



# HRCT and histopathological evaluation of fibrosis and tissue destruction in IPF associated with pulmonary emphysema

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## KEYWORDS

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## Summary

Idiopathic pulmonary fibrosis has been associated with emphysema in cigarette smokers as a new clinical entity: combined pulmonary fibrosis and emphysema (CPFE).

In order to compare histomorphometrical, roentgenological and immunohistochemical aspects of usual interstitial pneumonia (UIP) with and without associated pulmonary emphysema, 17 patients with biopsy-proven UIP were evaluated. Morphometrical evaluation of lung parenchyma destruction was used to divide patients in two subgroups: emphysema/UIP ( $n = 9$ ) and UIP alone ( $n = 8$ ); four patients with biopsy-proven emphysema without fibrosis were also evaluated.

At HRCT scan, emphysematous lesions were prevalent in the upper fields of both emphysema/UIP and emphysema groups and the distribution of fibrotic lesions was similar in emphysema/UIP compared to UIP alone. The semiquantitative histopathological fibrotic score was also similar in emphysema/UIP and UIP alone. In addition, the expression of tumor necrosis factor (TNF)- $\alpha$ , matrix metalloproteinase (MMP)-2, MMP-9, MMP-7 and membrane type 1-metalloproteinase (MT1-MMP) by fibroblasts of myofibroblastic foci was similar in emphysema/UIP and UIP alone patients. In contrast, fibroblasts in areas of parenchymal destruction of emphysema/UIP expressed MMP-2, MMP-9, MMP-7 and MT1-MMP at variable but significantly

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higher levels when compared to emphysema subjects, in the presence of similar levels of TIMP-1, TIMP-2 and TNF- $\alpha$ .

Fibrotic and emphysematous lesions in emphysema/UIP patients appear to follow the roentgenological and histopathological patterns expected for either UIP or emphysema. Interstitial fibroblast activation is more pronounced in the areas of lung destruction in emphysema/UIP compared to those with emphysema alone, as for exaggerated tissue remodeling.

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## Introduction

Idiopathic pulmonary fibrosis (IPF) is a lung disorder variably associated with cigarette smoking whereby about half of the subjects characterized by the pathological pattern of usual interstitial pneumonia (UIP)<sup>1</sup> are reported as smokers or former smokers.<sup>2</sup> Emphysema is the most common cigarette smoke-related disorder.<sup>3</sup> It has been defined as an abnormal, permanent enlargement of airspaces distal to the terminal bronchiole without obvious fibrosis,<sup>4</sup> however, the work of Thurlbeck and colleagues has suggested that both destruction and repair abnormalities may take place concomitantly in the emphysematous lung,<sup>5</sup> as smoker's irregular enlargement of airspaces is accompanied not only by loss of elastic tissue but also by collagen deposition.<sup>6</sup> In this regard, experimental animal studies suggest that collagen and elastin deposition may be considered interlaced events in a complex process leading to emphysema and to fibrosis,<sup>7,8</sup> i.e., diseases characterized by a profound disturbance of the lung architecture due to aberrant destruction and/or remodeling of the extracellular matrix (ECM). Matrix metalloproteases (MMPs) are known to play a central role in remodeling both through ECM cleavage and by generating bioactive mediators. Among them, MMP-2, MMP-7 and MMP-9 are highly expressed in IPF which are found in the alveolar and basal bronchiolar epithelial cells and the fibroblastic foci.<sup>9</sup> In emphysema, the expression of MMP2, MMP9 and membrane type 1-MMP (MT1-MMP) has been observed in epithelial cells, fibroblasts, and alveolar macrophages, and they are thought to play a role in the rupture of the elastic fibers.<sup>10–12</sup> MMPs may therefore be important both in IPF and in emphysema, thus in IPF and emphysema combined.

Earlier clinico-roentgenological and experimental studies of pulmonary fibrosis associated with emphysema have described pulmonary fibrosis as a "second disease", superimposed upon pre-existing emphysema.<sup>13</sup> More recently, Cottin and co-workers have described a rather large series of patients with a variety of fibrotic disorders associated with emphysema, thereby identifying a new disease entity called "combined pulmonary fibrosis and emphysema" (CPFE), an entity which can be recognized at computerized tomography.<sup>14</sup>

Since no correlative analysis of the roentgenological, histomorphometrical and immunohistochemical features of combined fibrosis and emphysema has been reported so far, this study was undertaken to immunohistochemically characterize the presentation of IPF associated with emphysema in order to correlate histo- and HRCT-morphometrical findings with immunohistochemical data in an attempt to investigate whether the concomitant pulmonary

fibrosis may modify the destruction and remodeling patterns of associated pulmonary emphysema.

