

FDA-Approved Antitussive Dextromethorphan Enhances Antifibrotic Effectiveness of Pirfenidone In Bleomycin-Induced Mice and Patients With Idiopathic Pulmonary Fibrosis

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Research

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**FDA-approved Antitussive Dextromethorphan Enhances Antifibrotic
Effectiveness of Pirfenidone in Bleomycin-induced Mice and Patients
with Idiopathic Pulmonary Fibrosis**

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Background: We aimed to investigate whether dextromethorphan (DM), an antitussive, can improve the antifibrotic efficacy of pirfenidone in treating idiopathic pulmonary fibrosis, a fatal interstitial lung disease characterized by progressive and irreversible respiratory failure.

Methods: A bleomycin-induced mice pulmonary fibrosis study and an open label randomized clinical trial were performed to evaluate the effectiveness of pirfenidone combined with DM.

Results: In the animal study, pirfenidone combined with DM protected mice against bleomycin-induced pulmonary fibrosis with better capabilities than pirfenidone alone or DM alone indicated by lung histologic analysis and hydroxyproline levels. In the clinical study, pirfenidone plus DM markedly mitigated pulmonary functions (FEV1 and FVC) decline and ameliorated chest HRCT imaging scores (ground glass opacities and reticulation) of patients with IPF than pirfenidone alone at one year after administration. There were no significant differences in adverse reactions between pirfenidone-DM group and pirfenidone group.

Conclusions: DM significantly potentiates antifibrotic effectiveness of pirfenidone in a mouse IPF model and patients with IPF and does not increase side effects of pirfenidone.

Keywords: Idiopathic pulmonary fibrosis, Pirfenidone, Dextromethorphan, Bleomycin, Pulmonary function testing, High resolution computed tomography, HRCT

1. Background

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, and fatal interstitial lung disease (ILD) of unknown etiology. It is characterized by the destruction of alveolar structure and abnormal deposition of extracellular matrix in the alveolar cavity and lung interstitium. Patients with IPF develop chest tightness, shortness of breath, and ultimate respiratory failure. The median survival time after diagnosis of

IPF is only 2-3 years [1]. At present, the disease progression cannot be completely reversed unless lung transplantation [2].

Many factors-related to inflammation, such as interferon- γ (IFN- γ), interleukin (IL)-4, transforming growth factor- β (TGF- β), α -SMA, and oxidative stress, have been found involved the pathogenesis of IPF, leading to the abnormal proliferation of fibroblasts and myofibroblasts and the aberrant deposition of extracellular matrix in the lung [3]. Decreased IFN- γ and increased IL-4 promote fibroblast proliferation and collagen deposition [4]. TGF- β initiates epithelial-mesenchymal transition (EMT) of alveolar epithelial cells via enhancing the production of mitochondrial reactive oxidative species (ROS) [5]. Myofibroblasts expressing α -SMA cannot apoptosis normally. Oxidative stress can damage epithelial cells, upregulate the expression of a variety of profibrotic mediators and cytokines, and make lung cathepsins and anti-proteases imbalance, eventually resulting in a large amount of extracellular matrix deposition and the formation of pulmonary fibrosis. Therefore, ROS-generating NADPH oxidases (NOX), including Nox1, Nox2, and Nox4, play critical roles in the pathogenesis of IPF. For example, the genetic ablation of p47phox subunit of Nox2 protected mice against bleomycin-induced lung fibrosis [6].

Pirfenidone, a TGF- β synthesis inhibitor with anti-inflammatory, antioxidant and antifibrotic effects, was approved at 2014 by the Food and Drug Administration (FDA) to treat IPF [7]. It reduces the accumulation of hydroxyproline, the expression of smooth actin (α -SMA), collagen deposition, and fibroblast proliferation by inhibiting the TGF- β 1/Smad and PI3K/Akt signaling pathways. The 2015 ATS/ERS/JRS/ALAT guidelines conditionally recommend pirfenidone for the treatment of patients with IPF [8]. Although pirfenidone improves patients' symptoms such as dyspnea, it only moderately slows down pulmonary functional decline and disease progression [9]. How to improve the therapeutic effect of IPF still faces a formidable challenge.

Dextromethorphan (DM), a dextrorotatory enantiomer of the opioid agonist levorphanol, is a widely used over-the-counter antitussive drug approved by FDA [10]. In recent years, the inflammation-repression effects of DM have been gradually

recognized and discovered. DM reduces serum levels of proinflammatory mediator tumor necrosis factor alpha (TNF- α), IL-6, IL-1 chemokine monocyte chemotactic protein 1 (MCP-1), and macrophage inflammatory protein 2 (MIP-2) in mice or patients [11-13]. Low dose DM inhibits the expression and activity of microglial NOX2 [14]. The anti-inflammatory and antioxidant properties of DM imply that it could be applied to promote anti-fibrotic capacity of pirfenidone for IPF.

