Detection of Interstitial Pneumonia with Autoimmune Features and Idiopathic Pulmonary Fibrosis Are Enhanced by Involvement of Matrix Metalloproteinases Levels and Clinical diagnosis

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Research Article

Keywords: interstitial pneumonia with autoimmune features, idiopathic pulmonary fibrosis, matrix metalloproteinases, detection, clinical diagnosis

Posted Date: April 7th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1501736/v1

Abstract

Background: Higher detection of interstitial pneumonia with autoimmune features (IPAF), idiopathic pulmonary fibrosis (IPF) has significant diagnostic and therapeutic implications. Some MMPs have been reliable diagnostic biomarkers in IPAF and IPF in previous studies, yet relevant reliability remains to be recognized. Material and

Methods: 36 ILD patients, including 31 IPAF patients (Mean ± SD, 50.20 ± 5.10 years; 16 (51.6%) females) and 5 IPF patients (Mean ± SD, 61.20 ± 6.73 years; 1 (20.0%) females) were retrospectively enrolled. Serial serum samples were collected from corresponding patients between January 2019 and December 2020. Notably, Serum MMPs levels were measured by U-PLEX Biomarker Group 1(Human) Multiplex Assays (MSD, USA).

Results: A combination of MMPs, combinatorial biomarkers and was strongly associated with the above clinical subjects (AUC, 0.597 for Stability vs. Improvement and 0.756 for Stability vs. Exacerbation). Importantly, the AUC of MMP-12 reaches 0.730 ($P<0.05$, Stability AUC vs. Improvement AUC) while MMP-13 reaches 0.741 ($P<0.05$, Stability AUC vs. Exacerbation AUC) showed better academic performance than other MMPs in two comparisons.

Conclusions: Clinical risk factors and MMPs are strongly associated with either stratification of the degree of disease of progression of IPAF or in two IPAF and IPF independent cohorts. To our knowledge, this is the first to illustrate that MMP-12 and MMP-13 may be expected to become typical promising biomarkers in Improvement - IPAF and Exacerbation - IPAF, respectively.

Introduction

The interstitial lung diseases (ILDs), a heterogeneous set of diffuse parenchymal lung diseases, characterized by various degrees of inflammation of the pulmonary interstices, ultimately may result in pulmonary fibrosis and contribute to high morbidity and mortality. Interstitial pneumonia with autoimmune features (IPAF), an overlap classification between idiopathic interstitial pneumonia (IIPs), especially idiopathic pulmonary fibrosis (IPF), and connective tissue disease-associated interstitial lung disease (CTD-ILD)[1], currently, the proportion of IPAF varies between 7 and 34% of all ILDs mainly up to the group studied and the subjects recruited as the decades progressed.[2-4] Moreover, idiopathic pulmonary fibrosis (IPF) is also an interstitial lung disease/ a diffuse parenchymal lung disease characterized by chronic progressive pulmonary fibrosis generating a poor prognosis[5]. Interestingly, the argument about the survival and prognosis between interstitial pneumonia with autoimmune features (IPAF) and idiopathic pulmonary fibrosis (IPF) is still endless. Multiple studies have testified the difference between them both. A study by Oldham et al. demonstrated that the IPAF subjects showed worse survival than the CTD-ILD patients while displaying slightly better survival than the idiopathic pulmonary fibrosis (IPF) patients.[6] However, a resemble study by Ahmad et al. found no distinct difference among IPAF, IPF and CTD-ILD[2], yet a recent view emphasized that patients enrolled in the

study conforming with IPAF criteria prone to have a history of smoking similar to that of patients with idiopathic pulmonary fibrosis (IPF). [7]

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases of an enzyme family and as the main set that catalyzes the normal turnover of the extracellular matrix (ECM) and regulates the activity of a group of endogenous proteins[8]. When under normal physiological conditions, it is essential to maintain the balance of tissue abnormalities. As the decades progressed, MMPs have been found to be significant in the area of precision medicine in several diseases as they may be used as biomarkers to detect an individual's disease susceptibility, condition, or progression. [9-15]The article by Yoshikazu Inoue et al. showed the study data in IPF: increased levels of MMP-1 (serum), MMP-7 (serum, BALF, and induced sputum) and other MMPs recognized in IPF.[16-18] In particular, previous research showed that in patients with IPF, elevated MMP-7 was strongly connected with reduced survival. [19-21] Unfortunately, until now, studies describing a change in level between matrix metalloproteinases (MMPs) and IPAF's subjects of worldwide scope were scarce. Meanwhile, previous research showed that surfactant protein A (SP-A), Krebs von den Lungen-6 (KL-6), lactate dehydrogenase (LDH), C-reactive protein (CRP), and total immunoglobulin E(tIgE) are also related to matrix metalloproteinases (MMPs), but further confirmation is needed later. Consequently, this article aims to identify clinical risk factors, cytokines especially serum matrix metalloproteinases (MMPs) associated with the patients with IPAF and IPF, providing guidance for the subsequent clinical diagnosis and treatment.

Materials And Methods

Ethical Approval

This project was granted institutional review board approval by the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University, with approval number: GYFYY-2016-73. Human serum samples were used in accordance with the legislation in China and the wishes of donors, their legal guardians, or next of kin, where applicable, who had offered written informed consent to use the serum samples for future unspecified research purposes.

Study Design

A total of 36 patients: 31 with IPAF and 5 with IPF, are enrolled in the cross-sectional study. The relevant data collection has originated from First Affiliated Hospital of Guangzhou Medical University from January 2019 and December 2020, in Guangzhou, China. The above subjects were diagnosed by respiratory physicians using Guidelines for the Diagnosis and Treatment of Interstitial lung Diseases (including evidence that clinical findings, lung ventilation, diffusion function, pathological biopsy, and the exclusion of other known causes of ILD. Surgical lung biopsy may be performed if necessary.) Patients undergoing immunotherapy or with cancer, COVID-19, or more resembling infections were excluded from this study, while patients' sex, age, clinical information (including diagnosis [IPAF or IPF], serum biomarkers, pulmonary function test results (forced vital capacity [FVC], forced expiratory volume in 1 second [FEV1]), forced expiratory volume in 1 second [FEV1]/forced vital capacity [FVC], carbon

monoxide diffusing capacity[DLCO]) and blood cell test are involved in our study. Interstitial pneumonia with autoimmune features (IPAF) subjects (including Stability, Improvement, and Exacerbation) with HRCT scans performed for clinical indications[2, 22] and with serum samples (n = 31) were evaluated for IPAF. All subjects had medication available. The idiopathic pulmonary fibrosis (IPF) cohort consisted of patients who closely resemble the IPAF cohort evaluated for IPF through the First Affiliated Hospital of Guangzhou Medical University from January 2019 and December 2020. IPF subjects with HRCT scans[23, 24] available to be interpreted (n = 5) were included in this study. Baseline demographics, smoking history, history of drugs, and comorbidities were obtained from the medical records. A variety of researchers have previously reported subject characteristics of the IPAF and IPF cohorts. More details include matrix metalloproteinases (MMPs), surfactant protein A (SP-A), Krebs von den Lungen-6 (KL-6), lactate dehydrogenase (LDH), C-reactive protein (CRP), total immunoglobulin E (tIgE), and body mass index (BMI) are found in the subsequent supplement.

