Serum CA 15-3 is increased in pulmonary fibrosis

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ABSTRACT. *Background and aim of the work*: Carbohydrate antigen CA 15-3 is a glycoprotein whose expression, aberrant intracellular localization and changes in glycosylation have been associated with a wide range of cancers. Pulmonary fibrosis represents the final evolution of a chronic inflammation and is defined by the overgrowth of fibroblasts and exaggerated extracellular matrix deposition. The aim of the present study was to evaluate the possible diagnostic role of CA 15-3 in fibrosis in different idiopathic interstitial pneumonias. *Methods:* CA 15-3 was measured in serum samples from healthy subjects (n=25) and patients affected with idiopathic pulmonary fibrosis (IPF/UIP) (n=20), sarcoidosis (n=22) at different stages (I, II, and III) and systemic sclerosis (n=25). CA 15-3 protein expression was also evaluated by immunohistochemistry in 21 lung biopsies and in 6 primary lung fibroblasts cell lines. *Results:* The CA 15-3 serum levels were significantly higher in patients with IPF/UIP and with clinically advanced sarcoidosis (stage III). Serum CA 15-3 levels were slightly increased in patients with systemic sclerosis. No difference was observed between serum CA 15-3 levels in patients with sarcoidosis at stages I and II compared with control subjects. In IPF/UIP and in sarcoidosis at stage III elevated CA 15-3 serum levels significantly correlated with decreased total lung capacity, decreased diffusing capacity of carbon monoxide and high resolution computed tomography findings. Immunohistochemical analysis showed an intense specific CA 15-3 staining in fibroblasts within fibroblastic foci, surrounding sarcoid granulomas and in all cell cultures of lung fibroblasts from IPF/UIP lungs. *Conclusions:* Our results indicate that increased CA 15-3 levels are associated with pulmonary interstitial damage, fibroblast activity and progression to fibrosis of the lung. Therefore, CA-15-3 may be considered a sensitive marker useful in the identification of patients with advanced fibrosis and more severe prognosis. *(Sarcoidosis Vasc Diffuse Lung Dis 2009; 26: 54-63)*

KEY WORDS: Idiopathic interstitial pneumonias, sarcoidosis, systemic sclerosis, CA 15-3, immunohistochemistry, ELISA

INTRODUCTION

Pulmonary fibrosis is defined by the overgrowth, hardening and/or scarring of the lung related to exaggerated deposition of extracellular matrix components. It represents the final evolution of chronic inflammation triggered by a wide variety of stimuli (infection, autoimmune reaction, allergic response, chemical insults, radiation as well as many other unknown tissue injuries). Although current treatment for fibrotic diseases typically targets the

inflammatory response, a growing body of evidence indicates that the mechanisms driving fibro-genesis are distinct from those regulating inflammation. The key cellular mediator of the fibrosis seems to be the myofibroblast, which serves as a primary collagen-producing cell (1). This cell is considered the key cellular mediator of fibrosis. Pulmonary fibrosis is also the final stage of idiopathic interstitial pneumonias (IIPs), as well as clinically advanced sarcoidosis and systemic sclerosis (1).

IPF/UIP has been considered a progressive disease with no effective identifiable treatment at the present time other than lung transplantation. It is a devastating disease with a short-term prognosis worse than many well-differentiated human tumors (2).

Sarcoidosis is a chronic inflammatory disease characterized by a highly focused exaggerated immune response to an unknown antigen(s) in the target organs. It develops systemic non-caseating sarcoid granulomas, with hilar lympho-adenopathy and pulmonary infiltration, leading to fibroblastic activation and collagen deposition with lung parenchyma destruction (3-6).

Systemic sclerosis is a chronic multi-systemic disease of unknown etiology characterized by vascular changes and varying degree of lung fibrosis (7, 8).

