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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Abstract

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

Materials and Methods: In this study, 36 ILDs patients, including 31 IPAF patients (Mean ± SD, 50.20 ± 5.10 years; 16 [51.6%] females) and five IPF patients (Mean± SD, 61.20 ± 6.73 years; one [20.0%] females) were retrospectively enrolled. Serial serum samples were collected from patients with IPAF and IPF 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 and combinatorial biomarkers was strongly associated with clinical subjects in this study (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 performance than other MMPs in two comparisons.

Conclusions: Clinical risk factors and MMPs are strongly associated with either stratification of the 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.

KEYWORDS
clinical diagnosis, detection, idiopathic pulmonary fibrosis, interstitial pneumonia with autoimmune features, matrix metalloproteinases
1 | INTRODUCTION

Interstitial lung diseases (ILDs), a heterogeneous set of diffuse parenchymal lung diseases, characterized by various degrees of inflammation of the pulmonary interstites, 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), currently, the proportion of IPAF varies between 7% and 34% of all ILDs, which mainly up to the group studied and the subjects recruited as the decades progressed. 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. 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 to the difference between them both. A study by Oldham et al. demonstrated that the IPAF subjects showed worse survival than the patients with CTD-ILD while displaying slightly better survival than patients with IPF. However, a resemble study by Ahmad et al. found no distinct difference among IPAF, IPF, and CTD-ILD, 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 IPF.

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. 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. 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. In particular, previous studies showed that elevated MMP-7 was strongly connected with reduced survival in patients with IPF. Unfortunately, until now, studies describing a change in level between matrix metalloproteinases (MMPs) and IPF's subjects of worldwide scope were scarce. Meanwhile, previous research also 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 (IgE), and body mass index (BMI) are found in the subsequent supplement.

2 | MATERIALS AND METHODS

2.1 | Study design

A total of 36 patients: 31 with IPAF and five with IPF, are enrolled in the cross-sectional study. The relevant data collection originated from the 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 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 s [FEV1]], forced expiratory volume in 1 s [FEV1]/forced vital capacity [FVC], carbon monoxide diffusing capacity [DLCO]), and blood cell test are involved in our study. Moreover, IPAF subjects (including Stability, Improvement, and Exacerbation) with HRCT scans performed for clinical indications and with serum samples (n = 31) were evaluated for severity of IPAF. All subjects had medication available. The 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 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 (IgE), and body mass index (BMI) are found in the subsequent supplement.

2.2 | Pulmonary function tests

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

2.3 | Blood collection

In 36 patients with ILDs (IPAF and IPF), 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.

2.4 | Measurement of serum biomarkers levels

Serum MMPs levels included detection ranges: MMP1 (200–15,000 pg/ml), MMP2 (100–200,000 pg/ml), MMP3 (2000–1,000,000 ng/ml), MMP7 (100–200,000 ng/ml), MMP9 (100–200,000 ng/ml), MMP10 (2.06–1500 ng/ml), MMP12 (78–5000 ng/ml), and MMP13 (78–5000 ng/ml) were measured on a fully Hypersensitive multifactor electrochemiluminescence instrument, S-600 and U-PLEX Platform (MSD, USA) with an effective linear range up to 6 log, detection wavelength: 620 nm, detection accuracy: ±0.5 nm and sensitivity reached 0.05 pg/ml, which using the principle of electrochemiluminescence, the multi-channel high-throughput and high-sensitivity quantitative analysis of proteins, enzymes, receptors, antibodies, antigens, cells, and other biomolecules can be realized by electroexcitation of markers and it can be applied to the detection of cytokines and signaling pathway proteins, covering all ELISA detectable indicators: inflammatory factors, chemokines, growth factors, according to the manufacturer’s instructions. In addition, detection ranges of levels of KL-6 (50–10,000 U/ml), SP-A (8 ng/L–200 ng/L), LDH (1 U/L–450 U/L), CRP (0.0005–0.8 mg/dl), and lgE (2–5000kU/L) 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.

2.5 | Statistical analysis

All statistical analyses were performed using SPSS 26.0 and R (R Development Core Team). p values < 0.05 were considered statistically significant. In this study, 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 used clinical risk factors (age, sex, BMI, and smoking history) associated with IPAF in this study. Subsequently, we took selected biomarkers (MMPs, SP-A, KL-6, LDH, CRP, and lgE) into consideration for further investigation. Given the variability and potential data loss in this cohort, respiratory symptoms were excluded from our exploratory modeling. Likewise, the IPF cohort followed the same research as the IPAF.