## Methods and materials

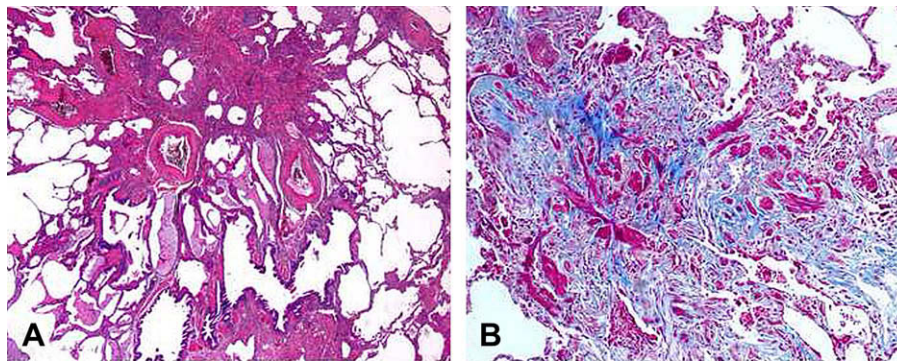
### Study population

Seventeen patients with IPF were included in the study. All met the ATS/ERS Consensus criteria for the diagnosis of IPF<sup>2</sup> as they had an HRCT scan consistent with the IPF pattern of pulmonary fibrosis (honeycombing with basal and peripheral reticular opacities and traction bronchiectasis, in the absence of peribronchovascular nodules, micronodules, isolated cysts and consolidation) and a histological pattern typical of UIP on lung biopsy obtained by video-assisted thoracoscopy. The initial evaluation included detailed history, including occupational activity, physical examination, chest X-rays and routine blood tests. Serum anti-neutrophil cytoplasmic antibody (P-ANCA), C-ANCA, extractable nuclear antigens (ENA), antinuclear, anti-mitochondrial and anti-DNA antibodies, rheumatoid factor, angiotensin converting enzyme and cryoglobulins were analyzed in order to rule out the diagnosis of collagen vascular diseases. Four patients presented mildly increased levels of anti-DNA antibodies.

The study was approved by the Institutional Bioethics Committee of the University of Roma "Tor Vergata".

### Histomorphometrical analysis

Tissue sections were stained with Haematoxylin–Eosin (Fig. 1A) and Masson's trichrome stains, and analyzed by two independent observers blind of the patient diagnosis. Fibrosis was scored by measuring the percentage of tissue with fibrotic changes on Masson's trichrome stained sections at 250 $\times$  magnifications and graded as follows: 0 (<25%); 1 (25–50%), 2 (50–75%); 3 (75–100%). Fibrosis was calculated in at least 10 randomly selected fields for each case; the number of fields to obtain a significant difference was calculated according to stereological formulae, as previously reported.<sup>15</sup> The parenchymal destruction index, expressed as the percentage of the pulmonary surface showing septal disruption, was microscopically evaluated using morphometric methods with computer-assisted image analysis, using Scion Image Software on Haematoxylin–Eosin stained sections at 250 $\times$  magnifications captured by a Hamamatsu camera (Hamamatsu City, Japan) connected to a Nikon microscope (Tokyo, Japan), as previously described.<sup>15,16</sup> At least 10 randomly selected fields were measured for each case and the number of fields to obtain a significant difference was



**Figure 1** (A) Haematoxylin–Eosin stained section of an emphysema/UIP patient's lung parenchyma, showing emphysematous areas adjacent to regions more closely resembling classic UIP at low magnification. (B) Masson's trichrome stain of a classic fibroblastic focus in fibrotic area of emphysema/UIP lung parenchyma fibrotic area. Original magnifications, A =  $\times 20$ , B =  $\times 250$ .

calculated according to stereological formulae.<sup>15</sup> The mean value of parenchymal destruction index calculated in all IPF cases was 39.8, thus the value of 40 was chosen to discriminate the emphysema/UIP group from the UIP alone group.

### Histomorphometrically defined study subgroups

After histopathological quantification of pulmonary emphysema, subjects with parenchymal destruction index greater than 40 were categorized as combined emphysema/UIP ( $n = 9$ ) and those with less than 40 as UIP alone ( $n = 8$ ). The emphysema/UIP group comprised three females and six males. They were all former smokers. Seven of them had been treated with corticosteroids, one with azathioprine and one with interferon-gamma. The UIP alone group included three females and five males. Five of them were current/former smokers and three never smoked. Six of them had been treated with corticosteroids. Four subjects with typical pulmonary emphysema were included in the study as disease controls. All had lung tissue examination as for lung cancer resection. They included one female and three males and all of them were former smokers.