Current available treatment is often ineffective against collagen deposition and new markers for evaluating the degree of disease pregression as well as new therapeutic targets are needed. CA 15-3, the product of the MUC1 gene, is a large transmembrane glycosylated molecule composed of three main domains, a large extracellular region, a membrane-spanning sequence and a cytoplasmic domain (9-12). Although the physiologic function of MUC1 is unclear, CA 15-3 has been implicated in cell adhesion, immunity, and metastasis (9-11). CA15-3 is a well known marker for the early detection of breast cancer recurrence and for assessing the efficacy of treatment for metastatic breast cancer (13). Elevated CA 15-3 serum levels have been previously reported in interstitial lung disease, associated with collagen diseases, such as dermatomyositis (DM) and polymyositis (PM), in patients without evidence of breast cancer (14, 15). These observations raise the possibility that serum CA 15-3 levels may represent a marker of pulmonary fibrosis and progression of disease. To test this hypothesis, we mesarued CA 15-3 levels in patients with different forms of lung damage and fibrosis in IPF/UIP, sarcoidosis and systemic sclerosis.

Materials and methods

1. Subjects

A total of 67 patients and 25 healthy controls were recruited for the study. Twenty patients were affected with IPF/UIP (14 smokers and 6 nonsmokers), twenty-two patients were affected with pulmonary sarcoidosis [6 stage I, (4 smokers and 2 nonsmokers); 8 stage II (5 smokers and 3 nonsmokers); 8 stage III, (4 smokers and 4 nonsmokers)]. Another twenty-five patients with systemic sclerosis (14 smokers and 11 nonsmokers) with lung involvement were added to the study. CA 15-3 serum levels were also assessed in 25 healthy subjects used as controls (15 smokers and 10 nonsmokers).

All patients underwent clinical examination, chest radiography, high resolution computed tomography (HRCT), lung function tests, fibreoptic bronchoscopy and some were subjected to lung biopsies (see below). All were checked for the presence of breast or other malignant tumors. Therefore, none of them, at the moment of the enrolment, had history or presence of cancer or previous treatment with corticosteroids or immunosuppressive drugs. All patients agreed to participate in the study and gave written informed consent.

2. Measurement of serum CA 15-3 levels by ELISA

The serum CA15-3 levels were measured using an ELISA commercial kit (ES300/Elecsys 2010; Roche Diagnostics). This method is a solid-phase, non-competitive immunoassay in which calibrators, controls and patient samples are incubated together with biotinylated anti-CA15-3 monoclonal antibody and horseradish peroxidase (HRP) labelled anti-CA15-3 monoclonal antibody in streptavidin coated microtiter strips. After washing, buffered substrate/chromogen reagent (hydrogen peroxide and 3,3',5,5' tetra-methylbenzidine) is added to each well and the enzyme reaction is allowed to proceed. During the enzyme reaction a blue colour will develop if antigen is present. The intensity of the colour is proportion-

al to the amount of CA15-3 present in the samples. The colour intensity is determined in a microtiter plate spectrophotometer at 620 nm. Calibration curves are constructed for each assay. The CA15-3 concentrations of patient samples are then read from the calibration curve.

3. HRCT

The study protocol required thin collimation (1-1.5 mm) images to be obtained from the lung using standard high-resolution technique in supine position. Two radiologists independently scored the baseline HRCT using a standardized form. The HRCT images were assessed for the presence and extent of abnormalities (ground glass attenuation, reticulation, honeycombing, centro-lobular nodules, other nodules, consolidation, and emphysema). For the purpose of this study, we have assessed the overall extent of fibrosis (i.e., the extent of reticulation and honeycombing), determined for the entire lung, using a 4-points scale (0 = no involvement, 1 = < 25% involvement, 2 = 26–50% involvement, 3 = 51-75% involvement, and 4 = 76-100% involvement) (16, 17).

4. Lung function tests

Lung function tests (LFTs) were carried out by a Fleish-type pneumotacograph with the Jager processing system (Jager, Italy). Testing was based on a complete VC (Vital Capacity) and FVC (Forced Vital Capacity) maneuver according to the ERS guidelines and repeated three times after adeguate instruction of the person being tested. Residual Volume (VR) and Total Lung Capacity (TLC) were obtained with the helium dilution method. Parameters were expressed as percentages of predicted values (CECA, 1983). Lung diffusing capacity for carbon monoxide (TLCO) completed the functional assessment.

5. Surgical biopsies and tissue preparation

Surgical lung biopsies were obtained from a total of 21 patients subdivided in patients affected by IPF/UIP (n=14) and sarcoidiosis III stage (n=2) as well as in 5 control patients (2 males and 3 females, age range 42-58 years) undergoing pulmonary resection for removal of benign lung tumors (5 amar-

tomas). Furthermore, two patients with systemic sclerosis who undersent surgical biopsy for diagnostic purpose were also recruited for immunohistochemistry. Specimens were dissected out and fixed in a buffered 10% formalin solution for 24 h. Fixed specimens were dehydrated in ethanol and embedded in paraffin. Serial 10 µm thick sections were obtained using a rotatory microtome, mounted on gelatine-coated cover slips and processed for immunohistochemistry (see below).