Receiver operating characteristic (ROC) curves were generated to determine whether combining these MMPs and other investigational biomarkers effectively identified patients with IPAF, including Stability AUC versus Improvement AUC and Stability AUC versus Exacerbation AUC, and then generated the area under the curve (AUC) for each biomarker of interest. Further, we determined whether the features of clinical significance were found by comparing the severity of progression on MMPs and other investigational biomarkers in the IPAF cohort. Regrettfully, the significance of clinical indications can be performed in the IPAF cohort but not yet evaluated in the IPF cohort so 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 patients with IPAF. Therefore, we grasped a risk situation for Stability AUC versus Improvement AUC and Stability AUC versus Exacerbation AUC in the IPAF cohort.

3 | 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 Exacerbation (Figure 1A). Of five 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 Stability, 2 (40.0%) in Improvement, and 2 (40.0%) in Exacerbation (Figure 1B). Baseline characteristics of IPAF and IPF cohorts are summarized in Table 1. In comparison amid the IPAF and IPF cohorts, patients with IPAF were inclined to be on medication (methylprednisolone, acetylcysteine, and pirfenidone). In contrast, there was no evident statistical significance in the IPF cohort.

3.1 | Clinical risk factors

Based on the t test, we found older age, female sex, BMI (>24), and even ever-smoker associated with IPAF and IPF (Table 1), among which, seems that when accepted medical treatment, the clinical performance of females is better than that of male in the severity of IPAF (Table 2, Figure 2).

3.2 | 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 all preferred Methylprednisolone. However, in the terms of outcomes for patients, methylprednisolone is not effective enough. Interestingly, acetylcysteine was used more frequently in patients with Stability-IPAF and Improvement-IPAF (Table 2).
3.3 | 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. In humans, this biomarker has a complex relationship with various disease processes, including atherosclerosis, hepatic fibrosis, and interstitial lung fibrosis. In accordance with the IPAF cohort, compared with Stability-IPAF, levels of MMP-2, MMP-3, MMP-7, MMP-9, MMP-10, and MMP-13 were significantly varied between Improvement-IPAF and Exacerbation-IPAF. Besides, 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 cohorts (Table 2). The AUCs of MMP-2, MMP-3, MMP-7, MMP-9, and MMP-13 for Stability AUC versus Improvement AUC and 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 versus Improvement AUC and Stability AUC versus 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 converse outcome between this comparison (Figure 3).

When adding MMPs to combinatorial biomarkers (SP-A, KL-6, LDH, CRP, tlgE), the ROC curve of Stability AUC-Improvement AUC followed similarly, but Stability AUC-Exacerbation AUC showed a stronger identification trend. Interestingly, the AUC of the MMP-12 ranged from 0.730 to 0.737 in Stability AUC versus Improvement AUC and Stability AUC versus Exacerbation AUC, with the strongest correlation in disease progression (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.[23,24] Due to patients with IPF enrolled being rare, the effect of MMPs in this cohort is needed to be proved in future studies.

3.4 | Other investigational biomarkers

Levels of KL-6 and SP-A significantly increased with the severity of IPAF, among which, KL-6 levels peaked in the IPAF cohort. Meanwhile, CRP and tlgE may be significantly stronger associated with IPF based on the t test (Table 1). However, owing to a small number of patients enrolled in the IPF cohort, this finding remains to be verified. Multivariable logistic regression analyses adjusting for 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, other three investigational biomarkers showed no obvious abnormalities (all within the normal range).

According to the stratification of IPAF’s severity, 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 versus Improvement AUC, the AUCs ranged from 0.537 to 0.710 with a combined AUC of 0.583; in Stability AUC versus Exacerbation AUC, the AUCs ranged from 0.379 to 0.600 with a combined AUC of 0.867 (Figure 4).

Interestingly, the correlation between MMPs and LDH showed a good effect based on previous studies; thus in our study, we performed a relevant analysis in order to explore the potential efficiency and found that MMP-2 and MMP-9 lead the above relevance whatever in all patients with IPAF or each stratification of IPAF’s severity (Figure 5).