### High-resolution computerized tomography (HRCT) scanning

Chest radiographs and HRCT scans were reviewed by an experienced thoracic radiologist unaware of patients' clinical details. HRCT images were scored for fibrosis and emphysema separately, using three sections taken at the level of the aortic arch (upper fields), the carina (medium fields) and 1 cm above the diaphragm (lower fields), as described by Kazerooni et al.<sup>17</sup> Each field was scored for fibrosis taking into account the following variables: ground-glass attenuation, interstitial thickening, subpleural cysts and generalized intraparenchymal cysts. Emphysema was defined as permeative destruction, without visible walls and without uniform distribution.<sup>4</sup> Bullae were defined as confluent areas of low density, arranged on a single layer, with a diameter of at least 1 cm, with a convex outline, thin walls and absence of lung tissue within the bulla.<sup>18</sup> The percentage of the lung involvement for each type of finding (ground glass, interstitial thickening, subpleural cysts,

generalized cysts, emphysema and bullae) was assessed visually and classified on a five point scale as 0 (absent), 1 (<25% of area involved), 2 (25–50% involved), 3 (50–75% involved), and 4 (>75% involved).

### Pulmonary function tests

Lung function testing included dynamic and static lung volumes (Master lab Jaeger, Würzburg, Germany) and arterial blood gas analysis. The normal spirometric values from the European Coal and Steel Community were used as reference.<sup>19</sup>

### Immunohistochemistry

The protein expression of MMP-2, MMP-9, MMP-7 and MT1-MMP of the tissue inhibitors of metalloproteinases (TIMP) TIMP-1 and TIMP-2 and of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) was investigated using immunohistochemistry; 4  $\mu$ m thick serial sections from each patient's lung biopsy were placed on Superfrost Plus slides (Menzel-Glaser, Braunschweig, Germany) and baked overnight at 60 °C. After deparaffinization in xylene and rehydration in graded concentrations of ethanol, sections were incubated in 0.3% hydrogen-peroxide–methanol to block endogenous peroxidases and rinsed with PBS.

Non-specific antibody binding was blocked by incubation with normal goat serum (Ylem, Avezzano, Italy; 1:20 in BSA 5%) for 30 min at room temperature. Antibody dilutions were 1:100 for mouse monoclonal antibodies anti-MT1-MMP (Oncogene, San Diego, CA), MMP-2 (Calbiochem, Cambridge, MA), MMP-7 (kindly provided by Prof. M. Chilosi, Verona, Italy), and rabbit polyclonal anti-MMP-9 (Neomarkers, Fremont, CA, USA), 1:10 for mouse monoclonal antibodies anti-TIMP-1 and TIMP-2 (Calbiochem, Cambridge, CA) and 1:100 for goat polyclonal anti-TNF- $\alpha$  (Santa Cruz Biotechnology Inc., Santa Cruz, CA) and all antibodies were incubated for 30 min. Sections were then incubated with specific Biotin-labelled secondary antibodies, followed by a streptavidin–horseradish peroxidases conjugate. Bound antibody was revealed with the use of the substrate 3,3'-diaminobenzidine. Positive (high-grade mammary carcinoma) and negative (non-specific IgG, 5  $\mu$ g/ml, instead of primary antibody) controls were included with each batch of sections to confirm the consistency of the analysis. Sections of

parenchyma from four non-smoking patients from lung cancer resection (mean age  $65 \pm 14.1$ ) with no clinicoradiological signs of emphysema were also used as negative controls. Interstitial fibroblasts were evaluated in thickened septa of emphysematous areas as well as in fibroblastic or myofibroblastic foci of emphysema/UIP patients; pneumocytes, macrophagic and capillary endothelial cells were morphologically excluded; cells of uncertain categorization areas were re-evaluated after routine AE1/AE3 cytokeratin, CD68 and Factor VIII immunostaining of serial sections (Diapath-Ventana system, Tucson, AZ, USA). Immunostaining was graded as follows: 0 (complete absence of or weak staining in <10% of cells); 1 (weak staining in <50% or strong staining in <10% of cells); 2 (weak staining in >50% or strong staining in <50% of cells); 3 (strong staining in >50% cells). Semiquantitative analysis was carried out by two observers without knowledge of the patient clinicoradiological status or of the antibody examined. For each case, at least 10 randomly selected high power fields were evaluated, as previously reported.<sup>20</sup>

## Statistical analysis

Clinic-radiological values are presented as mean  $\pm$  S.E.M. Distribution analysis was performed with the Kolmogorov–Smirnov test with the Dallal–Wilkinson approximation to Lilliefors's method for all variables. For multiple comparison among groups (functional, radiographical and histopathological variables) either one-way ANOVA followed by Tukey's test, or Kruskal–Wallis and Dunns tests, when appropriate, were used. Histomorphometrical and immunohistochemical results were analysed by Student's *t* test and expressed as arbitrary units  $\pm$  S.E.M. *P* values less than 0.05 were regarded as significant. Statistical analysis was performed with GraphPad Prism 4 (GraphPad Software, San Diego, CA).

