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Early View

Original article

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Risk factors associated with the development of interstitial lung abnormalities

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Abstract.

Background: Around 8-10% of individuals over 50 years, present interstitial lung abnormalities (ILA), but their risk factors are uncertain.

Methods: From 817 individuals recruited in our “Lung Aging Program”, 80 (9.7%) showed ILA and were compared with 564 individuals of the same cohort with normal HRCT to evaluate demographic and functional differences, and with 80 individuals, randomly selected from the same cohort for biomarkers. We evaluated *MUC5B* (rs35705950) variant, telomere length, and serum levels of matrix metalloproteinases (MMP)-1, -2, -3, -7, -8, -9, -12, -13, interleukin (IL)-6, surfactant protein (SP)-D, alpha-Klotho and resistin.

Results: Individuals with ILA were usually males ($p<0.005$), older than controls (<0.0001), smokers ($p=0.01$), with greater frequency of *MUC5B* rs35705950 (OR 3.5; CI95% 1.29-9.44, $p=0.01$), and reduced DL_{CO} and oxygen saturation. Resistin, IL-6, SP-D, MMP-1, MMP-7, and MMP-13 were significantly increased in individuals with ILA. Resistin (12±5 ng/ml versus 9±4 ng/ml; $p=0.0005$) and MMP-13 (357±143 versus 298±116, $p=0.004$ pg/ml), were the most increased biomarkers. On follow-up (24 ±18 months), 18 individuals showed progression which was associated with gastroesophageal reflux disease (OR 4.1 CI95% 1.2-12.9, $p=0.02$), and in females with diabetes mellitus (OR 5.3, CI95% 1.03-27.4, $p=0.01$).

Conclusions: Around 10% of asymptomatic respiratory individuals enrolled in our lung aging program show interstitial lung abnormalities. Increased serum concentrations of pro-inflammatory molecules and MMPs are associated with ILA.

Introduction

Several studies including large populations, mostly non-Hispanic white, have identified around 7-9% of individuals showing subclinical forms of interstitial lung abnormalities (ILA) as detected by chest high resolution computed tomography (HRCT) (1-3). ILA is defined as the presence of ground-glass opacities, reticular abnormalities, diffuse centrilobular nodules, honeycombing, traction bronchiectasis, non-emphysematous cysts, or architectural distortion involving at least 5% of non-dependent portions of the lung (4).

Recently, it was proposed that ILA can be subclassified as non-subpleural and non-fibrotic, subpleural non-fibrotic, and subpleural fibrotic with prognostic consequences (5).

Individuals with ILA may be asymptomatic, although they are more likely to report chronic cough or shortness of breath and may display reduced lung function compared with subjects without ILA (3). Recent reports on longitudinal studies have shown that ~40% of the ILA progress over approximately 5 years of follow-up displaying a higher rate of all-cause mortality, more likely associated with a respiratory cause when compared with those who did not have ILA (6, 7).

Some risk factors have been recognized so far, including occupational exposures to vapors, gas, dust and fumes, smoking, higher serum matrix metalloproteinase (MMP)-7 and interleukin-6, higher plasma concentrations of galectin-3, and the common promoter polymorphism (rs35705950) in MUC5B gene (1-3, 8, 9).

At the National Institute of Respiratory Diseases, we have an ongoing “Lung Aging Program” that includes respiratory asymptomatic subjects over 60 years

old (smokers and non-smokers), and from 817 individuals enrolled up to now, 80 (9.7%) showed interstitial lung abnormalities.

This study aimed to identify genetic, molecular, and environmental risk factors or comorbidities likely associated with the development of ILA in respiratory asymptomatic individuals and their clinical behavior.

Material and Methods

Study population

Respiratory asymptomatic volunteers aged 60 or older have been invited to participate in our "Lung Aging Program", initiated in Mexico City in March 2015. The present study included 817 adults that were recruited from 2015 until July 2019. From them, in addition to the 80 individuals with ILA, this cohort includes 45 subjects with emphysematous lesions, 48 with bronchiectasis, 49 with air trapping on expiratory phase, and 31 with miscellany changes (atelectasis, pleural thickening, etc). Only 564 had completely normal HRCT and were included as controls for demographic and functional comparisons.

The project was approved by the Scientific and Ethics committee of the Instituto Nacional de Enfermedades Respiratorias (C39-14), and all individuals signed a consent letter.