### 3.5 Combinatorial signature

A combination of clinical risk factors and MMPs is strongly associated with all IPAF severity. 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 the IPAF cohort (p < 0.05 for the difference between the curves) (Table 3, Figure 4).
TABLE 2  Baseline characteristics of IPAF subjects stratified by severity of IPAF.

| Variable                | IPAF Cohort | Stability (n = 14 [45.2%]) | Improvement (n = 9 [29.0%]) | Exacerbation (n = 8 [25.8%]) | p Value |
|-------------------------|-------------|-----------------------------|------------------------------|-------------------------------|---------|
| Demographics            |             |                             |                              |                               |         |
| Age, year               |             | 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 ± 1489                 | 9354 ± 1833                  | 8165 ± 5620                   | 0.165   |
| MMP-2                   |             | 147.037 ± 8712              | 169.612 ± 15.310             | 150.948 ± 19.505              | 0.400   |
| MMP-3                   |             | 21.378 ± 5264               | 30.362 ± 9814                | 32.063 ± 8502                 | 0.607   |
| MMP-7                   |             | 32.570 ± 6332               | 36.082 ± 4430                | 26.097 ± 8626                 | 0.389   |
| MMP-9                   |             | 105.518 ± 17.755            | 152.086 ± 28.858             | 144.759 ± 40.580              | 0.532   |
| MMP-10                  |             | 669.63 ± 97.15              | 588.37 ± 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)             |             | 1940 ± 599.02               | 1971 ± 623.62                | 1526 ± 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   |

Note: Data are presented as the Mean ± SD or number (%).
Abbreviations: BMI, body mass index; CRP, c-reactive protein; DLCO, diffuse lung carbon monoxide; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; IPAF, interstitial pneumonia with autoimmune features; IPF, idiopathic pulmonary fibrosis; KL-6, krebs von den lungen-6; LDH, lactate dehydrogenase; MMPs, matrix metalloproteinases; SP-A, surfactant protein A; tIgE, total immunoglobulin E.

3.6  |  Pulmonary function tests (PFTs) and Blood cell test

Whether comparison between IPAF and IPF or simply a contrast among the three stratifications of IPAF’s severity (p<0.05), apart from DLCO, which identified 36 patients with moderate interstitial lung diseases, the remaining PFTs including FVC and FEV1 showed no obvious clinical significance in two cohorts (Figure 6). No abnormality was found in the selected cytokines in the blood cell test in the IPAF and IPF cohorts.
FIGURE 2 Serum levels for each severity stratification of IPAF were in the selected MMPs. IPAF, interstitial pneumonia with autoimmune features.
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, IPAF and IPF differences and prediction of stratification of IPAF’s severity by investigational biomarkers are still ongoing; meanwhile, the five investigational biomarkers, consisting of SP-A, KL-6, LDH, CRP, tIgE, rarely enhance the ability of individuals to identify independently and in combination with these variables. Furthermore, this combined signature of clinical risk factors, MMPs, and other investigational biomarkers was tested in two cohorts and in IPAF subjects with stability, improvement, and exacerbation, respectively.

Several studies have given eloquent proof that IPAF is associated with age, female sex, and smoking status.\(^\text{4,7,25-29}\) In addition, we also found that KL-6 can reflect the disease progression and play a key role in the display of the degree of lung epithelial cell injury and fibrosis in patients with IPAF. In spite of MMPs combined with clinical risk factors that could detect the presence of IPAF in this study, it should be noted that MMP-7 and MMP-12 were weak differences between IPAF and IPF cohorts.

Elevated levels of SP-A, KL-6 have previously been associated with disease progression and reduced survival in IPAF and IPF cohorts. Given that a high proportion of patients with IPAF have typical radiological or histological patterns of interstitial pneumonia similar to the IPF, we hypothesized that biomarkers in IPF that predict clinical outcomes are also associated with IPAF, as well as we found significantly elevated levels of SP-A and KL-6 in both IPAF and IPF cohorts. When analyzed in combination with risk factors, combinatorial biomarkers along with 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 and KL-6 can identify between IPAF and IPF. Notably, though SP-A and KL-6 are the leading biomarkers in previous studies about IPAF, the diagnostic repeatability in other patients needs further clarification. At the same time, other potentially predictable inflammatory indications in body fluid may play a more significant role in the diagnosis of IPAF and should be further investigated.

The high prevalence of females and smoking are at higher risk of IPAF and IPF,\(^\text{4,7,27}\) thus continued support for early detection and prevention and advocacy for smoking cessation for all patients with such pulmonary diseases are critical components to manage individuals at risk of IPAF and IPF. 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 IPAF’s severity.