Here, we found that pirfenidone combined with DM showed the best potency in reducing fibrotic pathology compared with pirfenidone alone or DM alone in bleomycin (BLM)-induced pulmonary fibrosis mouse model. A following open label clinical trial showed that pirfenidone plus low dose DM significantly mitigated pulmonary function decline and ameliorated pulmonary imaging scores of patients with IPF than pirfenidone alone. In addition, the DM add-on therapy did not increase side effects of pirfenidone.

2. Methods

2.1. Preclinical study

2.1.1. Mouse model

C57BL/6J mice (Vital River Laboratory Animal Technology Co., Ltd, BeiJing, China) were obtained (male, 8~9 weeks old, average weight 23~25 g). They were hosted and bred in a specific pathogen free environment with access to food, water, and libitum.

2.1.2. Lung fibrosis model and drug treatment

Mice were divided into 5 groups of 8 animals each: control group, bleomycin group (BLM+NaCl), bleomycin+DM group (BLM+DM), bleomycin+pirfenidone group (BLM+PFD), and bleomycin+pirfenidone+DM group (BLM+PFD+DM). 1.5U (1.5mg/kg) bleomycin (Nippon Kayaku Co., Tokyo, Japan) was intratracheally administered to mice except the control group at day 0. Mice in different groups were pretreated with vehicle, pirfenidone alone, DM alone, or pirfenidone plus DM for one

day, and continuously administered to day 20. Pirfenidone (Beijing Kangdini Pharmaceutical Co., Ltd., China) was intragastrically administered once a day (100 mg/kg body weight). DM (Selleck biotechnology co., ltd, USA) was subcutaneously injected three times a day (10 ng/kg body weight). The control group and the bleomycin group were given the same volume of saline every day. The mice were sacrificed on D21 and lungs were taken out to prepare for histological staining and hydroxyproline assay.

2.1.3. Histologic analysis

Mice left lungs were dehydrated, paraffin embedded, and cut into 5- μ m sections, then stained with H&E and Masson Trichrome staining. The degree of inflammation and fibrosis of lung tissue was observed under an optical microscope. Pulmonary fibrosis area was quantified[15]. Slides were sampled with a random-start systematic sampling scheme using a 6-mm-grid randomly superimposed on the slide to indicate areas to evaluate. Digitized images of the slide were obtained using a $\times 2$ objective on an Olympus BH-2 microscope (Olympus America, Melville, New York, USA) with a Sony DXC970MD camera (Sony America, New York, New York, USA) and an IxTV capture card (IxMicro, San Jose, California, USA) on an Apple Macintosh G3 computer (Apple Computer Inc., Cupertino, California, USA). Images were then opened in Photoshop Elements (Adobe Systems Inc., San Jose, California, USA). The overall area of the lung was obtained by manual outlining. The area of the lung with fibrosis was then outlined and the area obtained. The pixels of total versus fibrotic tissue were then summed over each lung and a percentage was obtained.

2.1.4 Hydroxyproline assay

Collagen contents were evaluated with conventional hydroxyproline method in mice right lungs which had been cleared of blood [16]. Samples containing known amounts of purified collagen confirmed the capability of the assay to fully hydrolyze and recover hydroxyproline from collagen.

2.2. Human study

2.2.1. Patients

This study was a multicenter, open label, randomized study conducted by the Tianjin Medical University General Hospital, Tianjin Academy of Traditional Chinese Medicine Affiliated Hospital, Tianjin People's Hospital, and Tianjin Beichen Hospital all participated. This study was approved by the Ethics Committee of Tianjin Medical University General Hospital, China (No. IRB2020-YX-031-01). Informed consent was obtained for all study participants. All consecutive patients who had started pirfenidone treatment between May 2019 and December 2020 were candidates for our study. Patients were selected based on the following criteria: (1) The diagnosis of IPF was made in accordance with the 2018 ATS/ERS/JRS/ALAT guidelines [17] and patient's chest HRCT conforms to UIP pattern and possibly UIP pattern. (2) Glucocorticoids or immunosuppressants were not administered within 1 month. All patients took pirfenidone capsules (Beijing Kangdini Pharmaceutical Co., Ltd., 100mg/capsule, H20133376). An initial dose of 200 mg of pirfenidone was administered three times daily with meals (600 mg/day). Thereafter, the dose was gradually increased by 200 mg every 2 weeks to a maximum of 600 mg per dose (1800 mg/day). Dextromethorphan (DM) hydrobromide tablets (Shijiazhuang Yiling Pharmaceutical Co., Ltd., 15 mg/tablet, H20066348) were added with 7.5-15 mg once a day in pirfenidone-DM group. A total of 16 consecutive eligible patients were enrolled with 8 in pirfenidone group and 8 in pirfenidone-DM group.

