Respiratory Medicine 109 (2015) 1063–1068

Contents lists available at ScienceDirect

Respiratory Medicine

journal homepage: www.elsevier.com/locate/rmed

# Serum metalloproteinases 1 and 7 in the diagnosis of idiopathic pulmonary fibrosis and other interstitial pneumonias

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ARTICLE INFO

*Article history:*
Received 9 April 2015
Received in revised form
8 June 2015
Accepted 8 June 2015
Available online 12 June 2015

*Keywords:*
Metalloproteinases
Metalloproteinase 1
Metalloproteinase 7
Idiopathic pulmonary fibrosis
Idiopathic interstitial pneumonias
Sarcoidosis

ABSTRACT

*Introduction:* Accurate diagnosis of idiopathic pulmonary fibrosis (IPF) has important therapeutic and prognostic implications and would be greatly aided by reliable diagnostic biomarkers as IPF has sometimes overlapping features with other interstitial lung diseases (ILD).
*Objectives:* To explore the value of serum metalloproteinases (MMP) 1 and 7 levels in the differential diagnosis of IPF with other ILD.
*Methods:* MMP-1/7 serum levels were measured using Luminex xMAP technology in 139 patients- 47 IPF, 36 non-IPF Usual Interstitial Pneumonia (UIP), 14 idiopathic Nonspecific Interstitial Pneumonia (iNSIP), 29 secondary NSIP (secNSIP), 13 stage IV sarcoidosis- and 20 healthy controls, and compared using the Mann–Whitney U test.
*Results:* MMP-1 was significantly higher in IPF than non-IPF UIP ($P = .042$) and sarcoidosis ($P = .027$). MMP-7 was significantly higher in IPF than controls ($P < .001$), non-IPF UIP ($P = .003$), secNSIP ($P < .001$), and sarcoidosis ($P < .001$). The Area Under the Curve for IPF versus other ILD was 0.63 (95%CI, 0.53–0.73) for MMP-1, 0.73 (95%CI, 0.65–0.81) for MMP-7, and 0.74 (95%CI, 0.66–0.82) for MMP-1/MMP-7 combined. Sensitivity and specificity for MMP-7 cutoff = 3.91 ng/mL was 72.3% and 66.3%, respectively, Positive Predictive Values = 52.3% and Negative Predictive Values = 82.4%.
*Conclusions:* MMP-1 and particularly MMP-7 serum levels were significantly higher in IPF than in non-IPF UIP, the main entity in differential diagnosis. The value of these biomarkers as additional tools in a multidisciplinary approach to IPF diagnosis needs to be considered and further explored.


## 1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic fibrosing lung disease characterized by progressive functional deterioration and a median survival of 2–3 years from the time of diagnosis [1–3]. It is associated with a radiological and histological pattern of usual interstitial pneumonia (UIP) and is the most common form of idiopathic interstitial pneumonia [1,4]. Although its pathogenesis is largely unknown, IPF is assumed to be caused by aberrant tissue repair and remodeling following recurrent alveolar epithelial injury (the nature of which is currently unknown), leading to a persistent and progressive disordered fibroproliferation [2,5,6]. Since the UIP pattern is not pathognomonic, IPF is always a diagnosis of exclusion, with ruling out of interstitial lung diseases related to connective tissue lung disorders (CTD-ILDs), hypersensitivity pneumonitis (HP), and drug lung toxicity [1,7,8]. Moreover, in cases without typical radiological UIP features (e.g., possible UIP pattern), it can be very difficult to distinguish between IPF and conditions such as idiopathic or secondary fibrotic nonspecific interstitial pneumonia (NSIP) [1,7]. An accurate diagnosis of IPF is of key importance considering the therapeutic and prognostic implications, and surgical lung biopsy is required in cases with an inconclusive diagnosis [1,7]. Biopsy, however, is an invasive procedure, with attendant risks (e.g, acute exacerbation in IPF), and

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http://dx.doi.org/10.1016/j.rmed.2015.06.003
0954-6111/

furthermore, it is not feasible in patients with severe disease or serious comorbidities [1,9,10].

Both clinicians and patients, therefore, would benefit greatly from reliable IPF diagnostic biomarkers. While several recent studies have identified and suggested a potential diagnostic or prognostic role for certain peripheral blood biomarkers (e.g., Krebs von den Lungen 6 antigen [KL-6], glycoprotein, surfactant proteins A and D, chemokine CCL-18, vascular endothelial growth factor, and glycoprotein YKL-40), none of these candidate markers has been validated or clinically accepted [11,12].