Lung function measurements

Based on the advice of the ERS/ATS, pulmonary function testing was performed on a computerized spirometer (MasterScreen, Leibnizstrasse, Hochberg, Germany). The examination parameters included forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), and carbon monoxide diffusing capacity (DLCO).

Blood collection

In 36 ILD (IPAF and IPF) patients, the initial main symptoms included active dyspnea, diffuse infiltrating shadow on X-ray chest radiograph, restricted ventilation disorder, reduced diffusion (DLCO) function, hypoxemia, etc.

The fasting morning blood (5 mL) of the patients was collected through coagulation-promoting tubes within 24 h of the onset of the first respiratory symptoms. The collected samples were kept at room temperature for about 30 min and centrifuged at 3000 r/min for 10 min to obtain serum. Aliquots of serum were stored at − 80 °C to avoid repeated freeze-thaw.

Measurement of serum markers levels

Serum MMPs levels were measured on a fully Hypersensitive multifactor electrochemiluminescence instrument, S-600 and U-PLEX Platform (MSD, USA), according to the manufacturer's instructions. The levels of KL-6, SP-A, LDH, CRP, and tIgE were measured by commercially available assay kits according to the manufacturer's protocols. Samples that were above the upper detection limit were excluded from the analysis.

Statistical Analysis

All Statistical analyses were performed using SPSS 26.0 (Chicago, IL) and R (R Development Core Team, Vienna, Austria). P values less than 0.05 were considered statistically significant. T-test was used for

univariate analysis. In multivariate analysis, unadjusted and adjusted logistic regression models were used to evaluate the comparison between Stability and Improvement (or Exacerbation), respectively. The selected variables of interest (MMPs and other investigational biomarkers) were adjusted in the IPAF and IPF's logistic regression model. To evaluate the ability of a combinatorial signature to identify the presence of IPAF, we first evaluated clinical risk factors (age, sex, BMI, and smoking history) associated with IPAF in the literature[25-33]. Subsequently, we added selected biomarkers (MMPs, SP-A, KL-6, LDH, CRP, and tIgE) in this study. Given the variability and potential data loss in this cohort, respiratory symptoms were excluded from our exploratory modeling. The IPF cohort followed some similar research.

Receiver Operating Characteristic (ROC) curves were generated to determine whether combining these MMPs and other investigational biomarkers effectively identified subjects with IPAF, including Stability AUC vs. Improvement AUC and Stability AUC vs. Exacerbation AUC. We then generated the area under the curve (AUC) for each biomarker of interest. We determined whether the features of clinical significance were found by comparing the degree of disease progression on MMPs and Other Investigational Biomarkers in the IPAF Cohort. This combination of characteristics was tested in the IPAF cohort but not yet evaluated in the IPF cohort because the number of patients was insufficient for ROC curves. We believe that the utility of diagnostic tests derived from these variables lies in their ability to distinguish the severity of IPAF. Therefore, we grasped a risk situation for Stability AUC vs. Improvement AUC and Stability AUC vs. Exacerbation AUC in the IPAF cohort.

Result

Of 31 IPAF subjects enrolled, all of them are on medication in this cohort (Figure 1A), 16 (51.6%) had a history of medicine, 13 (41.9%) had comorbidities, 7 (22.6%) had a history of smoking, and 14 (45.2%) in Stability, 9 (29.0%) in Improvement and 14 (25.8%) in an Exacerbation (Figure 1A). Of 5 IPF subjects, all of them also undergo medication resembling the IPAF cohort, 4 (80.0%) had a history of medicine, 2 (40.0%) had comorbidities, and 1 (20.0%) had a history of smoking. On the strength of this assessment, 1 (20.0%) in a Stability, 2 (40.0%) in an Improvement and 2 (40.0%) in an Exacerbation (Figure 1B). Baseline characteristics of IPAF and IPF cohorts are summarized in Table 1. In comparing those amid the IPAF and IPF cohorts, IPAF patients were inclined to be on medication (Methylprednisolone, Acetylcysteine, and Pirfenidone). In contrast, there was no evident statistical significance in the IPF cohort.

Clinical risk factors

Based upon the T-Test, we found older age, female sex, BMI > 24, and even ever-smoker associated with IPAF and IPF (Table 1), among which, seeming that when received medical therapy, the clinical performance of females is better than that of male in stratification of the degree of disease of the severity of IPAF (see Table 2, Figure 2).

Medication use

In the IPAF cohort, subjects tended to use methylprednisolone (83.9%), followed by acetylcysteine and pirfenidone, whereas in IPF patients seemed to preferentially use acetylcysteine (80.0%) (Table 1). We also found that baseline characteristics of IPAF Subjects Stratified by Severity in Table 2 were all preferred Methylprednisolone. However, in terms of the result of patients of representation, the drug Methylprednisolone is not effective enough. Interestingly, Acetylcysteine was used more frequently in patients with Stability - IPAF and Improvement – IPAF (Table 2).

Matrix Metalloproteinases (MMPs)

Matrix metalloproteinases are a huge family of 23 endogenous zinc-containing proteases, including collagenase, gelatinase, matrix lysozyme, matrix elastase, and membrane matrix metalloproteinases.[34, 35] In humans, this biomarker has a complex relationship with various disease processes, including atherosclerosis, hepatic fibrosis, and interstitial lung fibrosis.[36] In accordance with the result of IPAF cohort, compared with Stability – IPAF, levels of MMP-2, MMP-3, MMP-7, MMP-9, MMP-10, MMP-13 were significantly varied between Improvement - IPAF and Exacerbation – IPAF while the corresponding biomarker to them may be found in this study. In addition, we observed a negative correlation between Levels of MMP-12 and the progression of Improvement - IPAF and Exacerbation – IPAF when compared with Stability - IPAF in IPAF specimens (Table 2). The AUCs of MMP-2, MMP-3, MMP-7, MMP-9 and MMP-13 for Stability AUC vs. Improvement AUC and Stability AUC vs. Exacerbation AUC were 0.683, 0.587, 0.512, 0.643, 0.603 and 0.491, 0.625, 0.634, 0.580, 0.741, respectively (Table 3). When using MMPs as a total factor, the AUC increased to 0.619 and 0.643 for Stability AUC vs. Improvement AUC and Stability AUC vs. Exacerbation AUC in the IPAF cohort ($P<0.05$ for the difference between the curves). Moreover, we found that MMP-2 and MMP-9 had a better utility in Stability AUC - Improvement AUC than Stability AUC - Exacerbation AUC, nevertheless, MMP-3, MMP-7, and MMP-13 obtained a contrary outcome between the above respective comparison (see Figure 3). When adding MMPs to combinatorial biomarkers (SP-A, KL-6, LDH, CRP, tIgE), the ROC curve AUCs of Stability AUC - Improvement AUC followed similarly, but Stability AUC - Exacerbation AUC showed a stronger identification trend. Interestingly, the AUC of the MMP-12 variable ranged from 0.730 to 0.737 in Stability AUC vs. Improvement AUC and Stability AUC vs. Exacerbation AUC, with the strongest degree of disease progression correlation (see Table 3).