2.2.2. Laboratory tests and pulmonary function testing

Blood routine test, C-reactive protein (CRP), liver and kidney function, and pulmonary function testing (PFT) of patients were collected before treatment, 6 months, and 1 year. Pulmonary function tests (PFT) were performed by standard techniques using CHESTAC-33 (Chest MI Co., Tokyo, Japan) and Fudac-77 (Fukuda Denshi, Tokyo). Pulmonary function test indexes included forced vital capacity (FVC), forced vital capacity percent (FVC%) predicted, forced expiratory volume in one second (FEV1), forced expiratory volume in one second percent (FEV1%)

predicted and diffusing capacity of the lung for carbon monoxide percent (DLCO%) predicted.

2.2.3. CT images

A high resolution CT scan (Lightspeed-64, GE, America) was used and CT images with 0.5 mm (or 1 mm) slice thickness were obtained (140 kVp and 200 mA). Each recorded extent of normal lung and each lesion (ground glass opacities (GGO), consolidation, reticular abnormality, honeycombing and emphysema) by 5% steps in three zones in each lung based on the method described by Best [18]. The upper zone, middle zone, and lower zone was defined as at or above the aortic arch, between the aortic arch and pulmonary veins, and at or below the pulmonary veins, respectively. The mean extent of three zones was calculated. HRCT scans were scored by two expert thoracic radiologists using the above method that assesses the degree of ground glass attenuation (HRCT alveolar score) and fibrotic change (HRCT interstitial score and Honeycomb score) [19]. Radiologists were blinded to the patient's diagnosis and grouping. Agreement between the radiologists was very good.

2.3. Statistical analysis

Statistical analyses were performed using GraphPad Prism 7 (GraphPad Software, La Jolla, California, USA). Multiple comparisons were performed using ANOVA tests with *post hoc* analysis. To compare demographic data and baseline clinical characteristics between pirfenidone group and pirfenidone-DM group, a Chi square test and Fisher's exact test for categorical variables and a Mann-Whitney U test for continuous variables were used as appropriate. The level of interobserver agreement between the two radiologists was evaluated by kappa statistic measure. *P* values < 0.05 were considered to indicate statistical significance.

3. Results

3.1. Pirfenidone combined with DM protects mice against bleomycin-induced pulmonary fibrosis

At 21 days after challenge, intratracheal injection of bleomycin resulted in the destruction of normal lung structures, markedly thickened alveolar septum, and mass filling of fibrous tissue indicated by H&E staining of mouse lung sections (Fig. 1A). The deposition of collagen fibers was largely increased in bleomycin-induced lung injury as shown by Masson's trichrome staining (Fig. 1A). Decreased alveolar damage and collagen fiber deposition were displayed in mice lung sections by administration of pirfenidone, DM, or pirfenidone combined with DM (Fig. 1A). The fibrosis focal area ratio by quantitative analysis in the four groups (BLM+NaCl, BLM+DM, BLM+PFD, and BLM+PFD+DM) was 0.097 ± 0.019 , 0.058 ± 0.047 , 0.056 ± 0.043 , and 0.031 ± 0.034 (mean \pm SD), respectively. Among these four groups, pirfenidone combined with DM significantly reduced the fibrosis focal area ratio caused by bleomycin (Fig. 1B).

Hydroxyproline (HPO), a non-essential amino acid found in collagen, plays a crucial role in collagen synthesis and serves as a biochemical marker of lung fibrosis. At day 21 after exposure to bleomycin or vehicle, lung hydroxyproline contents in the five groups (control, BLM+NaCl, BLM+DM, BLM+PFD, and BLM+PFD+DM) were 209.2 ± 23.5 , 400.1 ± 63.6 , 346.3 ± 59.3 , 317.7 ± 30.1 , and 302.7 ± 49.3 $\mu\text{g}/\text{lung}$ (mean \pm SD), respectively. The levels of lung hydroxyproline in BLM+DM group and BLM+PFD+DM group were significantly lower compared with bleomycin group (Fig. 1C). Together, the fibrosis focal area ratio quantification and hydroxyproline assay clearly indicated the potency of pirfenidone plus DM in alleviating bleomycin-elicited pulmonary fibrotic pathology.

3.2. Patient characteristics

Based on the findings in the mice experiments, we further studied whether DM could potentiate the treatment effectiveness of pirfenidone in patients with IPF. We enrolled 20 patients but 4 patients (2 from pirfenidone group and 2 from pirfenidone-DM group) died of infections. Therefore, 16 patients were statistically analyzed with PFTs and HRCT scores. There were no significant differences in gender, age, age at diagnosis, clinical-radiological diagnosis, smoking years, prior

treatment received steroids, and IPF treatment at baseline (Table 1). Three patients (50.00%) in the pirfenidone group and two patients (37.50%) in the pirfenidone-DM group showed the usual interstitial pneumonia (UIP) pattern on HRCT, but there was no statistical difference between the two groups (Table 1).

