Serum metalloproteinase-7 as a biomarker of progressive pulmonary fibrosis

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Shareable abstract (@ERSpublications)
MMP-7 levels are higher in patients with fibrotic non-IPF ILD who develop PPF, suggesting its potential as a biomarker. Identifying biomarkers such as MMP-7 may contribute to early treatment. https://bit.ly/3SrSizK

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Abstract

Introduction Progressive pulmonary fibrosis (PPF) corresponds to any fibrotic interstitial lung disease (ILD) other than idiopathic pulmonary fibrosis (IPF) that presents clinical, physiological and/or radiological evidence of disease progression similar to IPF. Matrix metalloproteinases (MMPs) have been implicated in the pathogenesis of pulmonary fibrosis and are associated with disease progression and reduced survival in IPF and other fibrotic ILDs. This study aimed to investigate the role of serum levels of MMP-1 and MMP-7 in patients with fibrotic non-IPF ILD as possible biomarkers of patients at risk of developing PPF.

Methods Newly diagnosed patients with fibrotic non-IPF ILD were included in this study. Serum levels of MMP-1 and MMP-7 were quantified at baseline and disease progression was monitored. PPF was defined according to the recent European Respiratory Society, American Thoracic Society, Japanese Respiratory Society and the Latin American Thoracic Society Clinical Practice Guidelines.

Results 79 patients with fibrotic non-IPF ILDs were included and classified as having PPF or non-PPF. Significantly higher levels of MMP-7, but not MMP-1, were detected in the PPF group (p=0.01). MMP-7 was independently associated with PPF (adjusted OR 1.263, 95% CI 1.029–1.551; p=0.026) after adjustment for sex, age and smoking history. A cut-off value of 3.53 $ng \cdot mL^{-1}$ for serum MMP-7 levels had a sensitivity of 61% and a specificity of 74% for predicting PPF in non-IPF ILDs.

Conclusions In patients with fibrotic non-IPF ILDs, serum MMP-7 levels were significantly greater in the subgroup of patients meeting the PPF criteria at follow-up. This can be considered and further investigated as a possible biomarker to identify fibrotic ILD patients at risk of PPF.

Introduction

Interstitial lung disease (ILD) comprises a range of lung diseases that affect the lung parenchyma through different patterns and behaviours of lung injury. Idiopathic pulmonary fibrosis (IPF) is one of the most common ILDs and is characterised by progressive pulmonary fibrosis (PPF). Several other ILDs may also present with progressive fibrosis. The course and prognosis can vary widely between patients, making it difficult to predict who will have a progressive phenotype similar to IPF [1].

PPF has been defined as an ILD with radiological evidence of pulmonary fibrosis, other than IPF, with at least two of the following three criteria occurring within the past year, without alternative explanation: worsening respiratory symptoms; physiological evidence of disease progression; and radiological evidence of fibrosis progression [2].

The identification of serum biomarkers that can predict the development of PPF in patients with fibrotic ILD is essential for the early initiation of treatment to prevent significant clinical and functional decline [3]. Matrix metalloproteinases (MMPs) are endopeptidases that have been implicated in lung fibrosis and play a role in extracellular matrix remodelling, wound healing and angiogenesis. In particular, MMP-1 and MMP-7 have been proposed as diagnostic biomarkers for IPF [4–6]. In addition, high levels of MMP-7 are associated with disease progression and reduced survival in IPF patients and patients with other ILDs, such as fibrotic hypersensitivity pneumonitis [7–11]. However, the utility of MMP-1 and MMP-7 measurements for studying progressive fibrosis in patients with ILDs remains unclear. This study aimed to determine the utility of serum MMP-1 and MMP-7 levels in identifying patients with fibrotic non-IPF ILDs who are at risk of developing PPF.

Methods

This prospective cohort study included patients with fibrotic non-IPF ILD from the ILD outpatient clinic at Centro Hospitalar Universitário São João (Oporto), a tertiary university centre in northern Portugal.