## Results

### Pulmonary function testing

Among the nine subjects with emphysema/UIP, four showed normal lung volumes while five had mildly reduced lung volumes; airflow was preserved in all subjects. Mild hypoxemia was present in four (Table 1). Among the eight patients with UIP alone, six presented mildly/moderately reduced lung volumes, one had very mildly reduced airflow ( $p > 0.19$  all comparisons, emphysema/UIP vs. UIP alone group, Table 1) and five showed mild hypoxemia ( $p > 0.79$  all comparisons, emphysema/UIP vs. UIP alone group, Table 1). All the four emphysema subjects had increased lung volumes ( $p = 0.024$  for RV and  $p = 0.017$  for TLC compared to the emphysema/UIP group; Table 1) with impaired airflow ( $p = 0.001$  compared to the emphysema/UIP group, Table 1) and mild to moderate hypoxemia ( $p = 0.51$  compared to the emphysema/UIP group, Table 1).

### HRCT evaluation

Fibrotic abnormalities were mainly represented by interstitial thickening and both subpleural and generalized cysts

**Table 1** Patients' demographical and functional characteristics

|                                    | Emphysema/<br>UIP | UIP            | Emphysema<br>UIP |
|------------------------------------|-------------------|----------------|------------------|
| Male/female                        | 6/3               | 5/3            | 2/2              |
| Age                                | $71 \pm 2.8$      | $65 \pm 4$     | $73 \pm 2$       |
| Smoker (ever/never)                | 9/0               | 5/3            | 4/0              |
| Corticosteroid<br>therapy (yes/no) | 6/3               | 6/2            | 0/4              |
| FVC (% pred)                       | $82 \pm 6$        | $69 \pm 8$     | $85 \pm 4$       |
| FEV <sub>1</sub> (% pred)          | $83 \pm 5$        | $71 \pm 8$     | $58 \pm 4$       |
| FEV <sub>1</sub> /FVC              | $85 \pm 2$        | $81 \pm 2$     | $53 \pm 4$       |
| RV % (pred)                        | $78 \pm 7$        | $65 \pm 8$     | $149 \pm 18$     |
| TLC (% pred)                       | $76 \pm 6$        | $63 \pm 6$     | $106 \pm 7$      |
| pH                                 | $7.4 \pm 0$       | $7.4 \pm 0.01$ | $7.4 \pm 0.01$   |
| PaO <sub>2</sub> (mmHg)            | $79 \pm 4$        | $73 \pm 5$     | $70 \pm 8$       |
| PaCO <sub>2</sub> (mmHg)           | $41 \pm 1$        | $37 \pm 1$     | $47 \pm 4$       |

in the emphysema/UIP as well as in the UIP alone subjects. In both groups, ground glass was modest in all lung fields. The distribution of fibrotic lesions in the upper, middle and lower fields of the lungs was not significantly different in the emphysema/UIP compared to the UIP alone group subjects ( $p > 0.07$ , all comparisons; Fig. 1B,D,F). HRCT non-bullous emphysematous abnormalities were seen in six out of the nine subjects in the emphysema/UIP group but in none of the UIP alone group. HRCT bullous emphysematous abnormalities were present in two subjects in the emphysema/UIP group. One of the subjects assigned to the latter group did not show any HRCT bullous or non-bullous abnormalities. In this group, emphysematous lesions were prevalent in the upper fields, although the difference was not statistically significant (Fig. 2A,C,E;  $p = 0.45$  upper+medium fields vs. lower fields). In the subjects with emphysema alone, there was a trend toward upper lobes predominance of HRCT non-bullous emphysematous abnormalities ( $p = 0.14$  upper+medium fields vs. lower fields) and no HRCT fibrotic lesions were seen.