3.3. Pirfenidone combined with DM mitigates pulmonary function decline for IPF patients

Pulmonary function tests (PFTs) were performed before medication, 6 months and 1 year. Before antifibrotic treatment and 6 months later, there were no significant differences in the performances of PFTs between pirfenidone group and pirfenidone-DM group (Table 2). Some PFT indexes of the two groups showed an overall downward trend, and DLCO%pred was not different between the two groups 1 year later (Fig. 2E). However, pulmonary function tests including FVC, FVC%pred, FEV1, and FEV1%pred in pirfenidone-DM group were statistically better at 1 year after treatment than those in pirfenidone group ($P<0.05$) (Fig. 2A-D). Therefore, pirfenidone combined with DM significantly mitigates pulmonary function decline compared with pirfenidone alone in patients with IPF.

3.4. Pirfenidone combined with DM ameliorated pulmonary imaging scores of patients with IPF

The inter-observer agreement between two radiologists with regard to changes in alveolar score, reticulation, and honeycombing was good (Cohen's kappa = 0.74 for alveolar score, $k = 0.78$ for reticulation, $k = 0.72$ for honeycombing). There was no significant difference in HRCT alveolar score, interstitial score and honeycomb score between the two groups of patients before treatment (Table 3). After 1 year of antifibrotic treatment, HRCT displayed that the alveolar score and interstitial score of patients in pirfenidone-DM group were markedly improved comparing to pirfenidone group ($P=0.035$, $P=0.037$, respectively) (Table3, Fig. 3 A, B), although honeycomb scores remained similarly between the two groups ($P=0.999$) (Table3, Fig. 3C). Thus, pirfenidone combined with DM significantly ameliorated pulmonary imaging scores

of patients with IPF. A patient's chest HRCT showed evidently alleviated ground glass opacities and reticulation upon 1 year administration of pirfenidone plus DM (Fig. 4).

3.5. DM did not increase adverse reactions of pirfenidone

2 patients (1 from pirfenidone group and 1 from pirfenidone-DM group) had gastrointestinal reactions, and 1 patient from pirfenidone group felt slight dizziness. There was no between-group difference in the risk of adverse reactions (t-test, $P>0.5$). The liver and kidney function and blood routine test of patients showed no statistical differences between two groups and no significant changes were observed before and after medication ($P>0.5$).

4. Discussion

The recommendations for pharmacotherapy in IPF patients was in clinical practice guidelines 2015, and pirfenidone were recommended conditionally for treatment as antifibrotic therapy [8] for it can only moderately slowed down the decline of lung function in patients with IPF. Since then, little clinical progress has been made. However, in the current study, DM significantly potentiates the antifibrotic efficacy of pirfenidone not only in mice induced by bleomycin but also in patients with IPF.

Someone propose that the core pathway that mediates fibrosis may be a better target to develop drugs for anti-fibrosis in multiple organ systems drug [21] and address that age-related redox imbalances has been recognized as one of these core approaches [22]. DM, as a broad and safe antitussive drug, has been found to have obvious anti-inflammatory and antioxidative effects in recent years. In order to explore the anti-fibrotic effect of the combination of pirfenidone and DM on IPF, we conducted this study and found that the combination of the two can significantly improve pulmonary fibrosis. In animal studies, compared with other groups, pirfenidone combined with DM can improve bleomycin-induced pulmonary fibrosis indicated by decreased proliferation of alveolar epithelium, production of fibroblasts, and levels of hydroxyproline. In clinical studies, we have observed that pirfenidone

combined with DM can improve the PFT of patients, delay the reduction of FEV1 and FVC, and mitigate the ground glass opacities and reticulation of HRCT.

During the development of IPF, oxidative stress refers to the imbalance between the production and removal of oxygen free radicals in the body or cells, which leads to an increase in reactive oxygen species (ROS). ROS regulates cell growth, proliferation and death by stimulating related genes, and promote the release of various inflammatory mediators and cytokines, such as transforming growth factor- β (TGF- β), etc. Then ROS promotes pulmonary fibrosis. TGF- β increases the production of mitochondrial ROS in different cells, which in turn will promote the apoptosis[23], epithelial-mesenchymal transition(EMT)[5], fibrosis gene expression and fibroblast differentiation [24]. Although the exact role of inflammation in fibrosis is still controversial, there is clear evidence that chronic inflammation also exists in IPF[25]. There are activated inflammatory cells in the lungs of IPF patients, such as alveolar macrophages and neutrophils[26]. These phagocytes may be involved in ROS-mediated epithelial cell damage. In addition, the lung tissues of IPF patients showed accumulation of activated non-proliferative T cells and B cells, with follicular dendritic cell aggregation[27, 28]. These inflammatory cells produce a large number of cytokines, such as TGF- β , TNF- α , IFN- γ , IL-4, IL-10, IL-12, IL-1, MCP and so on. They form a complex cytokine network, regulating the apoptosis of alveolar epithelial cells and the proliferation of mesenchymal fibroblasts, and ultimately facilitating the formation of lung fibrosis[4, 29].