6. Isolation of human pulmonary fibroblasts

Primary fibroblast cell cultures were established from 6 IPF/UIP patient lung biopsies and from histologically normal areas of surgical lung specimens from patients undergoing lung resection. Lung tissue speciments were subjected to enzymatic digestion by incubation at 37°C with collagenase and protease. Purity of isolated lung fibroblasts was assessed by crystal violet and by immunofluorescent staining using monoclonal antibody against human fibroblasts. Once isolated the cells were rinsed in DMEM and re-suspended in the same culture medium supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 µg/ml streptomycin, and 2 mM L-glutamine.

Fibroblasts that grew under these conditions were sub cultured, upon reaching confluence, by trypsinization and either used for experiments or kept as frozen stock at -80°C. Fibroblasts were cultured in tissue culture flasks at 37°C in a humidified atmosphere of 5% CO_2. Culture medium was changed every 2 days until cells reached confluence. To ensure maximum reproducibility, cultures were grown for no more than six passages after recovery from frozen stocks and cell viability was monitored by trypan blue dye exclusion. Cytospin centrifuged fibroblasts, from the cell cultures, were fixed in 4% formalin (freshly prepared from para-formaldehyde powder), for 60 min at 22°C, on electrostatic slides and used for immunocytochemical purposes (see below).

7. Immunohistochemical and immunocytochemical detection of CA 15-3 immunoreactivity

Sections from lung biopsies of patients with IPF/UIP and sarcoidosis as well as slides with ad-

herent fibroblasts from primary cell cultures were used for the immunocytochemical detection of the CA 15-3 expression. For this purpose, anti-CA 15-3 mouse monoclonal antibody (Clone: DF3, Dako-Cytomation Inc. USA) was used at a dilution of 1:50. The deparaffinized sections were treated with heat prior to the immunohistochemical staining procedure.

Briefly, consecutive sections were exposed to the antibody alone. Optimal antisera dilutions and incubation times were assessed in a series of preliminary experiments. After incubation, slides were rinsed twice in phosphate buffer and exposed for 30 min. at 25°C to anti-mouse secondary antibodies diluted 1:100. The product of immune reaction was revealed using 0.05% 3,3-diaminobenzidine in 0.1% H_2O_2 as a chromogen. Sections were then washed, dehydrated in ethanol, mounted in a synthetic mounting medium and viewed at a light microscope. Endogenous peroxidase activity was blocked by H_2O_2, whereas non-specific IgG binding to glass and tissue was prevented by adding a 3% fetal calf serum to the incubation medium. The background of immune reaction was evaluated by incubating some sections with a non-immune serum, followed by processing with secondary antibodies (non specific binding).

The intensity of the immunoreaction developed within different lung structures was assessed microdensitometrically with an IAS 2000 image analyzer (Delta Sistemi, Rome, Italy) connected via a TV camera to a light microscope. Briefly, sections were examined at a final x200 magnification. The system was calibrated taking as zero the background of the non specific reaction. Ten μm2 areas were delineated in each reaction by a measuring diaphragm. The intensity of immune staining (test control value) was assessed by a program of the image analyzer expressing the intensity of immune reaction in arbitrary units.

9. Statistical analysis

An open source software was used for statistical analysis (R 2.4.0; AT&T now Lucent Tecnologies; www.project.org). Data from patients are presented as mean and standard deviation or as median and range. Firstly, the data were tested to determine their normal distribution (Shapiro-Wilks test) and the homogeneity of variance (homoscedasticity) across groups (Bartlett's test) in order to perform ANOVA analysis. All CA 15-3 values, except in UIP, had a normal distribution. Data of CA 15-3 were logarithmically transformed before ANOVA analysis among groups; then, differences between two groups were assessed by Tukey test. TLCO data and HRCT scores were statistically evaluated with the Kruskall Wallis test and differences between groups were evaluated by the Dunnett test. CA 15-3 data were correlated to TLCO values and HRCT score by Pearson's correlation. CA 15-3 immunoreactivity in lung sections was evaluated applying ANOVA analysis among groups and then the Tukey test for the differences between groups. A p< 0.05 was considered as cut-off for statistical significance.