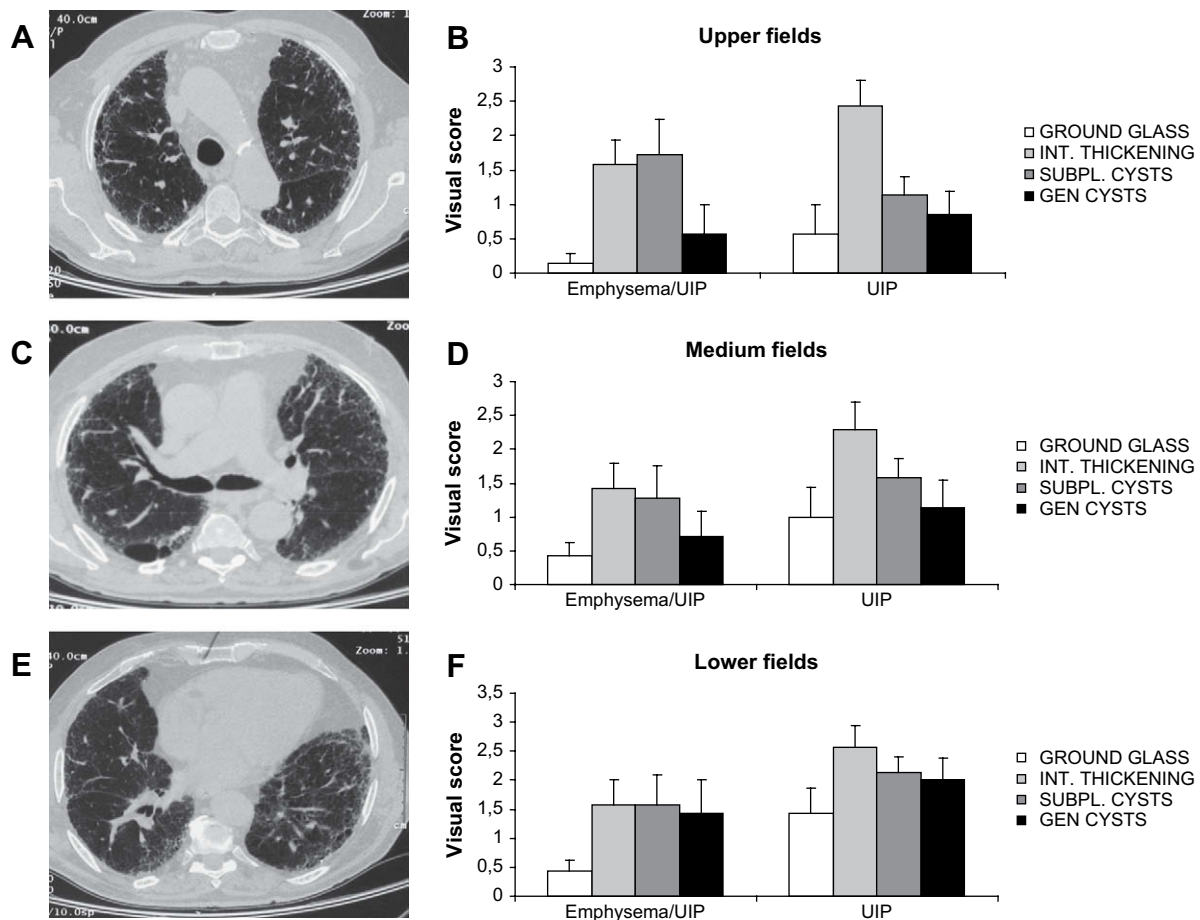
### Morphometry and immunohistochemistry

The fibrotic score, microscopically calculated on Masson's trichrome stained sections (Fig. 1B), was not different in the emphysema/UIP and UIP groups (Table 2), while it was significantly lower in the emphysema group ( $p = 0.002$ ), as expected. Conversely, the parenchymal destruction index was significantly higher in the emphysema than in the emphysema/UIP group ( $p = 0.007$ , Table 2). Obviously, parenchymal destruction index was also significantly higher in the emphysema/UIP group than in the UIP group ( $p = 0.002$ , Table 2).

TNF- $\alpha$  expression was present in macrophages and in the epithelium of terminal bronchioles, but it was almost absent in fibrotic areas, without differences between the UIP and the emphysema/UIP group ( $0.37 \pm 0.12$  and  $0.44 \pm 0.13$ , respectively). In the emphysema group, TNF- $\alpha$  expression was almost absent ( $0.1 \pm 0.1$ ).