In patients with IPF, after individual treatment and various immunotherapies, the epithelial cells and blood vessels in the lung are damaged, resulting in cell destruction, uncontrolled repair, and the development of progressive pulmonary fibrosis. Combining various hypotheses, when exposed to pathogenic factors, once fibrosis has formed, anti-fibrosis treatment may be initiated. Previously, progressive fibrosis was mostly classified as ordinary pulmonary fibrosis. Once fibrosis occurs, immunosuppression is no longer effective, and anti-fibrosis therapy is needed to

dampen the decline in lung function during pulmonary fibrosis. Pirfenidone is the world's first therapeutic drug to obtain indications for idiopathic pulmonary fibrosis.

Human lung tissue is exposed to higher concentrations of oxygen than other organs, therefore being more susceptible to damage from oxidative stress. When there is too much ROS production or insufficient antioxidant capacity in the body, excessive ROS exists in tissues or cells, which can induce oxidative stress and inevitably lead to lung tissue damage and remodeling[30]. NOX2 is universally expressed and stable in the lung, and its expression will increase under inflammatory conditions[31]. The evidence of NOX2 in IPF mainly comes from animal models, which show that mice with NOX2 genetic defects can reduce AEC damage induced by bleomycin or carbon nanotubes[32]. In addition to the role of NOX2 in inflammatory cells, studies have shown that the NOX2/ROS/NLRP3 inflammasome signaling pathway can promote the occurrence of IPF[33]. DM is derived from non-opioid morphinans. As a widely used and safe over-the-counter cough suppressant, it has a history of more than 50 years[34]. Structurally, it belongs to morphine drugs. In view of its right-handed structure, DM has a weak affinity with opioid receptors, and it is difficult to form addiction. DM can specifically bind to the phosphorylated form of the subunit gp91 in NOX2, thereby inhibiting the catalytic activity of NOX2, thereby inhibiting the production of ROS. In addition to repressing the production of superoxide, DM also reduces the production of NO, thereby reducing pulmonary fibrosis. Previous studies have confirmed that DM has the effect of antagonizing the inflammation in the nervous system and preventing neurodegeneration. Moreover, DM significantly lower the production of superoxide in neutrophils.

As a functional examination, PFTs mainly reflect the damage to lung ventilation and ventilation function caused by disease. Although FEV1 and FVC are affected by many factors such as alveolar elasticity and airway resistance during expiration, pirfenidone combined with DM in this study is better than pirfenidone treatment in delaying the decline of FEV1 and FVC in IPF patients. Pulmonary diffusion function depends on the gas diffusion area in the lung, the thickness of alveolar capillary

membrane and the blood flow in the lung. The deterioration of lung diffusion during IPF is aggravated by the excessive proliferation of fibroblasts and the continuous deposition of extracellular matrix in alveoli and lung interstitium accompanied by pulmonary vascular remodeling and occlusion. In terms of diffusion function, DLCO%pred after treatment was no statistical difference between the two groups before and after treatment, although pirfenidone plus DM appeared to slightly increase DLCO%pred than pirfenidone alone.

High-Resolution Computed Tomography (HRCT) plays a central role in diagnosing and staging the severity of IPF[17, 35]. HRCT can accurately predict the presence of UIP when features of honeycombing in a basilar, peripheral distribution are present[2,36]. The overall extent of lung fibrosis on CT (combination of reticulation and honeycombing) is a proxy of disease severity, as well as a strong independent predictor of mortality in patients with IPF [18]. In our study, pirfenidone combined with DM anti-fibrosis therapy slowed down the decline of FVC and FEV1 in patients with IPF compared with single-agent pirfenidone treatment. After 1 year of treatment, it has a significant therapeutic effect on pulmonary function testing, but We have also observed that it has no significant improvement in improving the HRCT of IPF patients' honeycomb. The DLCO% of IPF patients decreased, and the pathological changes on HRCT mainly showed reticulation and honeycombing, which is consistent with the good correlation between reticulation and honeycombing and DLCO%.

Adverse reactions of pirfenidone are common and mainly gastrointestinal manifestations, skin diseases and dizziness [37]. The combination of pirfenidone and DM was well-tolerated by patients in this study and DM did not increase side effects of pirfenidone. The add-on therapy containing DM has exerts potent effects in many clinical settings. NEW YORK, Dec. 08, 2020, Very recently, Axsome Therapeutics announced positive results from a phase II clinical trial of AXS-05 in patients with major depressive disorder. Receiving AXS-05 (45 mg dextromethorphan-105 mg bupropion) twice daily for up to 12 months, patients experienced rapid reduction of suicidal ideation and functional improvement with good safety.

Conclusions

DM significantly potentiates antifibrotic effectiveness of pirfenidone in a mouse IPF model and patients with IPF and does not increase side effects of pirfenidone. The efficacy and safety of the combination of pirfenidone and DM for patients with IPF warrants further verification by the double-blind randomized controlled trial (RCT).