Patients

Newly diagnosed patients with fibrotic ILD were selected between 2014 and 2015. Initial diagnoses were reassessed according to the current international guidelines. Patients who were diagnosed with IPF according to the established guidelines of the European Respiratory Society (ERS), American Thoracic Society (ATS), Japanese Respiratory Society (JRS) and the Latin American Thoracic Society (ALAT) were excluded [12]. The diagnosis of connective tissue disease (CTD)-associated ILD was established when interstitial pneumonia was identified in a CTD setting and diagnosed according to the European League Against Rheumatism (EULAR) recommendations [13]. Hypersensitivity pneumonitis was defined according to the ATS/JRS/ALAT 2020 Clinical Practice Guidelines [14]. Sarcoidosis was diagnosed according to the Official ATS 2020 Clinical Practice Guidelines [15]. Nonspecific interstitial pneumonia (NSIP) and unclassifiable fibrotic ILD were identified according to the 2013 ATS/ERS official statement [16].

Thoracic high-resolution computed tomography (HRCT) scans were interpreted by two radiologists, and pathology samples were analysed by two pathologists, all with expertise in ILD. A multidisciplinary panel of pulmonologists, radiologists and pathologists established all the diagnoses.

Samples

Blood samples were taken by venipuncture at the time of the diagnostic evaluation. Serum MMP-1 and MMP-7 levels were quantified using a human multiplex analysis assay (R&D Systems, Inc., Minneapolis, MN, USA) at baseline. This assay employs Fluorokine MAP multiplex technology (Luminex Corporation - Diasorin Inc., Stillwater, OK, USA), which integrates sandwich immunoassay principles with fluorescent-bead technology. Standards and serum samples were diluted 10-fold with calibrator diluents and added to pre-wet filter-bottomed microplates. Fluorescent beads with 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, followed by a 1-h incubation at room temperature, another wash and a 30-min incubation with a streptavidin–phycoerythrin conjugate. After a final wash, the beads were resuspended in wash buffer and analysed using a Luminex 200 instrument. Fluorescent intensity was processed with the Luminex 100 Integrated System version 2.3. A standard curve with known MMP concentrations enabled the quantification of each metalloproteinase, with minimum detectable doses for MMP-1 and MMP-7 being 0.0044 $ng \cdot mL^{-1}$ and 0.0169 $ng \cdot mL^{-1}$, respectively.

Outcomes

Patients' clinical, radiological and pulmonary function outcomes were monitored. Patients were divided into two groups according to clinical, radiological and lung function progression over time: those with and without the PPF criteria. PPF was defined according to the 2022 ATS/ERS/JRS/ALAT Clinical Practice Guideline when at least two of the following three criteria were met within 1 year, without an alternative explanation: worsening of respiratory symptoms; physiological evidence of disease progression given by an absolute decline in forced vital capacity (FVC) of at least 5% or an absolute decline in diffusing capacity for carbon monoxide (D_{LCO}) of at least 10%; and radiological evidence of disease progression [2].

Statistical analysis

Continuous variables are expressed as median (interquartile range). Normality of the distributions was assessed using the Shapiro–Wilk test. The Mann–Whitney U-test was used for pairwise comparisons of the distributions of the serum MMP-1 and MMP-7 levels between patients who met the PPF criteria and those who did not. Logistic regression models adjusted for age, sex and smoking history were applied to analyse independent associations. Receiver operating characteristic (ROC) analysis was used to determine the area

TABLE 1 Characteristics and diagnoses of patients with progressive pulmonary fibrosis (PPF) and non-PPF

	PPF n=33	Non-PPF n=46	p-value
Median (IQR) age, years	66 (52–69)	61 (54–73)	0.672
Female sex	23 (69.7)	37 (80.4)	0.274
Smoking history	9 (27.3)	10 (21.7)	0.573
Diagnosis			
Connective tissue disorder ILD	12 (36.4)	23 (50.0)	0.229
Fibrotic hypersensitivity pneumonitis	13 (39.4)	10 (21.7)	0.088
Sarcoidosis	5 (15.1)	8 (17.4)	0.791
Idiopathic nonspecific interstitial pneumonia	2 (6.1)	5 (10.9)	0.458
Unclassifiable fibrotic ILD	1 (3.0)	0 (0)	0.235
Radiological pattern of UIP	14 (42.4)	16 (34.8)	0.435

Data are presented as n (%), unless otherwise stated. IQR: interquartile range; ILD: interstitial lung disease; UIP: usual interstitial pneumonia.

under the curve (AUC) for serum MMP levels and the ability to discriminate between PPF and non-PPF patients. The Youden index was performed to identify the optimal cut-off value for predicting PPF. In addition, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for the identified cut-off values. The association between the usual interstitial pneumonia (UIP) pattern and the development of PPF was assessed using a chi-squared test, patients with idiopathic NSIP or sarcoidosis were excluded from the analysis. Cox regression was applied to analyse overall survival. A p-value <0.05 was considered statistically significant.