Results

1. Serum CA 15-3 levels

Baseline characteristics of the four patients groups are shown in table 1 and they are compared with healthy controls. In particular, total lung capacity (TLC), lung diffusing capacity for carbon monoxide as percentage of predicted, HRCT score and CA 15-3 levels are shown as mean ± standard deviation or as median (range) and statistically evaluated.

The mean serum CA 15-3 level in the group of healthy subjects was 18.08 ± 5.67 U/ml comparable to that previously reported (18, 19). The mean serum CA 15-3 level in the cohort of patients with IPF/UIP was 121.07 ± 44.2 U/ml, higher than that of healthy controls, systemic sclerosis and sarcoidosis at stage III (p<0.05) (Table 1). Furthermore, sarcoidosis groups at stage III and systemic sclerosis showed CA 15-3 serum levels more elevated as compared to healthy controls (72.18 ± 20.79 and 35.38 ± 11.72 respectively; p<0.05). In sarcoidosis at stage I-II no significant changes of CA 15-3 serum levels from normal values were documented.

Considering all four groups of patients, a negative correlation was found between serum CA 15-3 level and TLCO percentage (Figure 1; p<0.01). On the contrary, a significant positive correlation was revealed between CA 15-3 serum levels and HRCT score (Figure 2; p< 0.01). Also TLCO % was statistically correlated with HRCT score. These correla-

Table 1. Baseline characteristics of healthy controls, IPF/UIP, sarcoidosis and systemic sclerosis patients

		Hc	IPF/UIP	S(I-II)	S(III)	SS
Subjects (M/F)		25 (12/13)	20 (12/8)	14 (8/6)	8 (3/5)	25 (9/16)
Age	❖	42.72±9.6	61.85±12.4	40.8±7.08	45.25±7.7	45.7±9.7
Onset (years)	❖		1.4±0.9	2±2.5	2.2±2.6	3.5±2.8
TLC (% predicted)	❖	102±6.5	52.4±6.7*	85.6±12*°	61±7.6*	79.1±5*°
TLCO (%)	❖	96.88±6.9	46.37±16.08*	89.71±10.69	63.77±26.9*	68.55±18.55*
	➢	94 (89 ;110)	50.9 (14 ; 72.6)	90.6 (74 ; 108)	53.3 (34 ; 103.9)	66.1 (24 ; 90.3)
HRCT score	❖	0	3.1±1.02	0.39±0.56*	2±1.07*	1.02±0.95*
	➢		3 (1 ; 4)	0 (0 ; 1.5)	2 (1 ; 4)	1 (0 ; 3)
CA 15-3 (U/ml)	❖	18.08±5.67	121.07±44.2*	23±8.37°	72.18±20.79*°	35.38±11.72°
	➢	18 (7 ; 30)	108 (60.8 ; 205)	22.5 (8 ; 35.2)	70.5 (42.9 ; 108.8)	30 (17.7 ; 60.7)