A modified questionnaire used in the PLATINO study (10) was applied. PLATINO is a composite instrument that includes sections of the following questionnaires: ATS/DLD, ECRHS II, Lung Health Study, and some questions about place and time of residence, and has been validated in Spanish (10). From this questionnaire, we obtained the information on smoking, occupational exposure, comorbidities, economic impact, and exposure to intra-household

pollutants. Regarding smoking, subjects were categorized as never smokers, former smokers, or current smokers.

Body mass index (BMI, kg/m2) was calculated from weight and height squared, and underweight (BMI <18.5) and obese (BMI >40) individuals were not included. Other exclusion criteria included: inability to complete the walking functional tests and spirometry, presence of chronic non-respiratory diseases without medical control (diabetes mellitus, systemic arterial hypertension, hypothyroidism, epilepsy, etc.), or ever treated with chemotherapy or radiotherapy.

Routine laboratory studies including hematic biometry, blood chemistry, lipid profile, and acute phase reactants were registered.

Pulmonary Function Tests

All individuals underwent pulmonary function tests. Forced vital capacity (FVC) and forced expiratory volume in the first second (FEV1) were obtained by spirometry, and DL_{CO} (diffusing capacity of the lungs for carbon monoxide) was measured using Easy One Pro LAB. The percentage of the predicted value for DL_{CO} (DL_{CO}%) was adjusted for altitude according to ATS/ERS guidelines. Also, all subjects performed the 6-minute walking test (6MWT).

High-Resolution Computed Tomography

HRCT analysis was done with helicoidal tomography (Somatom, definition AS 128 detectors double-energy, Siemens) and individuals were scanned in a supine and prone position. Prone position was included because dependent abnormalities may be misleading unless persistent in the prone position.

HRCTs were prospectively and independently reviewed blindly by two

radiologists experienced in interstitial lung diseases as defined elsewhere (1-4). The kappa value for interobserver variability for ILA diagnosis was 0.61.
In discordant cases, the final diagnosis was performed by consensus.
HRCT findings were retrospectively classified as subpleural fibrotic, subpleural non-fibrotic, and non-subpleural non-fibrotic ILA (5). Subpleural fibrotic ILA included predominantly reticular abnormalities of subpleural localization with or without architectural distortion with traction bronchiectasis or honeycombing.

Serum biomarkers

Serum was obtained from peripheral blood samples by centrifugation at 4000 rpm at 4°C for 20 min. The serum was collected and stored at -80°C until use.
Serum levels of matrix metalloproteinase 7 **(**MMP-7), MMP-1, interleukin 6 (IL-6), surfactant protein (SP)-D, alpha-Klotho, and resistin were quantified by ELISA (R&D Systems, Minneapolis, MN) according to manufacturer's protocols. Alpha-Klotho was also measured by ELISA (Immuno-Biological Laboratories, IBL Minneapolis, MN).
Serum concentrations of MMP-2, MMP-3, MMP-8, MMP-9, MMP-12, and MMP-13 were determined by Luminex. For each sample, 50 μL of serum was used to measure MMP levels using a Human Premixed Multi-Analyte Kit, for simultaneous detection of multiple human biomarkers, LXSAHM (R&D Systems, Minneapolis, MN) using a Bio-Plex 200 array reader (Bio-Rad, Hercules, CA) according to the manufacturer's instructions. For data acquisition, Bio-Plex Manager Software, version 4, was used (Bio-Rad, Hercules, CA).

Genotyping

Genotyping was performed on genomic DNA extracted from 10 ml of peripheral blood using the commercial extraction kit BD tract isolation kit (Maxim Biotech,

San Francisco CA, USA). DNA was quantified by absorption spectrophotometry at 260 nm wavelength. The minor-allele of the single-nucleotide polymorphism (SNP) rs35705950 from MUC5B promoter, and rs2736100 SNP in the telomerase reverse transcriptase (TERT) gene was determined using TaqMan-specific probes for allelic determination.

The Polymerase chain reactions (PCRs) were carried out in a 25-μl mixture, which contained 2 μl of DNA (10 ng) and 12.5 μl of Master Mix 2X PCR (Applied Biosystems). The conditions of the PCRs were, 2 min at 95° C followed by 40 cycles of 15 s at 95° C and 1 m at 60°C.