Results

We included 79 patients with fibrotic non-IPF ILD: 44.3% (n=35) with CTD-ILD, 29.1% (n=23) with fibrotic hypersensitivity pneumonitis, 16.5% (n=13) with sarcoidosis, 8.9% (n=7) with idiopathic NSIP and 1.2% (n=1) with unclassifiable fibrotic ILD. The median age was 66 years (52–72), 75.9% (n=60) were female and 75.9% (n=60) were never smokers. A chest computed tomography scan revealed a UIP pattern in 37.9% (n=30) of patients and an NSIP pattern in 45.6% (n=36) of patients.

During a median follow-up period of 9 years (7–10), the following PPF criteria were observed in 33 patients (41.7%): 16 with all three progression criteria, 10 with symptomatic and physiological or radiological progression and 7 with physiological and radiological progression (tables 1 and 2). There was no correlation between the significant decrease in FVC (at least 5%) or D_{LCO} (at least 10%) and serum levels of MMP-1 (p=0.591 and p=0.925, respectively) or MMP-7 (p=0.904 and p=0.654, respectively). The PPF group had a median MMP-1 level of 2.69 ng·mL^{-1} (1.85–4.86) and the non-PPF group had a median level of 2.63 ng·mL^{-1} (1.54–4.10), with no statistically significant differences (p=0.421). MMP-7 levels were significantly greater in the PPF group than in the non-PPF group: 3.85 ng·mL^{-1} (2.40–5.94) *versus* 2.41 ng·mL^{-1} (1.71–3.63), p=0.01.

Using a binary logistic regression model, MMP-7 at baseline was found to be independently associated with PPF and remained significant after adjustment for sex, age and smoking history (adjusted OR 1.26, 95% CI 1.03–1.55; p=0.026). A cut-off of 3.53 ng·mL^{-1} showed a sensitivity of 61% and a specificity of 74% for detecting PPF (AUC 0.669), with a PPV of 72% and an NPV of 56% (figure 1).

Serum levels of MMP-1 or 7 were not significantly different between the UIP and non-UIP groups (the median serum MMP-1 levels in patients with UIP was 2.71 ng·mL^{-1} *versus* 2.63 ng·mL^{-1} without UIP,

TABLE 2 Criteria for inclusion in the progressive pulmonary fibrosis (PPF) group

	PPF n=33	Non-PPF n=46
Worsening of respiratory symptoms	29 (87.9)	7 (15.2)
Physiological evidence of disease progression	27 (81.8)	6 (13)
Radiological evidence of disease progression	25 (75.8)	3 (6.5)

Data are presented as n (%).

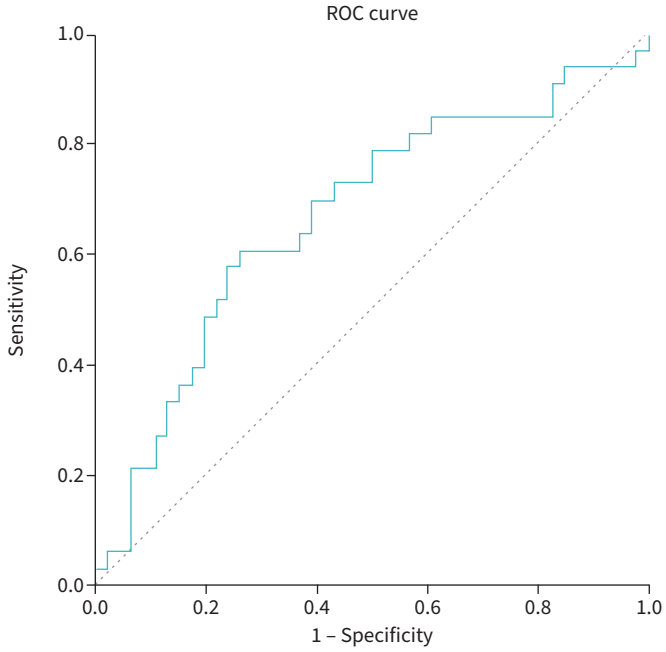

FIGURE 1 Receiver operating characteristic (ROC) curve for serum MMP-7 levels and ability to discriminate between progressive pulmonary fibrosis (PPF) and non-PPF patients.

p=0.739, and median serum MMP-7 levels in patients with UIP was 3.08 ng·mL^{-1} *versus* 3.23 ng·mL^{-1} without UIP, p=0.740). Furthermore, PPF evolution was independent of the presence of the UIP pattern (X^2=1.205 p=0.435).