Hc: Healthy controls; IPF/UIP: idiopathic pulmonary fibrosis; S(I-II) and S (III): sarcoidosis stage I-II or III; SS: systemic sclerosis.
TLC: Total lung capacity; TLCO: lung diffusing capacity for carbon monoxide, HRCT score: high resolution computed tomography score.
TLC: ANOVA analysis among groups ($p<0.001$) and then Tukey test: * $p<0.05$ Hc *vs* pts group - ° p< 0.05 IPF/UIP *vs* S(I-II) and SS
CA 15-3: ANOVA analysis among groups ($p<0.001$) and then Tukey test: * $p<0.05$ Hc *vs* ;IPF/UIP and S(III) - ° p< 0.05 IPF/UIP *vs* S(I-II) and S (III) and SS
TLCO: Kruskall-Wallis test among groups ($p<0.001$) and then Dunnett test between Hc and another group: *= p< 0.05
HRCT score: Kruskall-Wallis test among pts groups ($p<0.001$) and then Dunnett test between IPF/UIP and another group:
*= p< 0.05; ❖ mean ± standard deviation ➢median (range)
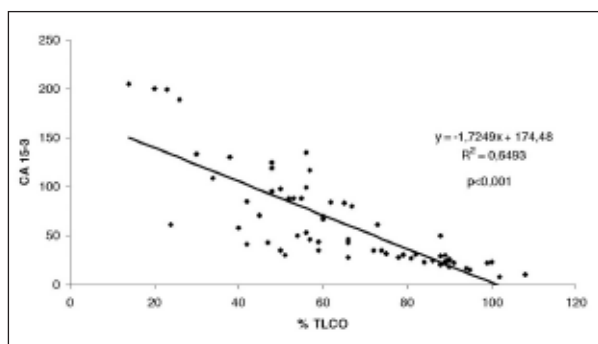
Fig. 1. CA15-3 levels (U/ml) in comparison with %TLCO (Pearsons' correlation) in 67 patients with sarcoidosis (stage I-II; III), IPF/UIP and systemic sclerosis
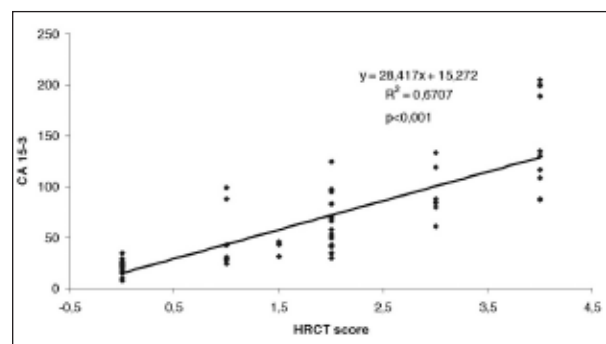
Fig. 2. CA15-3 levels (U/ml) in comparison with HRCT score (Pearsons' correlation) in 67 patients with sarcoidosis (stage I-II; III), IPF/UIP and systemic sclerosis

tions were also found when IPF/UIP, S(III) and SS groups were examined one by one (table 2).

CA 15-3 had a sensitivity of 75% and a specificity of 88.9% for separating patients with IPF/UIP and sarcoidosis at stage III from patients with sarcoidosis at stage I-II and systemic sclerosis. Its positive predictive value was of 59% while the negative predictive value was 61% for a cut-off<31U/ml.

No cases of lung or breast cancer were observed in our population of IPF/UIP patients although it has been reported in the literature that the clinical course of a discrete number of patients with IPF/UIP is complicated by lung cancer.

2. Correlation between CA 15-3 serum level and common blood and BAL markers of disease

No significant correlation between serum CA 15-3 and specific BAL and serum markers of disease was found in any group of patients examined. In particular, no statistical correlation was documented between CA 15-3 levels and total and differential BAL

Table 2. Correlation between CA15-3 serum levels and TLCO or HRCT score and between HRCT score and TLCO in four groups of patients suffering from IPF/UIP, sarcoidosis at stage I-II or III and systemic sclerosis.

	IPF/UIP (r)	S (I-II) (r)	S(III) (r)	SS (r)
Ca15-3 *vs* TLCO	-0,92***	-0,9***	-0,80*	-0,835***
Ca15-3 *vs* HRCT score	0,486*	0,636*	0,79*	0,798***
HRCT score *vs* TLCO	-0,50*	-0,656*	-0,72*	-0,926***

IPF/UIP: idiopathic pulmonary fibrosis; S(I-II) and S (III): sarcoidosis stage I-II or III; SS: systemic sclerosis.
CA 15-3: serum CA 15-3 level; TLCO: lung diffusing capacity for carbon monoxide, HRCT score: high resolution computed tomography score.
Pearson's correlation (r): * and ***: $p < 0.05$ and $p < 0.001$

cell count, BAL lymphocytes, CD4+/CD8+ ratio or angiotensin converting enzyme (sACE) level. Furthermore, in systemic sclerosis no correlation was demonstrated between CA 15-3 serum levels and auto-antibody tests performed, such as anti-endothelial cell antibodies (AECA), von Willerbrand factor antigen (vWfAg), anti-centromere antibodies (ACA) or anti-topoisomerase antibodies (TOPO) these last two being related to lung fibrosis (data not shown).