Abbreviations

IPF: idiopathic pulmonary fibrosis; PFD: pirfenidone; DM: dextromethorphan; FEV1: forced expiratory volume in one second; FEV1%pred: forced expiratory volume in one second as percentage of predicted volume; FVC: forced vital capacity; FVC%pred: forced vital capacity as percentage second as percentage of predicted volume; DLCO: diffusing capacity of the lungs for carbon monoxide; ROS:reactive oxygen species

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Nana Liu and Jie Huang performed research, collected data and wrote the manuscript.

Yubao Wang performed statistical analysis. Yunze Du collected data. Jau-Shyong Hong analyzed and interpreted data. Jing Feng and Wen Ning designed research and performed research. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Experiments in mice were approved by the local committee for animal welfare. For the human study, the protocol has been approved by the Ethics Committee of Tianjin Medical University General Hospital, China (No. IRB2020-YX-031-01). This study is registered for Chinese Clinical Trial Registry (Registration Number: ChiCTR1900023727). Written informed consent for participation in the study was obtained from all the patients.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Table 1. Patients demographics and clinical characteristics.

Characteristics	Pirfenidone (N =8)	Pirfenidone+DM (N =8)	<i>P</i> *
Female—n (%)	2 (25)	3 (38)	.590
>60—n (%)	5 (63)	6 (75)	.590
Age at diagnosis—years	65 (58-73)	66 (57-75)	.553
Clinical-radiological diagnosis—n (%)	5 (63)	6 (75)	.590
Smoking history—pack years	12 (1-23)	10 (1-19)	.400
UIP pattern on HRCT—n (%)	4 (50)	3 (38)	.614
Prior treatment received steroids—n (%)	1 (13)	2 (25)	.522
IPF treatment at baseline			
Oxygen therapy at baseline—n (%)	3 (38)	2 (25)	.590
Hypertension—n (%)	2 (25)	1 (13)	.522
Diabetes mellitus—n (%)	1 (13)	2 (25)	.522
GERD—n (%)	2 (25)	1 (13)	.522
Emphysema—n (%)	2 (25)	1 (13)	.522

Values are expressed as numbers and (%) or median and ranges as appropriate. To compare demographic data and baseline clinical characteristics between two groups, Chi square test for categorical variables.

Table 2. Changes pulmonary function tests (PFTs).

PFT	Pirfenidone (N =8)	Pirfenidone+DM (N =8)	<i>P</i> *
FVC —L			
at diagnosis	3.03 (2.60–3.59)	2.97 (2.40–3.65)	.999
6 months	2.42 (2.10–3.14)	2.78 (1.88–3.68)	.744
1 year	1.79 (1.55–2.18)	2.60 (1.68–3.49)	.038
FVC —% pred.			
at diagnosis	79.03 (56.40–90.30)	76.57 (59.60–93.30)	.999
6 months	70.73 (47.30–80.60)	75.25 (54.60–93.50)	.999
1 year	54.70 (41.30–61.50)	73.73 (53.50–91.20)	.044
FEV1 —L			
at diagnosis	2.34 (1.59-3.16)	2.36 (1.68-2.89)	.999
6 months	2.10 (1.46-2.66)	2.40 (1.73-3.02)	.952
1 year	1.61 (1.13-1.98)	2.39 (1.76-3.02)	.044
FEV1 —% pred.			
at diagnosis	83.15 (57.50–94.20)	86.15 (68.20–110.70)	.999
6 months	76.75 (53.90–89.30)	86.67 (57.80–116.30)	.906
1 year	64.28 (45.60–73.30)	88.28 (55.40–118.90)	.049
DLCO —% pred.			
at diagnosis	62.87 (42.60–78.40)	67.57 (47.50–75.80)	.999
6 months	55.43 (33.10–82.70)	61.70 (48.00–83.70)	.999
1 year	45.75 (20.10–77.60)	59.10 (40.50–8450)	.508

Values are expressed as median and ranges as appropriate. FVC, Forced Vital Capacity; FVC%pred, forced vital capacity percent predicted; FEV1, forced expiratory volume in one second; FEV1%pred, forced expiratory volume in one second percent predicted; DLCO%pred, diffusing capacity of the lung for carbon monoxide percent predicted.

Table 3. Changes in CT characteristics.

CT	Pirfenidone (N =8)	Pirfenidone+DM (N =8)	<i>P</i> *
Alveolar score—% (GGO)	31.67 (13.33-53.33)	33.33 (6.67-66.67)	.965
Alveolar score—% 1 year	37.50 (20.00-53.33)	20.00 (0.00-26.67)	.035
Interstitial score—% (reticulation)	40.83 (33.33-53.33)	55.0 (33.33-86.67)	.124
Interstitial score—% 1 year	56.67 (40.00-66.67)	38.33 (13.33-53.33)	.037
Honeycomb score—% (honeycombing)	32.50 (20.00-46.67)	30.84 (13.33-46.67)	.976
Honeycomb score—% 1 year	29.17 (6.67-60.00)	28.33 (0.00-60.00)	.999

Values are expressed as numbers and (%) or median and ranges as appropriate.