The matrix metalloproteinases (MMPs) belong to the zinc-dependent endoproteases that participate in extracellular matrix remodeling, wound healing, and angiogenesis and have been implicated in the pathogenesis of IPF [13,14]. Microarray studies have shown several members of the MMP family to be highly upregulated in IPF lungs, and elevated protein expression (MMP-1,2,3,7,8,9) has been found in the bronchoalveolar lavage fluid (BALF) and blood of IPF patients [13,15–20]. However, based on the available data, MMP-1 and MMP-7 are the most significantly overexpressed proteins in the lungs of patients with IPF compared with healthy controls [14]. Moreover, using a panel of 49 plasma proteins in a group of IPF patients, Rosas et al. [19] found increased concentrations of both MMP-1 and MMP-7, suggesting not only a determinant role in IPF pathogenesis but also potential utility as biomarkers in the differential diagnosis of this disease.

Although significant differences have been described for MMP-1 and MMP-7 expression in IPF compared with other ILDs, namely sarcoidosis and HP (mostly subacute forms), a direct comparison has never been made with conditions that pose the main challenge in the differential diagnosis of IPF [19]. The aim of our research was to compare serum MMP-1 and MMP-7 levels in IPF, non-IPF UIP, and fibrotic NSIP.

## 2. Material and methods

### 2.1. Patients and controls

We included 139 patients followed at the Interstitial Lung Diseases outpatient clinic at Centro Hospitalar São João, a tertiary referral center serving patients mostly from the north of Portugal. The patients were classified into 3 groups: IPF ($n = 47$), non-IPF UIP ($n = 36$), and fibrotic NSIP ($n = 43$). The non-IPF UIP group included 21 patients with HP (12 with bird fancier's disease, 6 with suberosis, and 3 with unknown etiology), 13 with CTD (7 with systemic sclerosis, 5 with rheumatoid arthritis, and 1 with undifferentiated CTD), and 2 with amiodarone lung toxicity. In the fibrotic NSIP group, there were 14 patients with idiopathic NSIP and 29 with NSIP secondary to an underling CTD (13 with systemic sclerosis, 8 with rheumatoid arthritis, 3 with Sjögren syndrome, 2 with mixed CTD, 2 with dermatopolymyositis, and 1 with systemic lupus erythematosus). Additionally, we included 13 patients with stage IV sarcoidosis and a control group of 20 healthy individuals (mean [SD] age of 70.3 [6.4] years, 9 females [45%]) with no history of lung disease or respiratory symptoms, or evidence of other diseases by chest radiography. Written informed consent was obtained from all individuals, and the study was approved by the hospital's ethics committee.

Patients with IPF were diagnosed according to the 2011 European Respiratory Society (ERS)/American Thoracic Society (ATS)/Japanese Respiratory Society/Latin American Thoracic Society Guidelines [1]. CTDs were diagnosed following The European League Against Rheumatism (EULAR) Recommendations [21]. All patients with NSIP underwent surgical lung biopsy, with application of the 2002 ATS/ERS International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias [22]. CTD-ILD diagnosis was based on high-resolution computed tomography (HRCT) findings, BALF features, and a previous CTD diagnosis [23]. HP was diagnosed using the criteria proposed by Lacasse et al. [24] Eleven (84.6%) of the 13 patients with sarcoidosis had histological confirmation in a compatible clinical and radiological context and the other 2 fulfilled criteria established in the consensus statement on sarcoidosis by the ERS/ATS/World Association of Sarcoidosis and Other Granulomatous Diseases (WASOG) [25], namely compatible clinical and radiographic features, a BALF CD4/CD8 lymphocyte ratio of >4.0, and a 2-year observation period to exclude other medical conditions. All radiological exams were evaluated by 2 radiologists and pathologic samples were analyzed by 2 pathologists, all trained in ILD evaluation. All the diagnoses were discussed and established by a multidisciplinary group including lung physicians, radiologists, and pathologists.

### 2.2. Blood samples

Blood samples were collected by venipuncture using Terumo Venosafe Serum-Gel tubes. Serum was separated within 30 min of blood collection following centrifugation for 10 min at 400× *g*. The serum samples were stored in aliquots at −80 °C until use.