| Variable | IPAF Stability AUC versus Improvement AUC | IPAF Stability AUC versus 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                                     |
| tlgE     | 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: CRP, c-reactive protein; IPAF, interstitial pneumonia with autoimmune features; KL-6, krebs von den lungen-6; LDH, lactate dehydrogenase; MMPs, matrix metalloproteinases; SP-A, surfactant protein A; tIgE, total immunoglobulin E.
FIGURE 3  Area under the curve (AUC) for Stability-Improvement and Stability-Exacerbation comparison of MMPs and combination biomarkers in the IPAF cohort. IPAF, interstitial pneumonia with autoimmune features; MMPs, matrix metalloproteinases.
The high incidence of Exacerbation-IPAF and adverse clinical outcomes in Table 2 highlight the urgency of effective risk stratification for patients with IPAF, while for those at risk for disease progression, thanks to a large number of disease modifiers and biologics available and novel anti-fibrotic therapies, early detection of Exacerbation-IPAF may lead to meaningful changes in clinical outcomes. Despite we did not value the association of molecular characteristics with disease progression, we have identified meaningful investigational biomarkers selected that are strongly associated with IPAF, particularly in patients with Improvement-IPAF, MMP-1, MMP-2, MMP-9, MMP-12 have been shown to predict disease progression and survival. In addition, resembling our findings in IPAF, levels of MMP-1, MMP-3, MMP-7-MMP-12, and MMP-13 seem to strengthen 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 yield 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 IPF progression and thereby have a potentially positive impact on the severity stratification of patients with IPF in the future.

In brief, we found that MMP-12 had a significant association with Improvement-IPAF, while MMP-13 predicted a specific association with Exacerbation-IPAF, yet more evidence in this finding has yet to be confirmed. MMP-13 is a crucial interstitial collagenase, important in bone remodeling and liver injury. However, the correlation 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. Corry, D.B., et al.30 clearly reveal that in a model of atopic lung disease, both MMP-2 and MMP-9 affected the inflammatory response of lung disease; however, no obvious outcome was found in this study. A report on the enzymological properties of MMPs by McQuibban, G.A., et al.31 additionally showed that MMPs not only acted on extracellular matrix elements but 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.30 Consequently, MMP-13 may really get IPAF to progress into Exacerbation-IPAF in patients with IPAF.

Surprisingly, Satish K Madala et al.32 found that MMP13 activity increased when MMP-12 was absent. IL-13-mediated increased expression of dependent MMP-13 in MMP-12 deficient mice in his experimental model also suggests that MMP-12 may play an essential counter-regulatory role in regulating IL-13-dependent tissue fibrosis, and MMP-12 seems to play its part in reducing fibrosis by modulating the activity of other MMP-13. 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 has been described in several live models, to some extent, providing some evidence for our recent findings.

Recently, MMPs have an interesting correlation with LDH chasing our eyes in some studies, also shown in this study, MMP-2 and MMP-9 get a stronger positive correlation with LDH compared to the other. For instance, Bronckers IM et al.33 demonstrate that serum MMP-2 and MMP-9 levels were positively correlated with LDH in myositis patients with interstitial lung diseases. Most importantly, Memedovski Z et al.34 even prove that MMP-2 and MMP-9 may be negatively correlated with LDH. Taken together, the debate between MMP-2, MMP-9, and LDH did not end soon, and we believe more evidence will be found in no time.

Some limitations were included in our study. First, there was a limited number of subjects in the IPF cohort, though 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 on 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 two cohorts and also in each stratification of IPAF’s severity. Finally, five investigational biomarkers (SP-A, KL-6, LDH, CRP, tlgE) have been tested in two cohorts respectively, noteworthy, inherent limitations such as efficacy in all experimental biomarkers, especially reproducibility and generalization. There is no doubt that more investigations are needed to progress to further clarify the correlation between the combination of characteristics and meaningful clinical outcomes. We are looking forward to 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. The latest proven subject is periostin, which is in fashion in interstitial lung diseases, particularly in idiopathic pulmonary fibrosis, and we have finished further exploration immediately. Fortunately, periostin is very much in line with current research, as IPAF is also a common typing in ILD. Given the strong diagnostic efficacy of IPF, we will try to measure the potential

**FIGURE 6** Analysis of the difference of various indicators at onset in IPAF and IPF cohorts. IPAF, interstitial pneumonia with autoimmune features; IPF, idiopathic pulmonary fibrosis.
efficacy of periostin in patients with IPAF for the next step. We hold a belief that any query about the limitations of this realm would be clarified in future studies.

5 | 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, TlGE, to some extent, identify the existence of Improvement and Exacerbation in the IPAF cohort. Unfortunately, further studies on the IPF cohort 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.

AUTHOR CONTRIBUTIONS

Mingshan Xue, Runpei Lin: acquisition of data. Mingtao Liu, 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.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data are contained within the article or supplementary material.

CONSENT TO PARTICIPATE

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.

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