Notably, in the IPF cohort, a great deal of previous research into MMPs has focused on levels of MMP-3, MMP-7, MMP-8, MMP-9, MMP-12, and MMP-13.[37, 38] Owing to the fact that the number of subjects in this cohort is rare, more experiments are needed to be proved in future studies.

Other investigational biomarkers

Levels of KL-6 and SP-A significantly increased with severity of IPAF, among which, KL-6 levels peaked in the IPAF cohort. Meanwhile, CRP and tIgE may be significantly stronger associated with IPF based on the T-test (Table 1). However, due to a small number of patients in the IPF cohort, this conclusion remains to be verified. Multivariable logistic regression analyses adjusting for the above
five investigational biomarkers in IPAF Subjects stratified by severity of IPAF are presented in Table 2. In

addition to KL-6, SP-A increased significantly, and the other three investigational biomarkers showed no obvious abnormalities (all within the normal range).

Given the degree of disease stratification of IPAF, AUCs for the five investigational biomarkers ranged from 0.379 to 0.710, and these combinatorial biomarkers were 0.583 and 0.867, respectively (Table 3). In Stability AUC vs. Improvement AUC, the AUCs ranged from 0.537 to 0.710 with a combined AUC of 0.583, in Stability AUC vs. Exacerbation AUC, the AUCs ranged from 0.379 to 0.600 with a combined AUC of 0.867 (see Figure 4).

Combinatorial signature

A combination of clinical risk factors and MMPs is strongly associated with IPAF, including Stability, Improvement, and Exacerbation. Importantly the addition of the five investigational biomarkers SP-A, KL-6, LDH, CRP, and tIgE significantly increased the AUC to 0.756 for comparison of Stability AUC - Exacerbation AUC in IPAF cohort ($P < 0.05$ for the difference between the curves) (Table3, Figure 4). [27, 39-49]

Pulmonary function testing (PFT) and Blood cell test

Whether IPAF is compared with IPF, or simply a comparison among the three stratifications of the severity of IPAF ($p<0.05$), apart from DLCO, which identified 36 patients with moderate interstitial lung disease, the remaining PFT items, including FVC, FEV1 showed that the patients in two cohorts did not show significant clinical significance in PFT (Figure 5). No abnormality was found in the selected cytokines in the blood cell test in the IPAF and IPF cohorts.

Discussion

In this study, clinical risk factors (older age, female sex, smoking history) and MMPs were strongly associated with IPAF and IPF. A biomarker signature composed of matrix metalloproteinases, pulmonary epithelial cell chemokines (surfactant protein A and Krebs Von den Lungen-6), lactate dehydrogenase, C-reactive protein, and total immunoglobulin E significantly strengthens this association. Unfortunately, our studies on predicting IPAF and IPF differences and stratification of the degree of disease of the severity of IPAF through investigational biomarkers are still undergoing, meanwhile, the five investigational biomarkers signature, consisting of SP-A, KL-6, LDH, CRP, tIgE, rarely enhances the ability of individuals to identify independently and in combination with the above variables. Importantly, this combined signature of clinical risk factors, MMPs, and investigational biomarkers was tested in two separate cohorts and individuals of IPAF subjects with Stability, Improvement, and Exacerbation.

Several studies have given eloquent proof that IPAF is associated with age, female sex, and smoking status[4, 7, 50-54]. In addition, our group et al. found that KL-6 can reflect the progression of the disease and play a key role in reflecting the degree of lung epithelial cell injury and fibrosis in IPAF. [39, 41]. Although this study showed that MMPs combined with clinical risk factors could detect the presence of

IPAF, it should be noted that MMP-7, MMP-12 were weak differences between the presence of two cohorts.

Elevated levels of SP-A, KL-6 have previously been associated with disease progression and reduced survival in IPAF and IPF patients[31, 55-64]. Given that a high proportion of patients with IPAF have typical radiological or histological patterns of interstitial pneumonia similar to IPF[61, 65-68], we hypothesized that the biomarkers in IPF that predict clinical outcomes are also associated with IPAF. In this study, we found significantly elevated blood levels of SP-A, KL-6 in two separate IPAF and IPF cohorts. When used in combination with these risk factors, this combination of biomarker signature, along with clinical risk factors and MMPs, significantly enhances the ability to differentiate IPAF from IPF. To the best of our knowledge, our study further confirms that the combination of SP-A, KL-6 can identify the presence of IPAF and IPF. Notably, although SP-A and KL-6 are the primary biomarkers of previous studies on IPAF, their repeatability in patients needs further clarification. At the same time, other potentially predictable inflammatory regulators in body fluids may play a more significant role in the prognosis of IPAF and should be further investigated.

The high prevalence of females and smoking places these individuals at higher risk of IPAF and IPF[4, 7, 52] and continues to support the importance of smoking cessation for all patients with this pulmonary disease as a critical component of managing individuals at risk of IPAF and IPF [69]. Our studies outlined here provide a better understanding of the clinical and molecular characteristics of IPAF and highlight the potential role of novel biomarkers in identifying the stratification of the degree of severity of IPAF.

The high incidence of Exacerbation - IPAF and adverse clinical outcomes in Table 2 highlight the need for effective risk stratification methods of patients with IPAF. In patients with IPAF who are at risk for disease progression, early detection of Exacerbation - IPAF may lead to meaningful changes in clinical outcomes due to a large number of disease modifiers and biologics available, and novel anti-fibrotic therapies[70]. Although our study did not value the association of molecular characteristics with disease progression, we have identified meaningful investigational biomarkers selected that are strongly associated with the presence of IPAF. Especially in patients with Improvement - IPAF, MMP-1, MMP-2, MMP-9, MMP-12 have been shown to predict disease progression and survival. In addition, resemble our findings in IPAF, MMP-1, MMP-3, MMP-7 MMP-12, MMP-13 biomarkers seem to improve the predictive ability for IPF. The reality is that the limited number of subjects and the application of diagnostic algorithms that combine these clinical risk factors with MMPs and investigational biomarkers yields strong positive and negative likelihood ratios for IPF. Studies of larger cohorts of IPF with detailed clinical phenotypes and longitudinal follow-up are needed to reduce the risk of likelihood ratios. These future studies may eventually help better understand the significance and rate of progression of IPF and thereby have a potentially positive impact on the stratification of severity of patients with IPF in the future.