MMPs expression was weakly positive or absent in interstitial stromal tissue as well as in lymphocytes and neutrophils but was present in interstitial fibroblasts and,





**Figure 2** Typical HRCT scan of an emphysema/UIP patient. (A) Fibrotic reticular lesions, prevalently subpleural, and extended emphysema, are seen in the upper fields. (C) Carina: coarse reticular lesions with subpleural cysts, predominantly in the left lung, and bullous emphysema at the apex of the inferior lobe and in the lingula. (E) Lower fields: coarse reticular lesions with subpleural and intraparenchymal cysts are seen bilaterally. Distribution of fibrosis visual scores in the upper (B), medium (D) and lower (F) fields in emphysema/UIP and UIP alone patients, respectively.

with greater intensity, in pneumocytes. MMPs were instead expressed at higher levels in the fibroblastic foci of both UIP and emphysema/UIP (Fig. 3). In addition, interstitial fibroblasts present in the areas of parenchymal destruction in emphysema/UIP expressed MMP-2, MMP-7, MMP-9 and MT1-MMP with higher, although variable, intensity than in emphysema ( $p < 0.02$ ,  $p < 0.001$ ,  $p < 0.01$  and  $p < 0.03$ , respectively) (Fig. 3). The different expression of MMPs in

the two groups above was observed in the presence of similarly low levels of TIMP-1 ( $0.19 \pm 0.1$  and  $0.25 \pm 0.14$ ;  $p > 0.05$ ) and TIMP-2 ( $0.37 \pm 0.12$  and  $0.3 \pm 0.14$ ;  $p > 0.05$ ). In contrast to the emphysema/UIP patients, in the UIP alone group the typical patchy areas of fibrosis and structural remodeling were alternating with apparently normal residual parenchyma, where MMP and TIMP immunoreactivities were similar to that of normal lung parenchyma of control tissue samples (not shown).

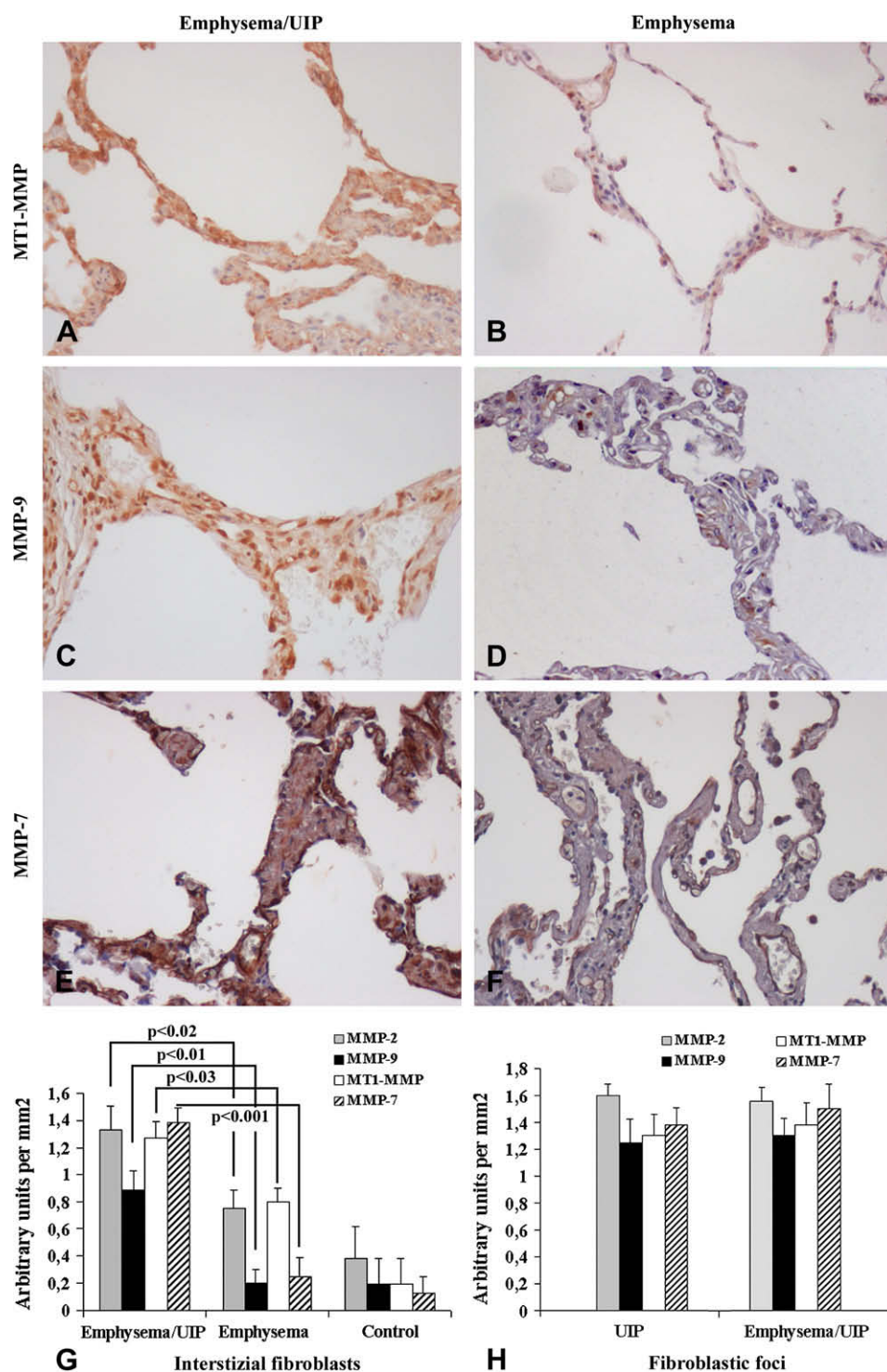
**Table 2** Radiographic and pathological findings characteristics