MMP-1 and MMP-7 were measured using a human multiplex analysis assay (R&D Systems, Inc.) according to the manufacturer's protocol. Briefly, the assay is based on the Fluorokine MAP multiplex technology (Luminex Corporation), which combines the principle of a sandwich immunoassay with fluorescent-bead-based technology. Appropriate standards and serum samples were diluted (10-fold) in calibrator diluents and added to pre-wet filter-bottomed microplates. Fluorescent beads containing MMP-1 and MMP-7 antibodies were added and incubated on a plate shaker for 2 h at room temperature. After washing, biotinylated detection antibodies were added to each well, incubated for 1 h at room temperature, washed, and incubated for a further 30 min with a streptavidin-phycoerythrin conjugate. After washing, the beads were resuspended in wash buffer and analyzed on a Luminex 200 instrument. Mean fluorescent intensity data were analyzed using the Luminex 100 Integrated System version 2.3. The generation of a standard curve using standard MMP concentrations allowed the measurement of each metalloproteinase individually. The minimum detectable dose for MMP-1 and MMP-7 was 4.4 and 16.9 pg/mL, respectively.

### 2.3. Statistical analysis

Analysis of variance or Kruskal–Wallis tests (for nonnormally distributed variables) were used to compare continuous variables and the $\chi^2$ test was used to compare proportions of demographic characteristics and MMP distributions according to diagnosis. The Mann–Whitney U test was used for pairwise comparison of the distribution of MMP-1 and MMP-7 serum levels in IPF patients versus controls and patients with other diagnoses. To calculate the associations of MMP-1 and MMP-7 independently with each disorder we computed logistic regression models adjusting for age, sex, and smoking history (never vs former or current smokers). A $P$ value of less than 0.05 was considered significant and all $P$ values were 2-sided.

Receiver operating characteristic (ROC) analysis was used to determine the area under the curve (AUC) for MMP-1, MMP-7, and MMP-1/MMP-7 combined with regard to IPF diagnosis versus any other diagnosis (excluding controls). Sensitivity, specificity, and positive and negative predictive values (PPV and NPV, respectively) were calculated for the cutoffs of MMP-1 and MMP-7, and the

Youden index (J = max[sensitivity + specificity−1]) was used to establish the best cutoff for IPF diagnosis.

## 3. Results

Patient demographics and characteristics according to diagnosis are presented in Table 1, together with statistically significant between-group differences based on the expected clinical characteristics of each group [21]. Healthy controls and IPF patients had a similar mean (SD) age (70.3 [6.4] vs 70.6 [9.5] years, $P = .865$) and sex distribution (9 females [45.0%] vs 17 [36.2%], $P = .686$) and similar proportions of smokers (11 [55.0%] vs 28 [62.2%], $P = .583$).

A significant difference was found for the mean serum concentration of MMP-7 but not MMP-1 across the groups (Table 1). MMP-1 concentration, however, was significantly higher in IPF than in non-IPF UIP ($P = .042$) and sarcoidosis ($P = .027$) (Fig. 1). MMP-7 levels were significantly higher in IPF patients than in controls ($P < .001$) and patients with non-IPF UIP ($P = .003$), secondary NSIP ($P < .001$), and sarcoidosis ($P < .001$) (Fig. 2).

In the multivariate logistic regression models for each diagnosis (Table 2), MMP-7 was independently associated with IPF (OR = 1.34; 95% CI, 1.17–1.54) and an inverse association was found for sarcoidosis (OR = 0.66; 95% CI, 0.45–0.96), ie, lower levels of MMP-7 were associated with the diagnosis of sarcoidosis. The association between MMP-7 and IPF remained significant after adjusting for sex, age and smoking history. No further significant associations were observed.

The AUC of the ROC curve for the diagnosis of IPF versus all other diagnoses was 0.63 (95% CI, 0.53–0.73) for MMP-1, 0.73 (95% CI, 0.65–0.81) for MMP-7, and 0.74 (0.66–0.82) for MMP-1 and MMP-7 combined (Fig. 3)

The best combination of sensitivity and specificity for MMP-7 was obtained for the cutoff of 3.91 ng/mL (72.3% and 66.3%, respectively), with a PPV of 52.3% and a NPV of 82.4% (Supplementary Table). As lower cutoffs were selected, higher NPVs were achieved, demonstrating better performance for each MMP in ruling out IPF.