Analysis of telomere length by quantitative real-time PCR

Relative telomere length was measured by quantitative polymerase chain reaction (qPCR) as previously described (11). Genomic DNA was extracted from blood samples and reactions were performed with the next reagents: Power SYBR® Green PCR Master Mix (Life Technologies, UK), RNase free water (SIGMA, UK), primer single gene (S) forward (36B4d F-300nM) (CCCATTCTATCATCAACGGGTACAA) and single-copy gene (S) reverse (36B4u R-300nM) (CAGCAAGTGGGAAGGTGTAATCC), primer Tel (T) Forward (900nM) (CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT), Tel (T) Reverse (900nM) (GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCC). The cycling profile was: 95ºC for 10 min; 95ºC for 15 s, 58ºC for 1 min, 72ºC for 30 s x 40 cycles; 95ºC for 15 s, 55ºC for 15 s and 95ºC for 15 s. Outlier values were excluded. The relative value for telomere length: telomere repeats copies number (T) to a single copy gene (S) (T/S) ratio was determined by comparison

with control calibration curves and was graphed as natural logarithm (LN) versus age.

Follow up

Individuals with ILA were followed every 6 months and examined with the same pulmonary function tests. Progression was determined when the individual presented two of the following conditions: 1) Decline of pulmonary function tests either FVC >10%, DLCO >15% or reduction of 50 meters in 6MWT; 2) initiation or worsening of respiratory symptoms; 3) An increase of more than 30% of lesions compared with the previous HRCT or the apparition of new lesions such as honeycombing or traction bronchiectasis, regardless of the percent of change of the lesions on the first scan (6). Briefly, the total lung area was divided into 3 zones (the upper zone, above the level of the carina; the middle zone, between the level of the carina and the level of the inferior pulmonary veins; and the lower zone, under the level of the inferior pulmonary veins). The extent of the abnormalities was evaluated visually for each lung zone and was scored based on a semi-quantitative estimate in percentage. The final percentage of involvement was obtained by averaging the three zones in the initial and follow-up HRCT. All paired CT evaluations were examined by two radiologists and progression was determined by consensus.

Statistical Analysis

Descriptive data are presented as frequency, mean and standard deviation (SD). Univariate analyses of baseline characteristics were performed with a t-test or chi-squared test as appropriate for the data. We performed a normality test with Kolmogorov-Smirnov. Values of $p<0.05$ were considered significant.

Bonferroni's correction was used to adjust for multiple testing and corrected p-values were considered where applicable. We used a logistic regression model to estimate odds ratios (ORs) with a 95% confidence interval (CI). Receiver operating curve (ROC) analysis was performed to evaluate the diagnostic performance of the biomarkers for detecting the presence of ILA. All analyses were performed using STATA version 15.0 (StataCorp, College Station, Texas, USA) and IBM SPSS Statistics for Windows, version 24.0 (Armonk, NY: IBM Corp, NY, USA).

Results

In this ongoing longitudinal Aging Lung Program involving respiratory asymptomatic individuals over 60 years, we found 80 individuals (9.7%) presenting interstitial lung abnormalities characterized by visual assessments of chest HRCT scans (**Figure 1**). The demographic and baseline characteristics of the 80 subjects with ILA were compared with 564 individuals of the same cohort with normal HRCT (non-ILA group). The results are summarized in **Table 1**. Individuals with ILA were significantly older (72$\pm$8 versus 69$\pm$8 years; $p<0.0001$) with male predominance (43% versus 26%; $p=0.005$), and former/current smokers ($p=0.01$). No differences were found in the presence of comorbidities usually associated with aging (e.g., hypertension, diabetes mellitus). Likewise, no differences were found regarding environmental or occupational exposure or in routine laboratory studies.

At baseline, compared with the 564 controls individuals with ILA displayed reduced pulmonary function tests associated with gas exchange, while no differences were found in FVC (**Table 2**).

High resolution computed tomography

Of the total 80 subjects with ILA, 24 (30%), exhibited a predominance of subpleural reticular opacities and 56 (70%) predominance of subpleural or central ground-glass attenuation. In all the cases the abnormalities were early/mild or moderate. According to the recent classification proposed by the Fleischner Society, 38 subjects (48%) showed subpleural non-fibrotic abnormalities, 24 (30%) subpleural fibrotic, and 18 (22%) non-subpleural, non-fibrotic abnormalities (5).