|                               | Emphysema/ UIP | UIP            | Emphysema      |
|-------------------------------|----------------|----------------|----------------|
| Ground glass                  | $0.3 \pm 0.1$  | $1.0 \pm 0.4$  | $0 \pm 0$      |
| Interstitial thickening       | $1.6 \pm 0.3$  | $2.4 \pm 0.4$  | $0 \pm 0$      |
| Subpleural cysts              | $1.5 \pm 0.4$  | $1.6 \pm 0.2$  | $0 \pm 0$      |
| Total cysts                   | $0.9 \pm 0.3$  | $1.3 \pm 0.3$  | $0 \pm 0$      |
| Bullae                        | $0.1 \pm 0.1$  | $0.1 \pm 0.1$  | $0 \pm 0$      |
| Emphysema                     | $0.5 \pm 0.2$  | $0.2 \pm 0.2$  | $1.4 \pm 0.4$  |
| Fibrosis (%)                  | $2.0 \pm 0.3$  | $2.1 \pm 0.2$  | $0.9 \pm 0.1$  |
| Parenchymal destruction index | $53.4 \pm 4.9$ | $28.0 \pm 2.9$ | $70.0 \pm 1.2$ |

## Discussion

The combination of IPF and emphysema, which has been recently defined by Cottin and co-workers as a new disease entity, CPFE, recognizable at computerized tomography,<sup>14</sup> has been described by a number of functional and radiographic studies in humans,<sup>21–23</sup> and it has been shown that in experimental animals exposure to cadmium<sup>7</sup> or over-expression of TNF- $\alpha$ <sup>8</sup> may lead to the development of concomitant pulmonary fibrosis and emphysema.

With regard to human disease, Hiwatari and co-workers described the development of fibrosis as a superimposed co-morbidity in patients with a known history of pulmonary emphysema,<sup>13</sup> in the context of this observation our



**Figure 3** Immunohistochemical and semiquantitative analyses of matrix metalloproteinases (MMPs) expression in lung parenchyma interstitial fibroblasts (A–F). Immunohistochemical investigation of lung parenchymal interstitial MT1-MMP (A, B) MMP-9 (C, D) and MMP-7 (E, F) in emphysema/UIP (A,C,E) and typical emphysema (B,D,F). (G) Bar graph showing semiquantitative analysis that reveals interstitial fibroblasts of parenchymal destruction areas of emphysema/UIP stain more than in those of typical emphysema subjects. (H) Bar graph of semiquantitative evaluation of MMPs expression in fibroblastic foci of lung parenchyma emphysema/UIP and UIP alone. Diaminobenzidine as chromogen; original magnification,  $\times 200$ .

morphometrical and immunohistochemical studies were undertaken to investigate whether the development of UIP in subjects upon pre-existing emphysema might play a role in the disease processes of CPFE.

Consistent with the observation of Hunninghake and colleagues,<sup>24</sup> the comparison of the roentgenological markers of fibrosis by visual quantification in emphysema/UIP patients and patients with UIP alone did not show any

significant difference in the type and distribution of fibrotic lesions. In both groups, IPF-type lesions were prevalent in the lower lobes but were also found in the subpleural regions of the upper lobes. Furthermore, the histopathological fibrosis scores were very similar in the two patient groups.

With regard to emphysematous abnormalities, the comparison of emphysema/UIP and emphysema cases confirmed the observation of Cottin and coworkers<sup>14</sup> that the spatial distribution of roentgenological markers of lung destruction was very similar in patients with emphysema or emphysema/UIP,<sup>14,22,25</sup> thus indicating that, as for fibrotic lesions, the morphology of emphysema in the emphysema/UIP subjects is similar to that of emphysema presenting alone.