3. Immunohistochemical detection of CA 15-3 immunoreactivity in fibroblasts from normal lung and IIP patients

In sections from control and fibrotic lung tissues, CA 15-3 positive staining was detected in epithelial cells (Figure 3 pictures A and B). A weak and scattered positivity was seen in alveolar epithelial cells, stromal fibroblasts and vascular smooth muscle cells in the normal lung. An inconstant CA 15-3 immunostaining was documented in interstitial lung fibroblasts in sections derived from systemic sclerosis (non specific pattern; Figure 3, picture D). In IPF/UIP lung sections, distinct population, of fibroblasts within fibroblastic foci displayed a specific cytoplasmic CA 15-3 immunostaining (Figure 3, picture E). No immunostaining was documented when the sections were pre-incubated with the secondary antibody alone (Figure 3, picture F, non specific NS). In pulmonary sarcoidosis, a specific but inconstant CA 15-3 immunostaining was demonstrated within fibroblasts surrounding sarcoid granulomas (Figure 3, picture G arrowheads). No

immunostaining was documented when the sections of sarcoid patients were pre-incubated with the secondary antibody alone (Figure 3 picture H, non specific NS).

In addition, specific CA 15-3 immunostaining was also documented in cytospin centrifuged fibroblasts from primary cell cultures from IPF/UIP lungs (Figure 3, Panel I) but not in normal fibroblasts (Figure 3, panel L). The microdensitometric evaluation of the immunoreactivity is summarized in Table 3.

Discussion

The aim of the present study was to study if serum CA 15-3 levels may represent a marker of pulmonary fibrosis and progression of disease in IPF/UIP, sarcoidosis and systemic sclerosis.

In the present study we demonstrated for the first time an increase in CA 15-3 serum levels in patients with pulmonary fibrosis, indicating that this previously known marker of breast cancer is also useful as a marker of fibrosis of the lung. In addition, we demonstrated that increased CA 15-3 serum levels correlate with the severity of lung involvement. CA 15-3 serum levels, in fact, are strongly increased in IPF/UIP, in stage III sarcoidosis and, to a lesser extent, in patients affected by systemic sclerosis with lung involvement, while they are normal in patients with sarcoidosis at stages I and II. Therefore our results suggest that CA 15-3 serum levels may also be considered as novel markers of lung interstitial damage.

Our results are in agreement with previous observation in which serum levels of CA 15-3 were found elevated in two cases of severe pulmonary fibrosis, associated with dermatomyositis and polymyositis (20).