The mortality rate was 46.8%, with a median overall survival of 7 (5–9) years. There was no correlation between the serum levels of MMP-1 or MMP-7 and mortality (p=0.696 and p=0.783, respectively) or overall survival (p=0.631 and p=0.170, respectively).

Discussion

Some fibrotic ILDs may exhibit a pattern of progressive fibrosis similar to IPF, posing significant challenges in the diagnosis and management of these patients. Due to their role in lung fibrosis, MMPs have been investigated as potential diagnostic or disease progression biomarkers. In our patient cohort, 42% met the PPF criteria, highlighting the significant burden of progressive disease in the context of fibrotic non-IPF ILD. MMP-7 showed a significant association with PPF, suggesting its potential as a biomarker for disease progression. A cut-off value of 3.53 ng·mL^{-1} for MMP-7 showed a specificity and PPV for PPF >70%.

PPF is characterised by radiological evidence of fibrosis and evidence of progression over time [2]. Several criteria for disease progression have been proposed. Most importantly, the INBUILD trial considered progression as having at least one of the following within 24 months: a relative decline in FVC of at least 10% of the predicted value, a relative decline in FVC of 5% to <10% of the predicted value along with worsening respiratory symptoms, an increased extent of fibrosis on an HRCT scan or both worsening of respiratory symptoms and increased fibrosis [3]. The 2022 ATS guidelines extrapolated the PPF criteria from IPF patient data due to their similar disease behaviour and prognosis and relied on different criteria related to worsening of symptoms and physiological and radiological evidence of progression within 1 year, which were the criteria used in our study [2].

There is an unmet need to discover biomarkers that can identify which patients with fibrotic non-IPF ILD are at risk of PPF. This could identify which patients may benefit from early antifibrotic treatment rather than waiting 1 or 2 years for clinical worsening, progression of lung fibrosis or a decrease in lung function to initiate treatment. While many investigations have been conducted in the search for potential biomarkers of fibrotic ILDs, most have focused solely on IPF. Certain studies have highlighted the importance of genetic biomarkers, such as the 52-gene risk profile, in predicting disease progression [17]. In addition,

other biomarkers have been investigated using proteomic analysis of peripheral blood or bronchoalveolar lavage fluid [18–20].

Subsequently, several studies have included patients with fibrotic non-IPF ILD. Some have used single-cell RNA sequencing to reveal disease-related differences in cell types within the lungs of patients with pulmonary fibrosis compared with controls [21, 22]. These studies included the expression of molecules associated with extracellular matrix deposition in fibrosis, such as MUC5B, and the expression of collagen by the $KRT5^{-}/KRT17^{+}$ epithelial cell population, which is highly enriched in patients with pulmonary fibrosis [21, 22]. However, most of the research not only relied on complex measurements for routine use but also included IPF patients and only a few PPF patients. More recent investigations on PPF have identified many other potential prognostic biomarkers, such as MMP-7 and MMP-12, cancer antigen-125, vascular cell adhesion molecule 1, Krebs von den Lungen-6 and surfactant protein-A and -D [10, 11].

Among these possible biomarkers, MMP-7 has been consistently highlighted as a promising biomarker for predicting mortality not only in IPF but also in PPF [7–11]. Research on MMP-7 initially focused on IPF, demonstrating the potential of the association of MMP-7 with surfactant protein-A and Krebs von den Lungen-6 antigen for predicting outcomes in IPF [4]. Subsequent studies have further validated its prognostic value as a predictor of disease progression [8, 9]. As research has progressed, MMP-7 has emerged as a key biomarker of IPF and has been proposed as a potential therapeutic target [11, 23]. Following the increased interest in IPF, the role of MMP-7, along with MMP-1, has been identified as a possible diagnostic biomarker for other fibrotic ILDs [5]. Their involvement in extracellular matrix remodelling and the modulation of bioactive mediators such as cytokines and chemokines further support their involvement in the pathogenesis of interstitial fibrotic diseases [6]. As a result, MMPs have gained increasing interest in the field of PPF and are being further investigated as diagnostic and prognostic biomarkers [10, 11].