According to the above data, our group found that MMP-12 had a significant association with Improvement - IPAF, while MMP-13 predicted a specific association with Exacerbation – IPAF, yet more reality in this finding has yet to be confirmed. MMP-13 is a crucial interstitial collagenase,

important in bone remodeling and liver injury. However, the relationship between MMP-13 and Exacerbation - IPAF remains unclear. This study found that the serum MMP-13 level in patients with Exacerbation – IPAF was nearly four times higher than in patients with Improvement - IPAF, suggesting that MMP-13 plays a positive role in patients with progressive IPAF. For example, in a model of atopic lung disease[71], both MMP -2 and MMP -9 have been shown to affect the inflammatory response of lung disease, but no significant effect was found in this study. In addition, a survey of the enzymological properties of MMPs[72] showed that MMPs not only acted on extracellular matrix elements, but also lysed cytokines and possibly inactivated them, which may indicate that MMP-13 may further aggravate the disease of patients with IPAF, arising our attention. Similarly, loss of MMP-13 has previously been reported to reduce liver damage and fibrosis in mice during cholestasis[73]. Thus, MMP-13 may really get IPAF into Exacerbation – IPAF in patients with IPAF.

Interestingly, Satish K Madala et al.[74] found that MMP13 activity increased when MMP12 was absent. In addition, IL-13-mediated increased expression of dependent MMP-13 in MMP-12 deficient mice in his experimental model suggests that MMP12 may play an essential counter-regulatory role in regulating IL13-dependent tissue fibrosis, and MMP12 seems to play its part in reducing fibrosis by modulating the activity of other MMP13. Based on the above description, we considered that MMP-12 would be a suitable biomarker for Improvement - IPAF. At the same time, we gained that the ability of matrix metalloproteinases to regulate multiple disease severity stratification-related cytokines have been described in several live models [75], to some extent, providing some evidence for our recent findings.

Some limitations were included in our study. Firstly, there was a limited number of subjects in the IPF cohort, although all data were available. Subject selection varies considerably in study timing and significant bias. To solve the possible bias that may result from subjects in the clinical process, we further explored IPF based upon comorbidities and medication history. We found no significant difference between ROC curves. This suggests that the IPF cohort results may be primarily caused by the number and medicine of patients with IPF and, therefore, more general or even limited value in this part. Secondly, these risk factors may decline in larger cohorts, especially when age, sex, and MMPs are stratified and included in clinical predictive models. However, previous results suggest that clinical risk factors and MMPs can independently identify the presence of two independent cohorts and stratification of severity in IPAF. Finally, although investigational biomarkers (SP-A, KL-6, LDH, CRP, tIgE) have been tested in independent cohorts, inherent limitations of effectiveness in all experimental biomarkers, especially where reproducibility and generalization remain [47]. More researches are needed to address further the correlation between this combination of characteristics and meaningful clinical outcomes. We are looking forward that a wider range of biomarkers and other variables of interest, such as routine blood test analysis and PFTs could be investigated in the near future. We believe that any query about the limitations of this realm should be clarified in future studies.

Conclusion

Overall, this study set out to gain a better understanding of clinical risk factors, MMPs associated with combinatorial biomarkers composed of SP-A, KL-6, LDH, CRP, tIgE, to some extent, identify the existence of Improvement and Exacerbation in the IPAF cohort. Unfortunately, studies on IPF could not be completed in this study. The results of this investigation show that MMP-12 and MMP-13 may possibly become representative biomarkers in Improvement - IPAF and Exacerbation - IPAF, respectively. These findings may facilitate the identification of IPAF at an earlier stage, potentially leading to decreased morbidity and mortality.

Abbreviations

ILD interstitial lung disease

IPAF interstitial pneumonia with autoimmune features

IPF idiopathic pulmonary fibrosis

MMPs Matrix Metalloproteinases

SP-A surfactant protein A

KL-6 Krebs Von den Lungen-6

LDH lactate dehydrogenase

CRP C-reactive protein

tIgE total immunoglobulin E

FVC forced vital capacity

FEV1 forced expiratory volume in 1 s

DLCO diffuse lung carbon monoxide

ROC receiver operating characteristic

AUC area under the curve

Declarations

Acknowledgements
Not applicable.

Author's Contributions: Mingshan Xue, Yifeng Zeng, Runpei Lin: acquisition of data. Mingtao Liu, Mingshan Xue, Teng Zhang, Baojun Guo, Youpeng Chen: concept, analysis and design. Mingtao Liu,

Mingshan Xue, : interpretation, and drafting of manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

Funding: This article was funded by the National Natural Science Foundation of China (Project No.: 81871736, 81960023), Bureau of Traditional Chinese Medicine Scientific Research Project of Guangdong (Project No.: 20192048), Science and Technology Innovation Committee Project of Guangzhou (Project No.: 201804020043), Key projects of Guangzhou Education Bureau (Project No.: 201831802), and Open Project of State Key Laboratory of Respiratory Disease (Project No.: SKLRD-OP-201803, SKLRD-OP-201809).

Availability of data and materials

Data is contained within the article or supplementary material.

Ethics approval and consent to participate

This project was granted institutional review board approval by the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University, with approval number: GYFYY-2016-73. Human serum samples were used in accordance with the legislation in China and the wishes of donors, their legal guardians, or next of kin, where applicable, who had offered written informed consent to use the serum samples for future unspecified research purposes.