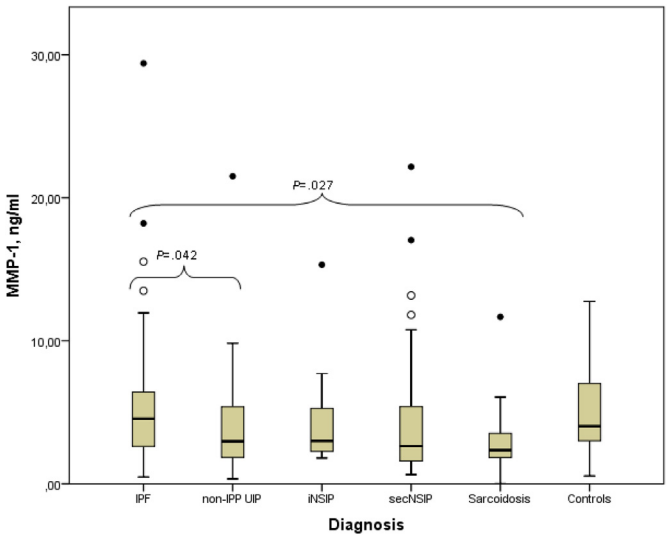


**Fig. 1.** White circles represent mild outliers and black circles represent extreme outliers. MMP-1 levels in patients and controls. Levels were significantly higher in IPF than in non-IPF UIP ($P = .042$) and sarcoidosis ($P = .027$). MMP indicates matrix metalloproteinase; IPF, idiopathic pulmonary fibrosis; non-IPF UIP, non-IPF usual interstitial pneumonia; iNSIP, idiopathic nonspecific interstitial pneumonia; secNSIP, NSIP secondary to a connective tissue lung disorder; UIP, usual interstitial pneumonia.

## 4. Discussion

We evaluated the potential value of MMP-1 and MMP-7 as diagnostic biomarkers for IPF by comparing serum concentrations in IPF patients and patients with other ILDs that pose a challenge in the differential diagnosis (non-IPF UIP and secondary and idiopathic fibrotic NSIP). Both MMP-1 and MMP-7 serum levels were significantly higher in IPF patients than in patients with non-IPF UIP and sarcoidosis. Moreover, MMP-7 levels were significantly higher in IPF patients than in patients with secondary NSIP and healthy controls (with a similar age, sex distribution and smoking habits). MMP-7 performed better than MMP-1 for the diagnosis of IPF, as measured by the AUC, with a cutoff of 3.91 ng/mL providing maximum sensitivity and specificity and an NPV of 82.4%.

IPF is the most severe of the idiopathic interstitial pneumonias, with a distinctive therapeutic approach and a poor prognosis. Consequently, a confident diagnosis and a clear distinction from other fibrotic ILDs have important clinical implications in terms of patient outcome and treatment options. Approximately one-third of IPF patients require surgical lung biopsy, which has inherent risks and is not always feasible [1]. Clinicians, therefore, need biomarkers not only to predict outcome and survival, but also to establish a reliable diagnosis.

An ideal biomarker should be easily accessible, amenable to reliable measurement, and suitable for use in longitudinal assessment [11,12]. Increasing evidence is being amassed on the potential value of MMPs in IPF [14,16]. Rosas et al. [19] analyzed 49 potential IPF serum markers and found 5 MMPs (including MMP-1 and MMP-7) among the 12 proteins that were differentially expressed. Moreover, MMP-1 and MMP-7 were significantly overexpressed in IPF patients compared with patients with sarcoidosis or chronic obstructive pulmonary disease (COPD), and enhanced MMP-7 expression was seen in both lung tissue and BALF.

In addition to experimental data [26,27], several investigations with lung tissue, BALF, and peripheral blood support a possible role of MMPs in the pathogenesis of IPF, namely through their