Given the longer natural history of smoker's emphysema,<sup>26–28</sup> it is reasonable to think that our results that IPF may develop upon an already anatomically altered lung,<sup>13</sup> hence one might, however, ask whether emphysematous and fibrotic lesions will progress independently or the development of IPF may modify the progression of emphysema.

Two sets of disease modifying factors have been examined in the present study: TNF- $\alpha$  and the MMP family of proteases. The role of the TNF- $\alpha$  gene in the pathogenesis of pulmonary fibrosis has been clearly demonstrated in the mouse model<sup>8,29,30</sup> and in human gene association<sup>31,32</sup> and cytokine expression studies.<sup>31,33</sup> In this study we were only able to demonstrate faint tissue expression of TNF- $\alpha$ , with no differences between macrophages or terminal bronchiole epithelial cells in emphysema/UIP and UIP. In the context of the demonstration of the importance of TNF- $\alpha$  in the pathogenesis of emphysema,<sup>8,34</sup> in addition to IPF, the present observation cannot exclude a role for TNF- $\alpha$  in the inflammatory process of emphysema or emphysema associated with UIP.

Matrix metalloproteinases (MMPs) are a family of structurally related enzymes that are capable of degrading all components of the extracellular matrix and basement membranes. The MMP family comprises over 20 members sharing 32–49% aminoacid similarity and has similar structural domains.<sup>35</sup> Expression of the MMPs has been observed in lung diseases leading to the destruction of lung parenchyma, such as IPF<sup>9</sup> and emphysema.<sup>36</sup> In experimental animal models and in human IPF, where heightened expression of MMP-2 and MMP-9 has been extensively described, these MMPs are thought to play a major role in the disruption of basement membranes and fibroblast invasion of the alveolar spaces.<sup>9</sup> In emphysema, upregulation of MMP-2 and MMP-9 has been observed in epithelial cells and alveolar macrophages, suggesting that their exaggerated expression may contribute to interstitial fibrillar collagen degradation and to the breakdown of elastic fibers.<sup>11</sup> In addition, the expression of MMP-7, that is primarily expressed by the abnormal bronchial epithelium in UIP,<sup>9,37</sup> as well as that of MT1-MMP, a membrane associated protein and an activator of pro-MMP-2 that play a pivotal role in the aberrant remodeling of the lung microenvironment and that of TIMPs, which are expressed<sup>38,39</sup> by epithelial cells and activated fibroblasts in the alveolar buds and fibroblastic

foci of IPF,<sup>35,40,41</sup> is thought to characterize the MMP expression pattern in UIP.

In this study, MMP-2, MMP-9, MMP-7 and MT1-MMP were found to be expressed at similar levels in the fibroblastic foci of UIP and emphysema/UIP patients, thus suggesting not only that the fibrotic lesions of emphysema/UIP and UIP share similar histopathological and roentgenological patterns, but also display similar activation patterns of profibrotic gene.<sup>37,39</sup> Furthermore, this study shows that, different from the areas of uninvolved lung parenchyma described in UIP next to the fibroblastic foci, the areas of tissue destruction of emphysema/UIP are characterized by the presence of interstitial fibroblasts expressing high levels of MMP-2, MMP-7, MMP-9 and MT1-MMP, which are not seen in patients with emphysema, but unaffected by pulmonary fibrosis, with no significant differences in TIMP levels. It is thus enticing to hypothesize that increased MMPs expression may play a role in accelerating the process of destruction and remodeling of emphysema in these patients with combined emphysema and IPF.<sup>6</sup> Is the activation of fibroblasts in the areas of tissue destruction of emphysema/UIP indicative of a more aggressive disease process, leading to the more precipitous course of CPFE? It has been reported that in CPFE patients respiratory failure occurs more rapidly and that pulmonary hypertension leading to cor pulmonale is more frequent.<sup>14</sup> Whether the observed exaggerated activation of mesenchymal cells affects the emphysematous process we cannot say. A limitation of the present study is represented by the limited size of available bioptic tissue samples, so that lung structure and biology, including morphometrical and immunohistochemical data, in UIP unaffected segments or lobes remain uninvestigated. This notwithstanding, the data indicate UIP and emphysema as two overlapping processes, whereby the UIP process appears to alter both normal and the emphysematous lung. In the context of the observation that all of the emphysema/UIP patients in this and in previous studies<sup>13,14,22,25</sup> were smokers it is enticing to hypothesize that, although the role of cigarette smoking in the pathogenesis of UIP has not been firmly established yet, tobacco smoke is likely to play an important role in the abnormalities characterizing parenchymal destruction in CPFE.

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## Conflict of interest

None of the authors have a conflict of interest to declare in relation to this work.

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