Gene polymorphisms and biomarkers

We examined the gene common variant of TERT (rs2736100), the gain-of-function MUC5B promoter variant (rs35705950), and biomarkers in ILA group (n=80) and 80 randomly selected subjects of the non-ILA group. The minor allele frequency of the MUC5B promoter polymorphism was found significantly associated with ILA (OR 3.5; CI95% 1.29-9.44, p=0.01). No differences were observed neither with the common TERT variant nor in telomere length.

We measured as putative biomarkers, the serum concentrations of several matrix metalloproteinases, as well as markers of inflammation, aging, and alveolar epithelial cell integrity. From all of them, we found that individuals with ILA displayed a significant increase in MMP-1, MMP-7, MMP-13, IL-6, SP- D, and resistin (**Table 3**). After Bonferroni's correction to adjust for multiple testing only MMP-13 and resistin remained statistically associated with the risk of ILA. ROC curves were constructed and the area under the curve (AUC), cut-off, sensitivity, and specificity were determined to analyze the diagnostic

performance of resistin, and MMP-13**.** After adjustment by age, gender, and DL_{CO}, only resistin was associated with ILA (AUC 0.74, OR= 1.12, IC95% 1.0-1.2, p=0.01).

Follow up

On follow-up (24 ±18 months), 18 individuals (23%) showed progression revealed by a decline in the pulmonary function tests and increased HRCT opacities in the whole-lung visual ILA score or initiation of symptoms (**Figure 2**). Lung functional deterioration (Delta) of progressor individuals are shown in **Supplementary Table 1**.

Three of these progressors showed changes suggestive of UIP-like pattern and therefore were included in our IPF program. In five that showed progression, the presence of some autoantibodies was revealed, and they were included in the Autoimmune-associated ILD program. Finally, three of the subjects showing progression died from non-respiratory causes.

We evaluated whether there were differences at baseline between progressive and non-progressive ILA. No association was found with pulmonary function and type of opacities on HRCT (e.g., subpleural fibrotic versus subpleural or non-subpleural non-fibrotic). Likewise, none of the biomarkers, MUC5B polymorphism, or demographic data showed an association with progression. Interestingly, progression was associated with gastroesophageal reflux (OR 4.1, CI95% 1.2-12.9; p=0.02), and in female with diabetes mellitus (OR 5.3, CI95% 1.03-27.4, p=0.01).

Discussion

Several population-based studies have demonstrated that around 8% of individuals over 50 years, mostly smokers, show interstitial lung abnormalities on HRCT (3, 4). However, the mechanisms implicated in its development remain unclear. Since different radiological features and diverse localization of the lung abnormalities are labeled as ILA, they likely represent diverse biopathological processes that will require longitudinal long-term observation of large cohorts at risk to determine etiology. In our study for example, after follow-up, three patients were diagnosed as IPF, and five as suffering from an autoimmune disease. Although studies on pathological correlates are scanty, a recent report showed that individuals with ILA were more likely to have subpleural fibrosis, fibroblastic foci, and atypical adenomatous hyperplasia compared with those without ILA, suggesting that ILA, in some cases, represents an early stage and/or mild form of pulmonary fibrosis (12). Certainly, these changes are not specific, and this retrospective study was performed in the context of lung nodule resections.

An important finding that emerges from the analysis of 4 large cohorts, is that interstitial lung abnormalities are associated with a greater risk of all-cause mortality, although the reasons are uncertain (7).

In the last 5 years, we have been recruiting respiratory asymptomatic individuals > 60 years, all residents of Mexico City (2240 meters altitude above sea level), and variable levels of air pollution. We found, similar to other populations with a different genetic background (e.g., non-Hispanic white participants from the general population, African-American participants from

COPDGene, and in a geographically and genetically isolated population from Iceland), and diverse environmental conditions, that around 10% of them presented ILA (compared to none of 61 young individuals, data non-shown), supporting that age is likely one of the driven forces of these interstitial lung abnormalities. In general, our results support the universality of this clinical problem, and that screening of aging populations may help to detect early subclinical stages of interstitial lung diseases, as has been suggested in other studies (13). Our findings also confirm that ILA is more frequent in older males, smokers, carrying the common variant MUC5B (rS35705950). This gain-of-function polymorphism has been associated with ILA and undiagnosed HRCT findings that are consistent with an early stage of pulmonary fibrosis (3, 14). More recently, GWAS of ILA in six different cohorts also confirmed the association with this MUC5B promoter variant and described novel genome-wide associations near IPO11 (rs6886640) and FCF1P3 (rs73199442) with ILA, and near HTRE1 (rs7744971) with subpleural-predominant ILA (15).