Our findings also suggest the potential use of MMP-7 as a serum biomarker to identify individuals with fibrotic non-IPF ILD at risk of developing PPF. Early detection and intervention in patients with PPF are critical given the morbidity and mortality associated with this condition. Our cohort included a diverse group of patients with fibrotic non-IPF ILDs, highlighting the heterogeneity within the ILD spectrum and the need for personalised approaches for diagnosis and treatment. As previously discussed, MMP-7 has been identified as a potential biomarker for IPF. Nevertheless, the 2018 ATS/ERS/JRS/ALAT guidelines for the diagnosis of IPF do not recommend the use of MMP-7 for the diagnosis of IPF due to the high rates of false-positive and false-negative results [12]. We previously reported a serum MMP-7 cut-off value of 3.91 $ng \cdot mL^{-1}$ with a PPV of 52.3% and NPV of 82.5% for the diagnosis of IPF [5]. In the present study, a cut-off value of 3.53 $ng \cdot mL^{-1}$ was calculated with a sensitivity of 61%, specificity of 74%, PPV of 72% and NPV of 56% for the detection of PPF. This suggests that isolated serum MMP-7 levels may have a high rate of false-positive and false-negative results but may improve diagnostic accuracy when used in combination with other indicators of disease progression.

Age, sex and smoking status are potential confounders when determining possible biomarkers of disease progression. Age has been associated with disease progression in patients with other ILDs, such as IPF [24]. Sex differences have also been observed [23]. Smoking is a well-established risk factor for several fibrotic ILDs and is associated with disease progression [23]. Despite the similar distribution between the PPF and non-PPF groups, the association between serum MMP-7 levels and PPF was adjusted for these confounding factors, and a significant association between MMP-7 levels and PPF behaviour remained after adjusting for these variables.

In our research, serum levels of MMP-1 and MMP-7 did not correlate with significant changes in FVC (at least 5%) or D_{LCO} (at least 10%), which differed from other studies. This discrepancy may be due to the smaller sample size in our study [8].

The UIP pattern has been identified as a risk factor for PPF [24]. In our study, the MMP-1 and MMP-7 levels did not differ with the radiological pattern of UIP. Furthermore, the UIP pattern was independent of the development of the PPF. This highlights that the identification of the UIP pattern alone is not sufficient to determine which patients will benefit from antifibrotic therapy, but a combination with worsening symptoms or a decrease in lung function is required, reinforcing the need for more precise biomarkers [3, 25].

In contrast with other studies, we did not find an association between the overall survival or mortality and the MMP-7 serum levels. However, previous studies only included IPF patients. The lack of association between MMP-7 and overall survival in the present study may be due to the relatively lower levels of

MMP-7 in non-IPF ILD patients and a slower progression over time. A longer follow-up time may be needed to address the precise impact of MMP-7 serum levels on survival of non-IPF ILD [7].

This study has several limitations. The small sample size could make it difficult to assess the associations between MMP-1 and MMP-7 with each of the different fibrotic non-IPF ILD included in the study. Further validation in larger, more diverse cohorts will be essential to establish the clinical utility and cut-off values of serum levels of MMP-7 in the PPF. The use of a single time point to quantify serum levels of MMPs, at the time of diagnosis in the study and at different stages or phases of disease progression, may also limit our understanding, as changes may occur over time [8]. Furthermore, understanding the influence of treatments on MMP-7 levels over time is crucial, as these changes may serve as predictors of antifibrotic therapy outcomes [26]. The inclusion of MMP-7, among other potential biomarkers, may be valuable for enhancing sensitivity and specificity. In addition, other MMPs that were not included in this study, such as MMP-12, may also have prognostic value [10].

Conclusions

PPF represents a significant clinical challenge. Our study aimed to investigate the potential of MMP-1 and MMP-7 as biomarkers for identifying patients with fibrotic non-IPF ILD at risk of developing PPF. The baseline serum MMP-7 concentration was significantly greater in the group of patients who developed PPF, suggesting that MMP-7 levels are a promising biomarker of fibrosis progression.

The identification of serum biomarkers is of significant clinical importance, as it may lead to early therapeutic intervention to potentially prevent the progression of pulmonary fibrosis. The independent association of MMP-7 with PPF, even after adjustment for confounders, highlights its potential. However, further validation is needed to establish its clinical utility and to determine appropriate cut-off values for predicting PPF.

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