [n=282]	10.8 ± 1.9	11.7 ± 1.7	p<0.005

Table 2: Baseline data compared between current or former smokers and non-smokers. Smokers were characterized by older age, an increase in the male to female ratio, a higher prevalence of emphysema, a higher FVC/DLco ratio, higher spirometric volumes and lower po2 levels. [Abbreviations: ILD = interstitial lung disease; PH = pulmonary hypertension {as judged by echocardiography)].

	Current/ex-smokers (n=132)	**Lifelong non-smokers (n=201)**	**Signficance**
Age (years)	57.2 ± 11.4	52.2 ± 13.7	p<0.001
Gender (M;F ratio)	48:84 (males 36%)	26:175 (males 15%)	p<0.001
Extent of ILD	Median 15% (1 – 84%)	Median 18% (1 – 77.5%)	p=0.14
Prevalence of emphysema	n = 26 (19.7%)	n = 15 (7.5%)	p<0.001
Prevalence of PH (n=311)	n = 19 (15.2%)	n = 28 (15.0%)	p=0.95
FEV1 (% predicted)	78.5 ± 20.0	72.9 ± 18.9	p<0.01
FVC (% predicted)	80.6 ± 23.1	72.8 ± 20.5	p<0.01
DLco (% predicted)	50.7 ± 17.9	51.8 ± 18.1	p=0.59
FVC/DLco ratio	Median 1.53 (0.79 – 3.40)	Median 1.43 (0.73 – 3.55)	p<0.001
pO2 (kpa) [n=282]	11.1 ± 1.9	11.8 ± 1.6	p<0.01

Table 3: The independent effects of the extent of interstitial lung disease, smoking status and the presence of emphysema on individual pulmonary function variables are shown, with adjustment for age and gender. Effects are expressed as regression coefficients (per unit change in the extent of ILD and as a global effect in current or former smokers, compared to life-long non-smokers, and for the presence of emphysema) with 95% confidence intervals in parentheses and p values. [Abbreviations: ILD = interstitial lung disease; Aa gradient = alveolar-arterial oxygen gradient].

	Extent of ILD	**Smoker (vs non-smoker)**	**Emphysema present**	**Equation R^2**
FEV1	-0.31 (-0.44, -0.19) p<0.0005	4.3 (0.0, 8.7) p<0.05	-0.5 (-6.7, 5.7) p=0.87	0.14
FVC	-0.43 (-0.56, -0.30); p<0.0005	6.0 (1.4, 10.6); p=0.01	3.6 (-3.0, 10.2) p=0.29	0.22
DLco	-0.58 (-0.68, -0.48); p<0.0005	- 0.8 (-4.2, 2.7); p=0.66	-12.4 (-17.3, -7.5) p<0.0005	0.37
pO2	-0.04 (-0.05, -0.03); p<0.0005	-0.26 (-0.63, 0.11); p=0.16	-0.53 (-1.02, -0.02) p=0.04	0.36
Aa gradient	0.04 (0.03, 0.05); p<0.0005	0.40 (0.01, 0.78); p=0.04	0.67 (0.15, 1.19) p=0.01	0.32

Table 4: Determinants of FVC/DLCO ratio. Effects are expressed as regression coefficients with 95% confidence intervals and p values.

	Extent of ILD	**Emphysema present**	**Pulmonary Hypertension**	**Smoking status**
FVC/DLCO	RC = 0.009; 95% CI 0.006,-0.011; p<0.0005	RC=0.55; 95% CI 0.41,0.69; p<0.0005	RC=0.25; 95% CI 0.11,-0.38; p<0.0005	RC=0.13; 95% CI 0.03,-0.22; p<0.01

Figure legends

Figure 1: DLco levels are shown in relation to the extent of disease on HRCT. Patients with emphysema (n = 41, closed circles) are compared to patients without emphysema (n = 295, open circles). The presence of emphysema had a significant effect on DLco levels with, on average, a reduction in DLco of 12.7% (i.e. approximately 25% of baseline levels) after adjustment for the extent of interstitial lung disease.

Figure 2: FVC levels are shown in relation to the extent of disease on HRCT. Patients with emphysema (n = 41, closed circles) are compared to patients without emphysema (n = 295, open circles). The presence of emphysema did not have a significant effect on FVC levels.

Figure 3: The FVC/DLco ratio is shown in relation to the extent of disease on HRCT. Patients with emphysema (n = 41, closed circles) are compared to patients without emphysema (n = 295, open circles). The presence of emphysema had a significant effect on the FVC/DLco ratio with, on average, an increase of 0.57 (i.e. approximately 35% of baseline levels), after adjustment for the extent of interstitial lung disease, age and the presence of pulmonary hypertension, as judged by echocardiography.