**Table 1**
Patients characteristics according to diagnosis.

| | UIP | | NSIP | | Sarcoidosis (n = 13) | Controls (n = 20) | *P* Value[a] |
|---|---|---|---|---|---|---|---|
| | IPF (n = 47) | Non-IPF (n = 36) | iNSIP (n = 14) | secNSIP (n = 29) | | | |
| Age, mean (SD), y | 70.6 (9.5) | 65.4 (11.5) | 62.4 (12.1) | 61.1 (9.5) | 53.9 (12.6) | 70.3 (6.4) | <.001 |
| Female, No. % | 17 (36.2) | 24 (66.7) | 10 (71.4) | 26 (89.7) | 7 (53.8) | 9 (45.0) | <.001 |
| Ex/current smokers, No. (%) | 28 (62.2) | 5 (13.9) | 4 (28.6) | 8 (27.6) | 4 (30.8) | 11 (55.0) | <.001 |
| Never smokers, No. (%) | 17 (37.8) | 31 (86.1) | 10 (71.4) | 21 (72.4) | 9 (69.2) | 9 (45.0) | |
| MMP-1, mean (SD), ng/mL | 5.79 (5.20) | 4.02 (3.85) | 4.43 (3.59) | 4.93 (5.25) | 3.22 (2.96) | 4.89 (3.11) | .200 |
| MMP-7, mean (SD), ng/mL | 5.79 (3.07) | 4.07 (2.73) | 4.32 (2.90) | 3.76 (2.62) | 2.39 (1.68) | 1.52 (0.77) | <.001 |

Abbreviations: iNSIP, idiopathic NSIP; MMP, matrix metalloproteinase; NSIP, nonspecific interstitial pneumonia; secNSIP, NSIP secondary to a connective tissue lung disorder; UIP, usual interstitial pneumonia.
[a] Comparing all groups.

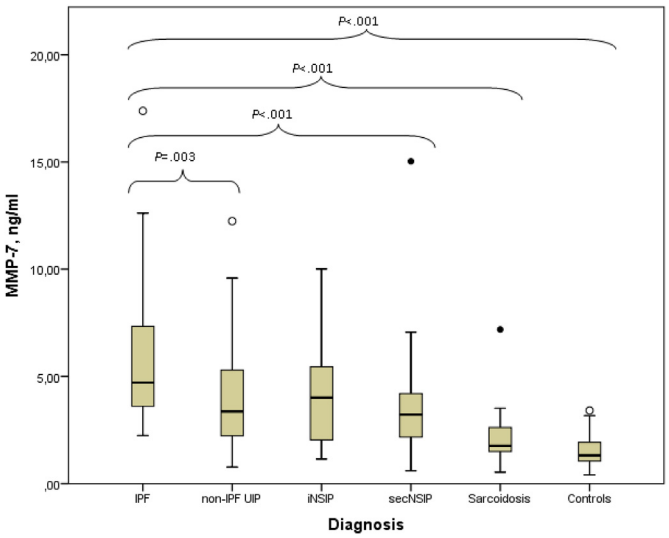


**Fig. 2.** White circles represent mild outliers and black circles represent extreme outliers. MMP-7 levels in patients and controls. Levels were significantly higher in IPF patients than in controls ($P < .001$), patients with non-IPF UIP ($P = .003$), secondary NSIP ($P < .001$), and sarcoidosis ($P < .001$). MMP indicates matrix metalloproteinase; IPF, idiopathic pulmonary fibrosis; non-IPF UIP, non-IPF usual interstitial pneumonia; iNSIP, idiopathic nonspecific interstitial pneumonia; secNSIP, NSIP secondary to a connective tissue lung disorder; UIP, usual interstitial pneumonia.

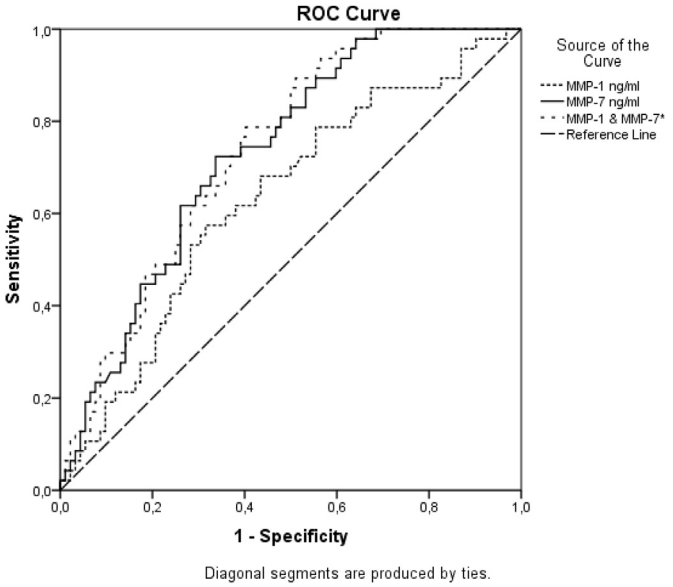


**Fig. 3.** Receiver operator characteristic (ROC) curves for MMP-1 and MMP-7 comparing idiopathic pulmonary fibrosis and all other diagnoses. *The combined variable (MMP-1 & MMP-7) is the predicted probability extracted from a logistic regression model fitted for IPF diagnosis vs other, using MMP-1 and MMP-7 as covariates.