In contrast, we did not find significant differences regarding the common gene variant of TERT that has been associated with an increased risk of sporadic IPF (16).

As putative biomarkers, we selected several matrix metalloproteases, IL-6, SP-D, alpha-Klotho, and resistin. MMP-7 has been found to increase in ILA, as well as in several interstitial lung diseases and, together with MMP-1 has been proposed as a biomarker of IPF (17, 18).

Likewise, SP-D, likely reflecting alveolar epithelial injury, is increased in several ILD (19, 20), and IL-6 is regarded as one of the main components of the so-called inflammaging process that may result in age-associated pathologies (21-

23). Finally, α-Klotho was selected because is an anti-aging molecule that decreases progressively with aging, and has been implicated in some aging-associated lung disorders (24, 25).

We corroborated that MMP-7 is significantly increased in ILA subjects, and found a marked increase in some new biomarkers such as MMP-1, MMP-13, SP-D, and resistin. After Bonferroni correction for multiple testing MMP-13 and resistin remained markedly increased in ILA subjects. In a very recent report, it was also found that higher serum resistin levels were associated with greater high-attenuation areas on computed tomography (26). Resistin is a small, cysteine-rich secretory protein, that among other functions is an inflammatory regulator (27).

MMP-13 plays important roles not only in extracellular matrix remodeling but also in the processing of numerous bioactive mediators such as growth factors, cytokines, and chemokines, modulating their activity or releasing them from extracellular matrix-bound stocks (28). Interestingly, higher levels of collagen biomarkers have been associated with ILA independently of gender, race, and smoking status (29). Together, these results suggest that extracellular matrix remodeling may occur early and even before the onset of clinically evident disease.

Several studies have demonstrated rates of imaging progression oscillating between 20-40% (30). In our study, a longitudinal follow-up of the subjects with ILA showed progression in around 20% in a period of 2-3 years characterized by impaired lung function and increased radiological abnormalities. Compared with those without progression, they showed a high prevalence of gastroesophageal reflux, and in female was associated with diabetes mellitus.

None of the genetic or molecular markers studied in our study showed an association with progression. This finding suggests that the biomarkers confirmed or revealed in our study may be useful as diagnostic biomarkers for early detection of ILA in respiratory asymptomatic subjects.

In other studies, it has been demonstrated that a definite pattern of fibrosis by HRCT, characterized by traction bronchiectasis and honeycombing, is associated with an increased risk of progression (31). However, older individuals with HRCT abnormalities suspected of usual interstitial pneumonia, mainly if they are symptomatic, are diagnosed in our institution as “probably early IPF in study” and are excluded from the ILA group.

Our study has several limitations. First, the small number of patients with ILA, compromises the statistical power for some measurements, for example, the relationship with telomere length. Likewise, the absence of differences in the TERT variant (rs2736100) between groups may likely reflect the limited number of subjects in the groups and the relatively high frequency of this variant in controls. Another limitation is the time of follow-up. Actually, the rate of imaging progression of ILAs range from 20% over 2 years (32) while maybe greater 50% over 5 years as reported in the AGES-Reykjavik study (31). Therefore, these results should be corroborated in larger samples and greater follow-up periods.

In summary, our findings suggest that a significant percentage of asymptomatic respiratory individuals over 60 years develop interstitial lung abnormalities. Increased serum concentrations of pro-inflammatory molecules such as resistin and matrix metalloproteinases like MMP-13 may play a pathogenic role and may help to identify individuals who may be at higher risk of developing ILA.

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Authors' contributions:

Designed the study: IBR, MS

Data collection: AKSP, KME, AAD

Methodology: LCG, RF, IBR

Sample processing: GRM, EM, IH, CB, MP, LCG

Review of clinical studies and provided input on clinical implications: IBR, MM, FJ, AP, MS

Administered the follow-up and analyzed the data: AKSP, AAD, RF, IBR, AP, MS

Writing- original draft: MS

Writing- review & editing: IBR, AP, MS

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Table 1
Demographic and clinical characteristics of the Study Population

Variable	ILA n=80	Control n=564	p-value
Age years, (SD)	72 ± 8	69 ± 8	<0.0001
Gender male (%)	34 (43)	149 (26)	0.005
Smoking (never/former/current)	31/40/9	302/161/101	0.01
Systemic hypertension (%)	28 (35)	224(40)	0.5
Diabetes Mellitus (%)	15 (19)	127(23)	0.5
Gastroesophageal reflux (%)	22 (28)	197(35)	0.2