Consent for publication

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

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Tables

Table 1. Baseline Characteristics of IPAF and IPF cohorts

	IPAF Cohort	IPF Cohort	p value
Variable	(n=31)	(n=5)	——
Demographics			
Age,yr	50.20 ± 5.10	61.20 ± 6.73	0.074
Sex, female	16 (51.6%)	1 (20.0%)	0.406
BMI	24.18 ± 0.64	24.74 ± 0.96	0.480
Ever-smoker	7 (22.6%)	1 (20.0%)	0.364
Medication use (ever)			
Methylprednisolone	26 (83.9%)	2 (40.0%)	0.556
Acetylcysteine	16 (51.6%)	4 (80.0%)	0.328
Pirfenidone	4 (51.6%)	1 (20.0%)	0.153
MMPs(ng/mL)			
MMP-1	7991.79 ± 1621.10	3677.75 ± 1237.85	0.208
MMP-2	154186.15 ± 8218.85	196109.60 ± 1237.85	0.071
MMP-3	21176.23 ± 4092.53	38908.00 ± 18859.30	0.599
MMP-7	31844.08 ± 4286.09	30645.80 ± 12364.32	0.909
MMP-9	108313.00 ± 13576.06	121551.00 ± 42797.25	0.982
MMP-10	596.70 ± 60.32	739.50 ± 159.65	0.599
MMP-12	555.33 ± 88.84	575.02 ± 96.04	0.150
MMP-13	288.11 ± 122.95	122.57 ± 72.26	0.807
Other investigational biomarkers			
SP-A (ng/mL)	54.89 ± 7.41	101.74 ± 54.07	0.240
KL-6 (U/mL)	2496.20 ± 893.25	882.60 ± 132.03	0.437
LDH (U/L)	219.45 ± 7.85	197.98 ± 7.85	0.815
CRP (mg/dL)	0.18 ± 0.10	0.35 ± 0.19	0.232
tIgE (kU/L)	17.82 ± 5.42	289.72 ± 255.40	0.862
Pulmonary function testing			
FEV1/FVC% of predicted	106.50 ± 1.70	104.15 ± 3.69	0.733

FEV1, % of predicted	73.46 ± 3.09	69.35 ± 9.57	0.604
FVC, % of predicted	71.74 ± 3.22	67.24 ± 8.55	0.766
DLCO, % of predicted	58.91 ± 58.91	46.94 ± 6.25	0.090

Abbreviations: IPAF, interstitial pneumonia with autoimmune features, IPF, idiopathic pulmonary fibrosis, BMI, body mass index, MMPs, matrix metalloproteinases, SP-A, surfactant protein A, KL-6, krebs von den lungen-6, LDH, lactate dehydrogenase, CRP, c-reactive protein, tIgE, total immunoglobulin E, FVC, forced vital capacity, FEV1, forced expiratory volume in 1 s, DLCO, diffuse lung carbon monoxide.

Data are presented as the Mean ± SD or number (%)

Table 2. Baseline Characteristics of IPAF Subjects Stratified by Severity of IPAF

	IPAF Cohort			p value
	Stability	Improvement	Exacerbation	
Variable	(n=14 [45.2%])	(n=9 [29.0%])	(n=8 [25.8%])	——
Demographics				
Age,yr	51.79 ± 3.45	51.56 ± 4.19	47.13 ± 6.33	0.930
Sex, female	7（50.0%）	6（66.7%）	3（27.5%）	0.480
BMI	24.28 ± 0.72	24.47 ± 1.74	24.05 ± 1.09	0.892
Ever-smoker	3（21.4%）	1（11.1%）	3（37.5%）	0.509
Medication use (ever)				
Methylprednisolone	11（78.6%）	8（88.9%）	7（87.5%）	0.463
Acetylcysteine	10（71.4%）	4（44.4%）	1（9.0%）	0.637
Pirfenidone	3（21.4%）	1（11.1%）	——	0.637
MMPs (ng/mL)				
MMP-1	7017.34 ± 1488.80	9353.6 ± 1832.79	8165.06 ± 5619.65	0.165
MMP-2	147036.93 ± 8712.38	169612.44 ± 15309.68	150947.75 ± 19505.10	0.400
MMP-3	21378.07 ± 5263.64	30361.89 ± 9813.56	32062.63 ± 8502.27	0.607
MMP-7	32570.29 ± 6332.31	36081.89 ± 4430.04	26096.63 ± 8625.66	0.389
MMP-9	105517.64 ± 17755.11	152086.44 ± 28858.42	144758.75 ± 40580.45	0.532
MMP-10	669.63 ± 97.15	588.3678 ± 83.55	714.88 ± 224.56	0.934
MMP-12	662.14 ± 135.61	425.19 ± 134.57	321.11 ± 80.11	0.087
MMP-13	348.59 ± 171.07	283.70 ± 242.40	865.60 ± 190.51	0.162
Other investigational biomarkers				
SP-A (ng/mL)	42.64 ± 3.61	80.03 ± 21.39	48.03 ± 11.63	0.240
KL-6 (U/mL)	1939.88 ± 599.02	1970.88 ± 623.62	1525.88 ± 650.06	0.437
LDH (U/L)	223.89 ± 11.39	226.14 ± 20.91	226.28 ± 14.77	0.815

CRP (mg/dL)	0.32 ± 0.09	0.55 ± 0.13	0.55 ± 0.19	0.232
tIgE (kU/L)	52.49 ± 21.47	124.13 ± 62.39	50.11 ± 13.40	0.862
Pulmonary function testing				
FEV1/FVC% of predicted	107.04 ± 2.49	106.85 ± 2.76	104.62 ± 3.60	0.977
FEV1, % of predicted	79.64 ± 4.78	68.49 ± 4.15	67.67 ± 4.24	0.102
FVC, % of predicted	76.75 ± 5.09	66.34 ± 4.28	67.30 ± 4.95	0.285
DLCO, % of predicted	57.69 ± 3.72	50.14 ± 4.04	68.85 ± 4.66	0.060
Blood cell ratio (%)				
Leukocyte	7.74 ± 1.05	7.94 ± 1.06	7.43 ± 0.80	0.806
Neutrophil	66.64 ± 3.52	70.08 ± 4.54	56.83 ± 1.83	0.073
Lymphocyte	23.06 ± 2.89	18.63 ± 3.26	31.85 ± 2.04	0.013
Monocyte	8.06 ± 1.31	8.89 ± 1.79	8.28 ± 0.64	0.494
Eosinophils	1.78 ± 0.35	1.99 ± 0.54	2.58 ± 1.13	0.930
Basophil	0.46 ± 0.08	0.41 ± 0.13	0.48 ± 0.07	0.935

Abbreviations: IPAF, interstitial pneumonia with autoimmune features, IPF, idiopathic pulmonary fibrosis, BMI, body mass index, MMPs, matrix metalloproteinases, SP-A, surfactant protein A, KL-6, krebs von den lungen-6, LDH, lactate dehydrogenase, CRP, c-reactive protein, tIgE, total immunoglobulin E, FVC, forced vital capacity, FEV1, forced expiratory volume in 1 s, DLCO, diffuse lung carbon monoxide.