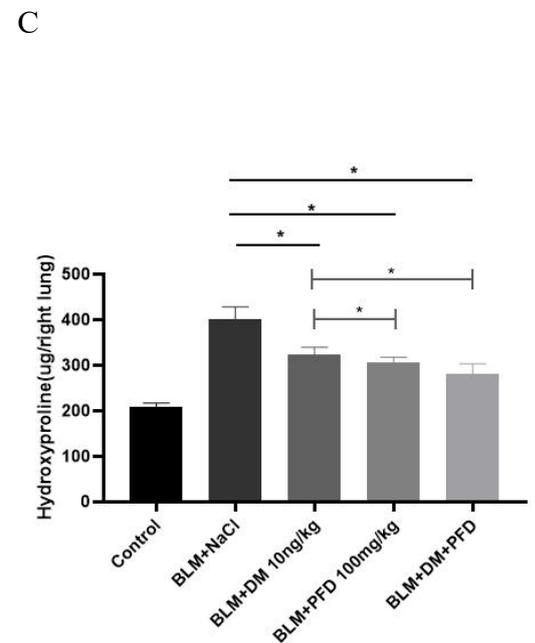
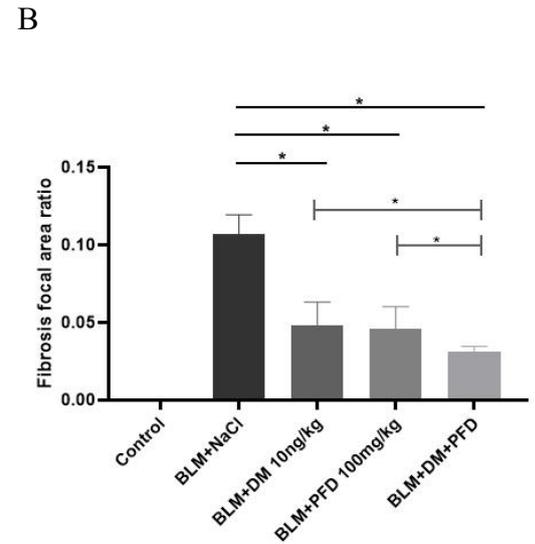
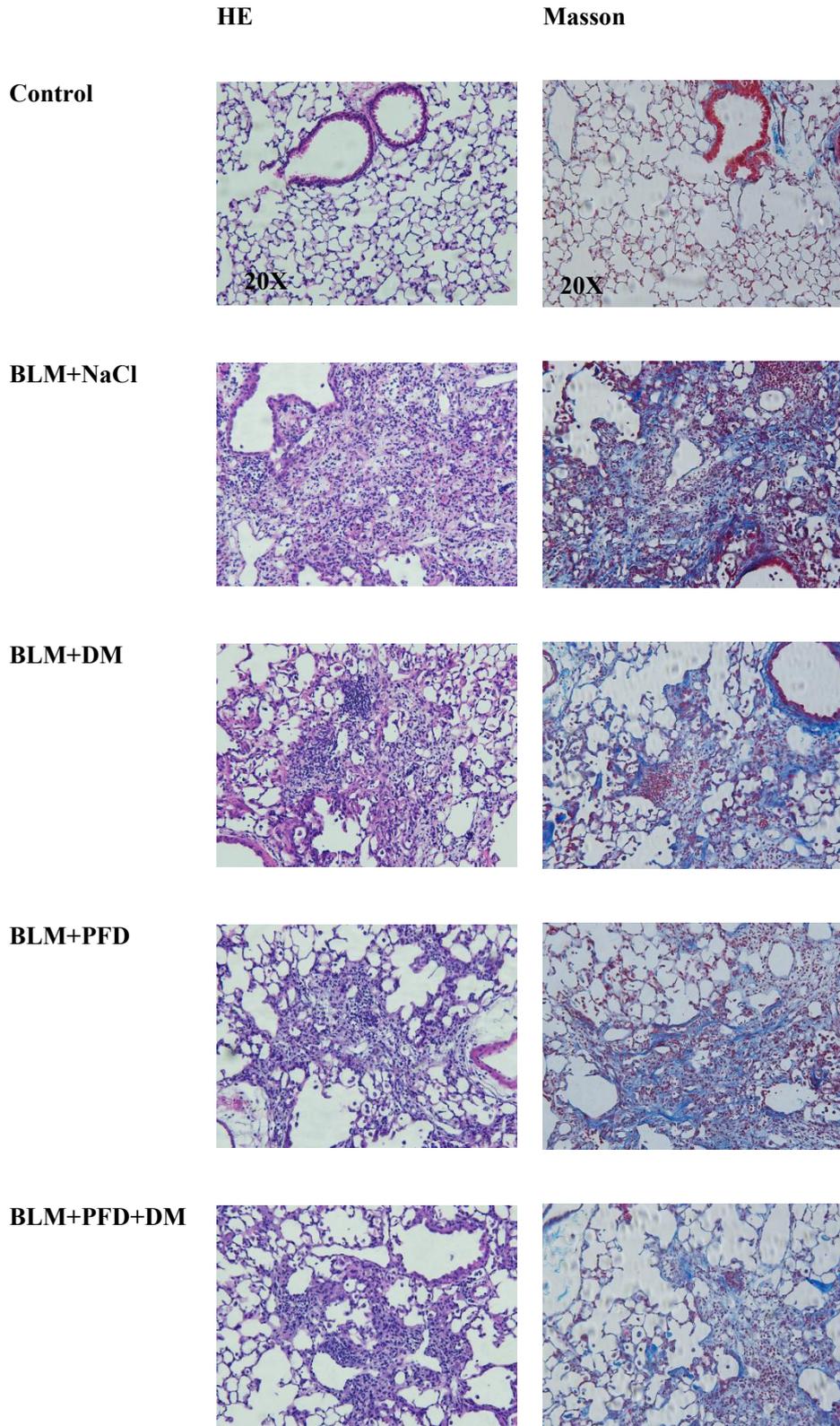
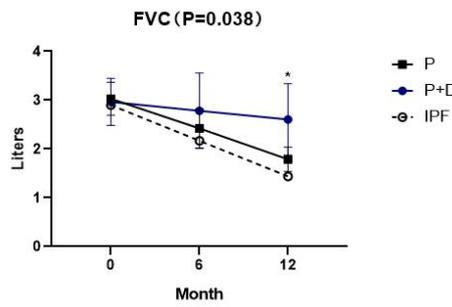