Table 2
Comparison of baseline pulmonary function tests between individuals with and without ILA

Variable	ILA n=80	Controls n=564	p-value
FVC (% predicted)	93 ± 17	95 ± 15	0.19
DL_{CO} adjusted (% predicted)	87 ± 18	102 ± 19	<0.0001
DL_{CO}/AV index	95 ± 29	109 ± 20	<0.0001
SpO2 at rest	94 ± 2	95 ± 2	0.0003
SpO2 post exercise	87 ± 9	91 ± 5	<0.0001
6MWT (meters)	407 ± 144	460 ± 112	0.0001

Table 3
Basal serum concentrations of biomarkers between groups

Biomarker	ILA n=80	Non-ILA n=80	p value	Corrected p value*
MMP-1 ng/ml (SD)	7 ± 4	6 ± 3	0.02	0.2
MMP-2 ng/ml (SD)	38± 4	37 ± 2	0.53	1.0
MMP-3 ng/ml (SD)	19 ± 11	17 ± 10	0.28	1.0
MMP-7 mcg/ml (SD)	6 ± 4	4 ± 2	0.008	0.09
MMP-8 ng/ml (SD)	4 ± 4	3 ± 3	0.28	1.0
MMP-9 ng/ml (SD)	14 ± 9	12 ± 8.2	0.32	1.0
MMP-12 pg/ml (SD)	30 ± 12	27 ± 10	0.16	1.0
MMP- 13 pg/ml (SD)	357 ± 143	298 ± 116	0.004	**0.04**
IL-6 ng/ml (SD)	15.7 ± 21	11.4 ± 15	0.04	0.4
SP-D ng/ml (SD)	10 ± 11	8 ± 6	0.04	0.4
alfa-Klotho pg/ml (SD)	735 ± 462	519 ± 133	0.99	1.0
Resistin ng/ml (SD)	12 ± 5	9 ± 4	0.0005	**0.006**
*corrected by Bonferroni adjustment; MMP=matrix metalloproteinase; SD=standard deviation; IL=interleukin; SP-D=surfactant protein D.				

Legends for figures

Figure 1. HRCT showing interstitial lung abnormalities in two asymptomatic respiratory subjects from the Lung Aging Program. A) central and subpleural reticular lesions in a male, 67 yr, non-smoker, without occupational or home exposures. B) predominant subpleural reticular abnormalities in a male 63yr, former smoker, without other exposures.

Figure 2. Representative HRCT images of progressing interstitial lung abnormalities. Images are from a single subject at the time of enrollment (male, 79 years, former smoker and welder, working in casting during 12 years, actual knife sharpener) and at 2-years follow-up. Panels A and B: Baseline and follow-up showing an increase of subpleural ground glass and reticular opacities. Panel C and D: Baseline and follow-up HRCT scans showing an increase of subpleural cystic lesions, reticular pattern and traction bronchiectasis (arrow).