Data are presented as the Mean ± SD or number (%)

Table 3. ROC curves for Respective Comparison of Degree of Disease Progression on MMPs and Combinatorial Biomarkers in the IPAF Cohort

	IPAF	
Variable	Stability AUC vs. Improvement AUC	Stability AUC vs. Exacerbation AUC
MMP-1	0.619	0.643
MMP-2	0.683	0.491
MMP-3	0.587	0.625
MMP-7	0.512	0.634
MMP-9	0.643	0.580
MMP-10	0.464	0.482
MMP-12	0.730	0.737
MMP-13	0.603	0.741
MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-10, MMP-12, MMP-13	0.619	0.643
SP-A	0.659	0.589
KL-6	0.607	0.585
LDH	0.562	0.571
CRP	0.710	0.379
tIgE	0.537	0.600
Combinatorial biomarkers	0.583	0.867
MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-10, MMP-12, MMP-13, Combinatorial biomarkers	0.597	0.756

Abbreviations: IPAF, interstitial pneumonia with autoimmune features, MMPs, matrix metalloproteinases, SP-A, surfactant protein A, KL-6, krebs von den lungen-6, LDH, lactate dehydrogenase, CRP, c-reactive protein, tIgE, total immunoglobulin E

Figures

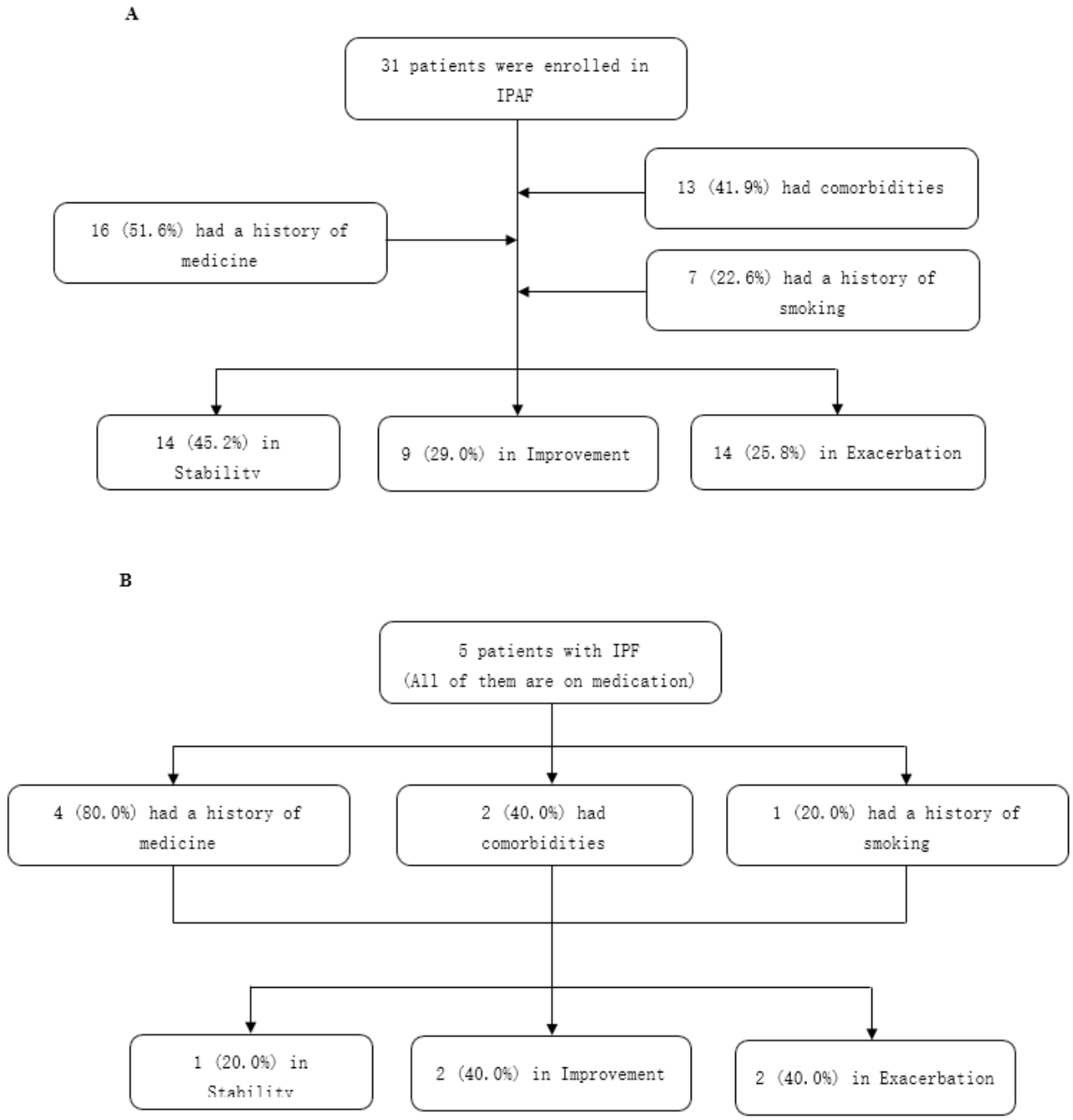

Figure 1

Study enrollment in IPAF (A) and IPF (B) cohorts. A flow diagram of study enrollment divides patients into groups according to clinical diagnosis, the history of medicine, comorbidities, history of smoking. Abbreviations: IPAF = interstitial pneumonia with autoimmune features, IPF = idiopathic pulmonary fibrosis.