contribution to extracellular matrix remodeling. In fact, MMPs are involved not only in the degradation of matrix components, but also in the modulation of a variety of bioactive mediators, such as cytokines and chemokines [13,14]. MMPs are regulated at transcriptional and posttranscriptional levels by inhibitors and inducers, creating a delicate balance in extracellular matrix remodeling [13,14]. Their expression, usually modest under physiological conditions, increases in repair processes such as wound healing, and uncontrolled MMP activity results in tissue damage and functional changes [14].

MMP-7, the smallest member of the MMP family, has a profibrotic profile with diverse biological functions, ranging from innate immunity to inflammation, apoptosis, and fibroproliferation [27,28]. It is also able to process numerous bioactive substrates and activate proteases, including itself. The release of preformed TGF-β from extracellular matrix is the main regulation process related to TGF bioactivity, one of the presumptive mediators in IPF pathogenesis. In an animal model, MMP-7 knockout mice treated with bleomycin did not develop lung fibrosis [29]. Additionally, MMP-7 has been found on the surface of epithelial cells and alveolar macrophages in IPF lung tissue, but not in healthy lung tissue [30,31]. Interestingly, enhanced levels of serum MMP-7 were also found in patients with asymptomatic IPF (although at lower levels than in symptomatic patients), pointing to its possible value as a marker for both early disease and progression [28]. In fact, BALF and serum MMP-7 levels showed not only a negative correlation with forced vital capacity and diffusing capacity but also an independent association with IPF mortality [3,19,20,31].

The potential role of MMP-7 as a differential biomarker in IPF is still unclear, particularly when clinically similar ILDs are involved. In fact, Rosas et al. compared IPF with sarcoidosis and COPD, which clearly have distinct clinical and radiological features. Although the study included patients with HP mostly with subacute presentation, the major confounding diagnosis is chronic advanced HP associated with fibrosis, particularly with UIP pattern. Our study, which showed significantly higher serum MMP-7 levels in IPF compared with non-IPF UIP, is the first, to our knowledge, to investigate the value of this marker in distinguishing IPF from other ILDs with the UIP pattern.

Vuorinen et al. [18] described a higher level of MMP-7 in the BALF of IPF patients compared with controls, with no significant differences for idiopathic NSIP or sarcoidosis. In our series, while we did not find significant differences for serum MMP- 7 levels between IPF and idiopathic NSIP, we did observe significantly

**Table 2**
Odds ratios (95% CI) for the association of MMP-1 and MMP-7 with each diagnosis.

| | IPF | Non-IPF UIP | NSIP | secNSIP | Sarcoidosis |
|---|---|---|---|---|---|
| MMP-1a | 1.07 (0.99–1.15) | 0.94 (0.85–1.04) | 0.98 (0.85–1.12) | 1.01 (0.92–1.10) | 0.86 (0.68–1.08) |
| MMP-1 adjusted[b] | 1.04 (0.96–1.13) | 0.95 (0.85–1.05) | 1.00 (0.88–1.14) | 1.06 (0.97–1.15) | 0.88 (0.68–1.13) |
| MMP-1 adjusted[c] | 1.05 (0.97–1.14) | 0.94 (0.85–1.04) | 1.00 (0.88–1.14) | 1.06 (0.96–1.15) | 0.88 (0.69–1.12) |
| MMP-7 | 1.34 (1.17–1.54)[a] | 1.00 (0.88–1.13) | 1.03 (0.86–1.23) | 0.95 (0.82–1.10) | 0.66 (0.45–0.96)[a] |
| MMP-7 adjusted[b] | 1.39 (1.18–1.63)[a] | 1.00 (0.87–1.14) | 1.06 (0.88–1.28) | 1.00 (0.85–1.17) | 0.72 (0.48–1.08) |
| MMP-7 adjusted[c] | 1.40 (1.18–1.66)[a] | 1.00 (0.87–1.14) | 1.06 (0.88–1.28) | 1.00 (0.85–1.17) | 0.73 (0.49–1.09) |

Abbreviations: IPF, idiopathic pulmonary fibrosis; MMP, matrix metalloproteinase; NSIP, nonspecific interstitial pneumonia; secNSIP, NSIP secondary to a connective tissue lung disorder; UIP, usual interstitial pneumonia.