Although no specific serum diagnostic or prognostic marker has been clearly identified so far, many different tumor-associated antigens have been suggested to be linked to interstitial lung diseases (21, 22, 23-26). The serum levels of KL-6 were elevated in 70-100% of patients with interstitial pneumonitis, with pulmonary fibrosis (either idiopathic or related to collagen-vascular disorders), hypersensitivity pneumonitis, sarcoidosis, and radiation pneumonitis (21, 22, 23-26). Moreover, KL-6 displays chemotac-
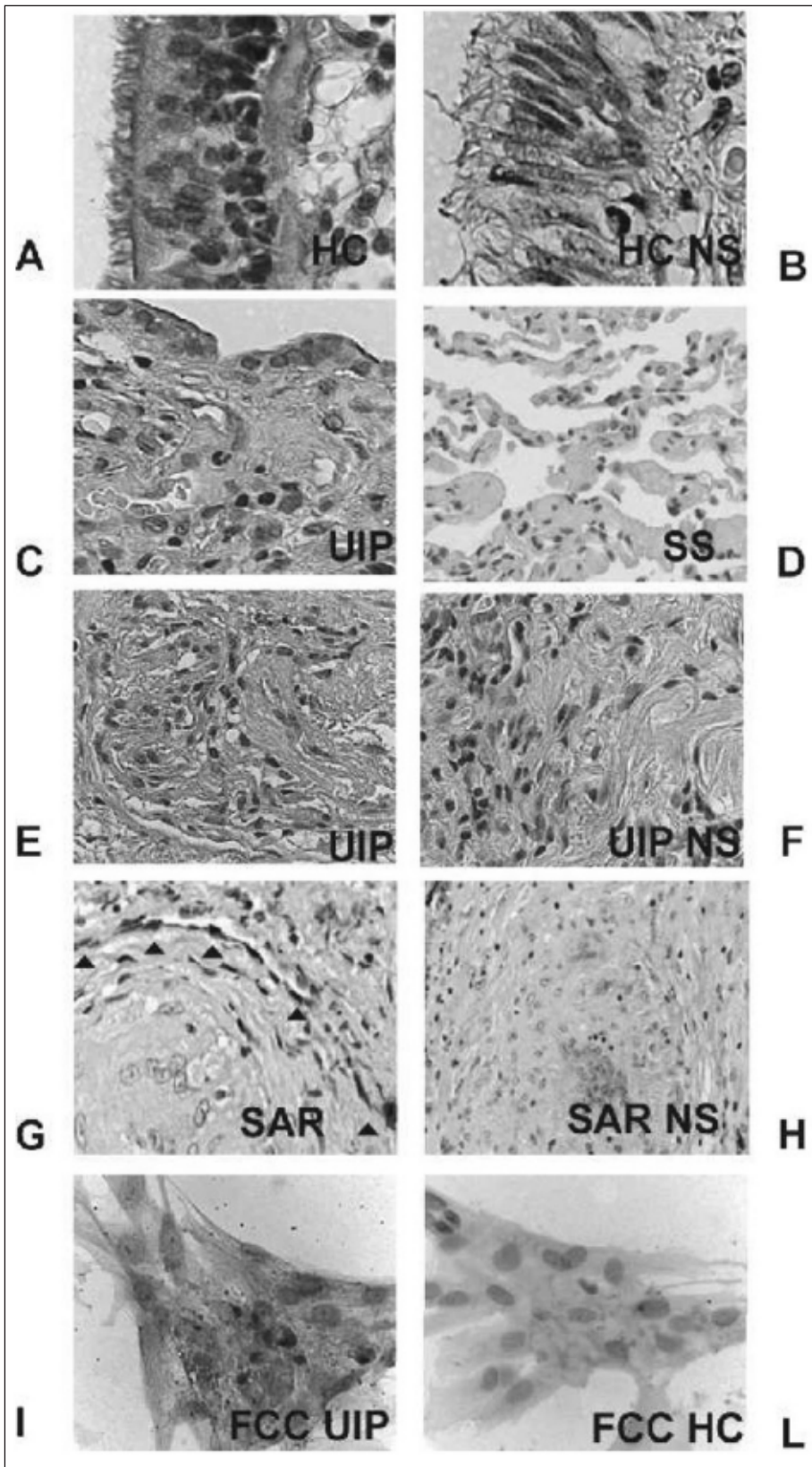
Fig. 3. Immunohisochemical detection of CA 15-3 staining in representative paraffin embedded section of lung biopsies from normal lung (HC), systemic sclerosis patients (SS), IPF/UIP patients (UIP) with or without primary antibody (non specific staining NS), sarcoidosis stage III (SAR) with or without primary antibody (NS) and cultured fibroblasts (FCC) from IPF/UIP and HC.

UIP: Idiopatic interstitial pneumoniae/usual interstitial pneumoniae;
SS: systemic sclerosis;
HC: normal lung;
SAR: sarcoidosis,
FCC: cultured fibroblasts;
NS: non specific.
Magnification: x 400

Table 3. Microdensitometric evaluation of CA 15-3 immunoreactivity in lung sections of healthy controls, IPF/UIP, sarcoidosis (stage III) and systemic sclerosis patients.

	Hc (2pts)	S(III) (2 pts)	SS (2 pts)	IPF/UIP (14 pts)
Ciliated epithelium	12.4 ± 3.3	18.4 ± 1.1[§]	14.2 ± 2.1	19.2 ± 1.6[*°]
Bronchial glands	6.5 ± 0.3	8.3 ± 0.6	7.8 ± 0.7	9.6 ± 1.0[* ^]
Interstitial lung Fibroblasts	9.2 ± 0.8	18.9 ± 1.9 [§ &]	14.2 ± 1.1 [ç]	21.3 ± 1.9[*^]
Hyperplastic type II alveolar cells				$6.3+2.6$

Hc: healthy controls; S (III): sarcoidosis at stage III; SS: systemic sclerosis; IPF/UIP: idiopathic pulmonary fibrosis.

Data are mean $\pm$ SEM and were obtained by examining three slides per each subject. Intensity of the immunostaining is expressed as arbitrary units proportional to the intensity of the staining measure microdensitometrically. For details see materials and methods section.