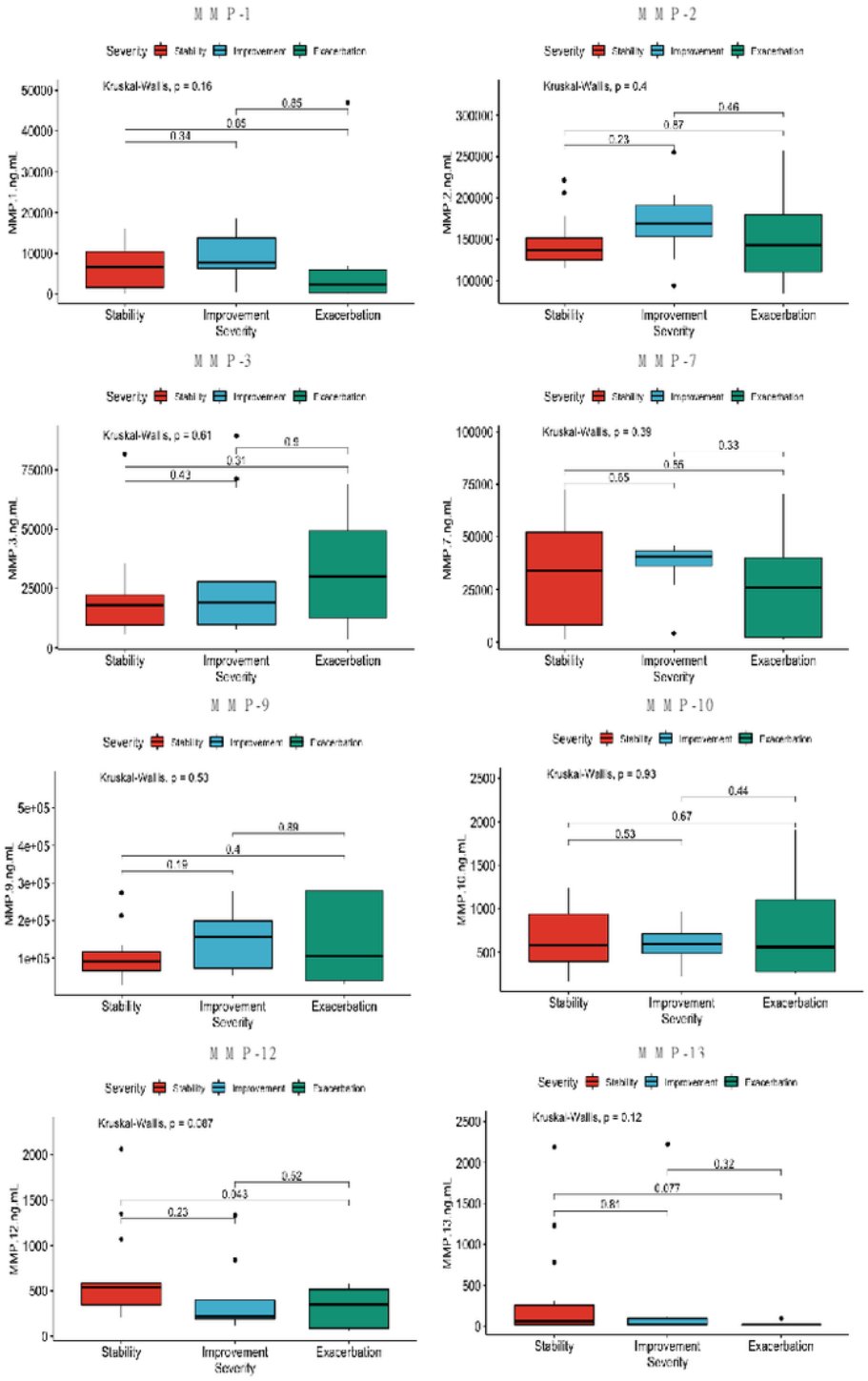

Figure 2

Serum levels for each severity stratification of IPAF were in the selected MMPs.

Abbreviations: IPAF, interstitial pneumonia with autoimmune features

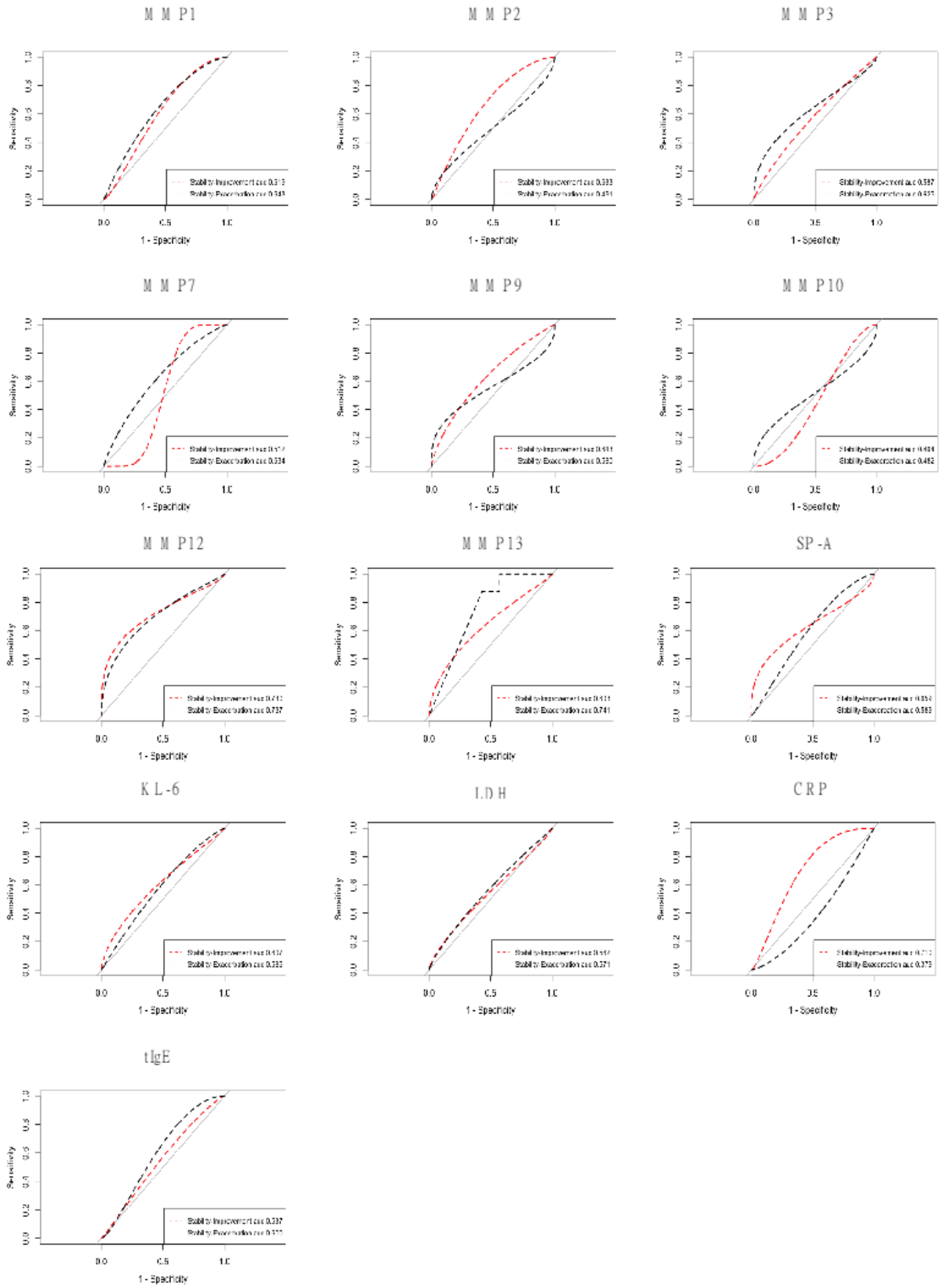

Figure 3

Area under the curve (AUC) for Stability-Improvement and Stability-Exacerbation comparison of MMPs and combination biomarkers in the IPAF cohort.