[a] $P < .05$.

[b] Adjusted for age and sex.

[c] Adjusted for age, sex, and smoking status (smoking status variable was dichotomized: former or current smokers vs never smokers).

higher levels in IPF than in NSIP with underlying CTD. Interestingly, similar gene expression patterns have been found in IPF and some NSIP cases, but there are clear differences when other ILDs that can nevertheless express an UIP or NSIP pattern are considered [15]. Moreover, all the patients with idiopathic NSIP included had thoracic HRCT features alike UIP instead of HP or organizing pneumonia like features, the two other possible patterns described in this entity. Vuorinen et al. showed lung MMP-7 immunoreactivity in some fibroblastic foci, vascular smooth muscle cells, and areas of hyperplastic epithelium in IPF samples; in NSIP samples immunoreactivity was observed in areas of inflammation beneath the alveolar epithelium. These findings suggest that MMP-7 may have relevant but different roles in the pathophysiologic processes involved in different ILDs, but the differences might not be quantitatively detectable. Moreover, although idiopathic NSIP is associated with a better prognosis, it is sometimes difficult to clearly distinguish from IPF on HRCT. Furthermore, the 2 entities have a similar BALF profile and some patients with IPF show NSIP areas in their surgical biopsy. The above observations suggest that IPF and NSIP may possibly be connected.

Our results concerning sarcoidosis contrast with those of Vuorinen et al. [18] since we found significant low MMP-7 levels in comparison with IPF. They are in agreement with Rosas et al. [19] and Zhou et al. [32] findings, but our study is the first to include only sarcoidosis patients with lung fibrosis. The majority of patients studied by Vuorinen et al. did not have significant lung disease, while those studied by Zhou et al. had stage II sarcoidosis. In another study, Huh et al. [33] found no significant differences in BALF MMP-7 levels between patients with IPF and cryptogenic organizing pneumonia (COP) although it included few patients and COP is rarely confused with UIP.

Like MMP-7, MMP-1 has multiple biological functions that may be involved in the pathogenesis of IPF, such as cytokine processing, cell migration and growth regulation. MMP-1 expression in IPF seems to be primarily in the alveolar epithelium and is not observed in fibroblastic foci or in the interstitial compartment, where interstitial collagens are secreted and accumulated, respectively [13,28]. It has also been suggested that MMP-1 might contribute to the formation of cystic spaces, resulting in the characteristic honeycombing pattern seen in IPF [14]. MMP-1 has been shown to be upregulated in the lung tissue of IPF compared to HP patients and healthy controls [15,34]. To our knowledge, ours is the first report to show significantly higher serum MMP-1 levels in IPF compared with non-IPF UIP. The significantly lower MMP-1 levels detected in sarcoidosis are consistent with the findings of Rosas et al. [19].

The relatively small sample size for some of the clinical entities considered in our analysis, particularly idiopathic NSIP, is the main limitation of this study. The cutoff values used were based on the distribution of MMPs in this particular sample of patients and thus could vary in other groups. Replications of our findings are therefore needed. While it is largely believed that a combination of biomarkers has a greater likelihood of becoming a reliable diagnostic tool for IPF than any single biomarker, combinations can be difficult to implement for methodological and economic reasons. Our data show that MMP-7 is clearly the best differentiator but, unlike previous studies, we found that its specificity improved only marginally when MMP-1 was added.

In conclusion, our results provide additional support for the potential value of serum MMP-1 and MMP-7 concentrations as diagnostic biomarkers in IPF. Since non-IPF UIP poses the main challenge in the differential diagnosis of IPF, the implications of significantly higher levels of MMP-1 and, in particular, MMP-7 in IPF patients need to be carefully considered as potential tools in the multidisciplinary diagnostic approach to IPF.

## Authors contribution

AM, LD and AM elaborated the study conception and design, analysis of data, manuscript preparation and revision.

MB and OS performed the laboratory proceedings.

NM and PM assisted the patients in the Interstitial Lung Diseases unit, cooperated in the data acquisition and drafted the manuscript.

DC participated in the data acquisition and performed the statistic evaluation.

All authors read and approved the final manuscript.

## Conflict of interests statement

The authors declare that they do not have any conflict of interests to declare.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.rmed.2015.06.003.

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