Ciliated epithelium   - ANOVA among groups: $p<0.001$
                                    - Tukey test: $p < 0.05$ [*] IPF/UIP vs Hc ; [°] IPF/UIP vs Ss ; [§] S III vs Hc

Bronchial glands   - ANOVA among groups. $p<0.001$
                                    - Tukey test: $p < 0.05$ [*] IPF/UIP vs Hc ; [^] IPF/UIP vs SS

IL fibroblasts   - ANOVA among groups $p<0.001$
                                  - Tukey test: $p < 0.05$ [*] IPF/UIP vs Hc ; [^] IPF/UIP vs SS ; [§] S III vs Hc; [&] S III vs SS ; [ç] SS vs Hc

tic activity (27), accelerates proliferation and inhibits the apoptosis of human lung fibroblasts (27). Finally, increased serum KL-6 levels have been associated with poor prognosis and increased risk of mortality in IIP patients (21). KL-6 is also involved in interstitial pneumonia associated with ANCA-related vasculitis (16) and with amiodarone-induced pulmonary damage (17). KL-6 and CA 15-3, belong to the same MUC-1 superfamily (9, 10, 15). It is interesting to note that the serum levels of KL-6 positively correlated with those of CA15-3 in patients with interstitial pneumonia, associated with collagen diseases (11). The reason for this association maylie in the common origin of these two molecules. Furthermore it is possible to hypothesize that the elevated serum levels of these antigens may be related to their increased secretion, by bronchial epithelium, bronchial glands and stromal cells with subsequent release into the bloodstream. We did not detect differences in the expression of CA 15-3 in alveolar epithelial cells, therefore, it is unlikely that these cells may be responsible, for this phenomenon.

Other serum proteins have been reported to be elevated in IIP patients, such as many components of the Wnt β-catenin pathway (29, 30) as well as in fibrotic lung tissue repair and in epithelial-mesenchymal transitions associated with lung tumor progression (31). An aberrant activation of Wnt downstream target genes was demonstrated in patients with IPF/UIP (29, 32). In this regard, it is interesting to note that MUC-1 was found to be able to bind directly to β-catenin and to coactivate transcription of the Wnt responsive cyclin D1 promoter (33).

It has been described that in IPF/UIP, sarcoidosis and systemic sclerosis patietns baseline percent-predicted TLCO correlated well with HRCT findings (34-37). TLCO is considered a good monitoring parameter and of particular interest, given that it is considered an independent predictor of mortality (34). In addition, the percent-predicted TLCO and the HRCT scores are considered as the sole independent predictors of 2-year survival in patients with IPF/UIP (37). The correlation between CA 15-3 and functional and radiological findings support the usefulness of CA 15-3 as an integral part of the evaluation of patients with suspected pulmonary fibrosis and further supports its role as a possible predictor of severity of the disease. Furthermore, the lesser degree of increase in CA 15-3 serum levels detected in systemic sclerosis may be related to a less severe fibrotic involvement of the lung in our patients (see functional and radiological findings) and probably with different mechanisms of lung damage and repair.

CA 15-3 monoclonal antibody has been shown to stain different cells in normal lung (stromal fibroblasts, glandular epithelial and vascular smooth muscle). A significant CA 15-3 immunostaining was clearly noticeable in both fibroblasts within fibrob-

lastic foci, in fibroblasts surrounding sarcoid granulomas and in primary cultures of lung fibroblasts from IPF/UIP patients. Although the functional role of CA 15-3 mediated cell signalling remains to be elucidated, it has been postulated that it may be implicated in several cell functions. It was reported that MUC1 mucin displays various pathophysiologic roles. It inhibits cell-cell adhesion, decreases the susceptibility of malignant cells to cytotoxic T cells and MUC1 itself may be a target molecule of HLA-unrestricted cytotoxic T cells (27). Furthermore, MUC1 interacts with fibroblast growth factor, receptor-3, a member of the receptor tyrosine kinase family, activated by basic fibroblast growth factor a potent mitogenic factor for myofibroblasts and fibroblasts proliferation. Therefore, CA 15-3 increase during fibrosis may play a role in modulating fibroblast intracellular signalling and fibro-proliferative response.

Our results suggest that circulating levels of CA 15-3 can be elevated in some patients with non-malignant diseases and that it may also be considered as a sensitive indicator of diffuse interstitial lung disease clinical course and lung involvement.

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