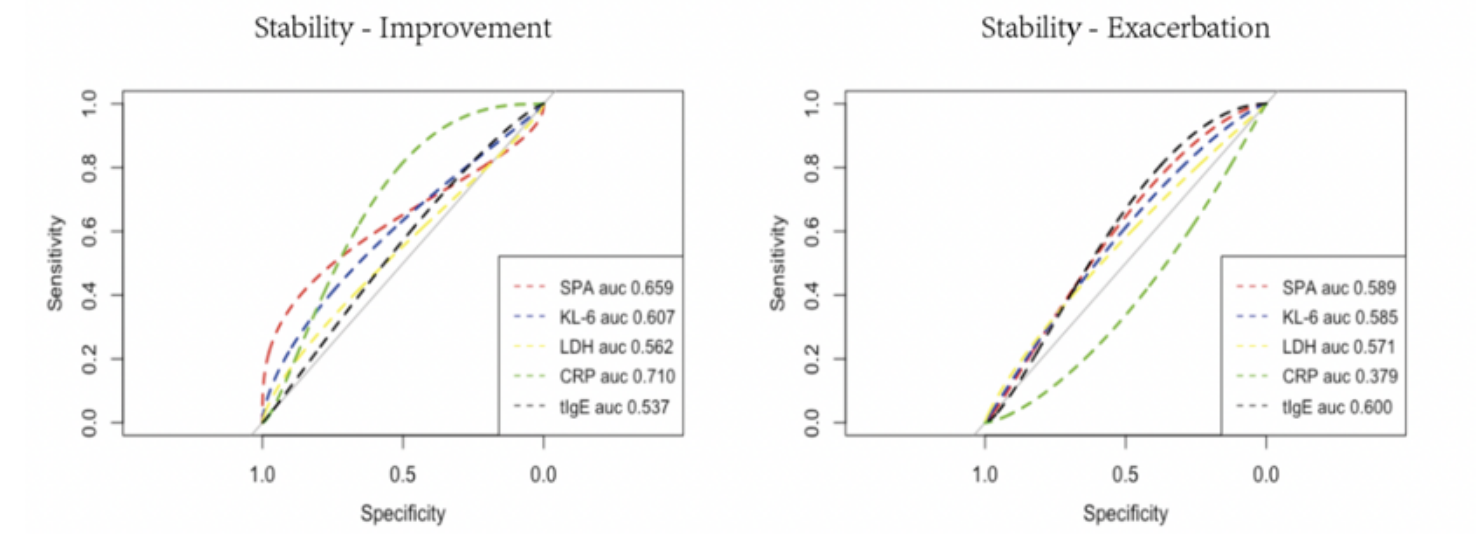

Figure 4

Area under the curve (AUC) of combinatory biomarkers of Stability - Improvement and Stability - Exacerbation in the IPAF study population.

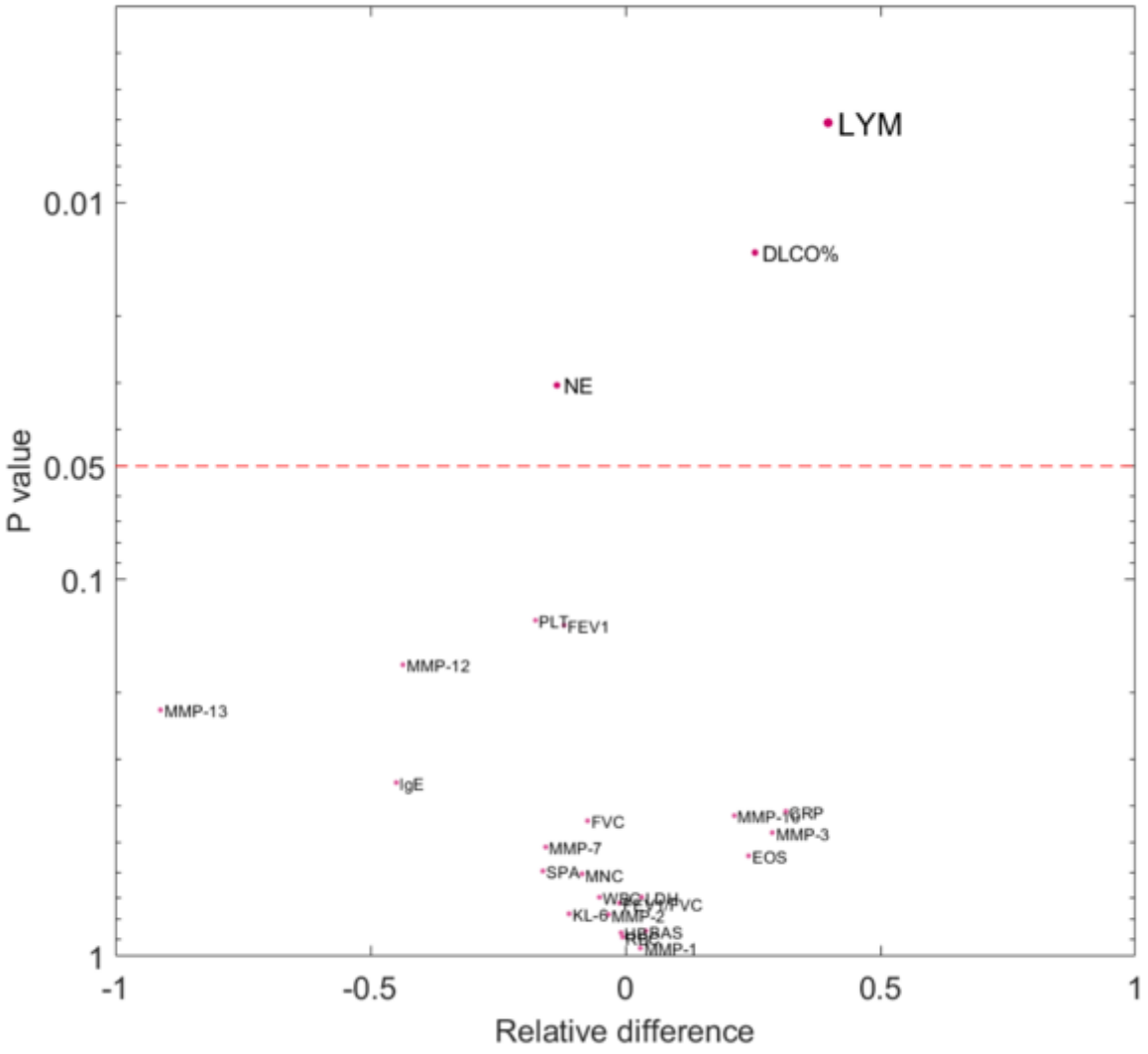

Figure 5

Analysis of the difference of various indicators at onset in IPAF and IPF cohorts