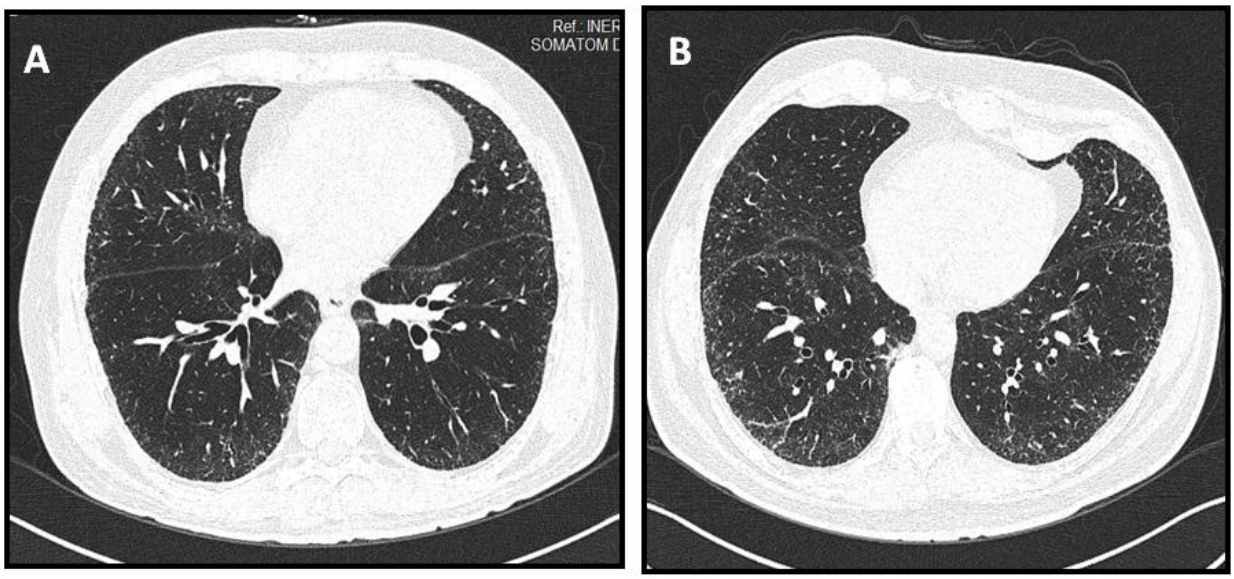

Figure 1.

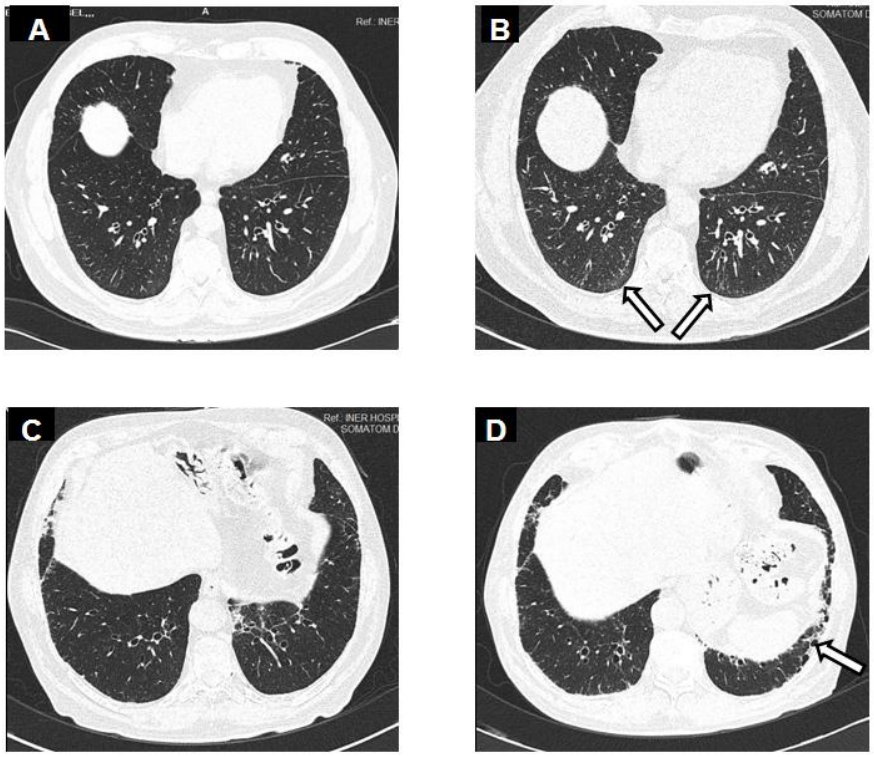

Figure 2

Table S1
Delta of pulmonary function tests and type of ILA according Fleischner classification in progressive individuals

Subject	Delta FVC% predicted	Delta DLCO % predicted	Delta Meters 6MWT	Fleischner* classification
1	-11	- 15	-20	SF
2	-13	-20	-100	SNF
3	-9	-13	-50	NSNF
4	-10	-21	0	NSNF
5	-3	-2	-160	SF
6	-6	-13	-290	NSNF
7	-10	-18	0	SNF
8	-8	-9	-160	NSNF
9	-12	-22	-68	SNF
10	-6	-6	-160	NSNF
11	-19	0	0	SNF
12	-4	-10	-330	SF
13	0	0	-50	SF
14	-2	-9	-58	SNF
15	0	-11	-185	SF
16	-6	-5	-200	NSNF
17	-7	-9	-240	SNF
18	0	-22	-36	SF

*SF= subpleural fibrotic, SNF= subpleural non-fibrotic, NSNF= non-subpleural non-fibrotic