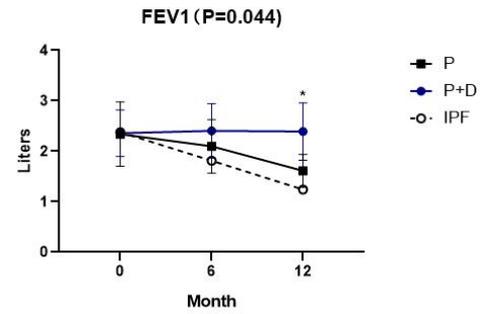
Figure 1. Pirfenidone combined with DM reduce bleomycin-induced pulmonary fibrosis in mice at D21. (A) As indicated by H&E staining of lung sections, the intratracheal injection of bleomycin led to the destruction of normal pulmonary architecture, the prominently thickening of alveolar septum and the mass filling of fibrous tissue. As illustrated by Masson's trichrome staining, the deposition of collagen fibers was largely increased in

bleomycin-induced lung injury. Pirfenidone combined with DM alleviated pulmonary pathological changes and reduced the production of collagen. Pirfenidone plus DM significantly decreased fibrosis focal area ratio (B) and hydroxyproline measurement in the lungs (C). Data are the mean \pm SD. Statistical analyses were performed by an ANOVA one-way test with Tukey's test for comparisons between groups. *, $P < 0.05$.

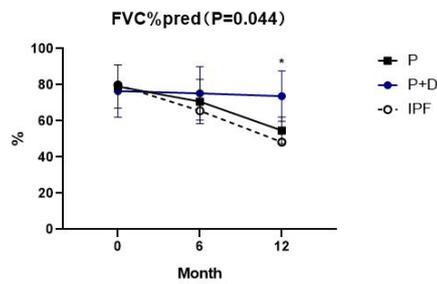
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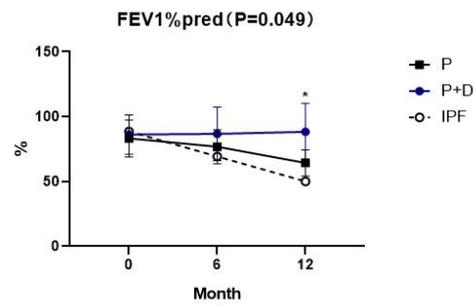
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D



E

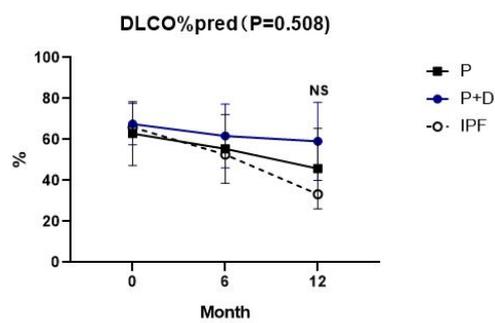


Figure 2. PFT indexes of two groups of patients. Except DLCO%pred, the comparison of FVC, FEV1, FVC%pred, FEV1%pred showed statistical differences between the two groups ($P_{FVC}=0.038$, $P_{FEV1}=0.044$, $P_{FVC\%pred}=0.044$, and $P_{FEV1\%pred}=0.049$). Reference baseline for lung function in IPF patients comes from other studies[20]. * $p<0.05$ and NS (no significance) compared to pirfenidone group. Two-way ANOVA followed by Bonferroni *post hoc* multiple comparison test was performed.

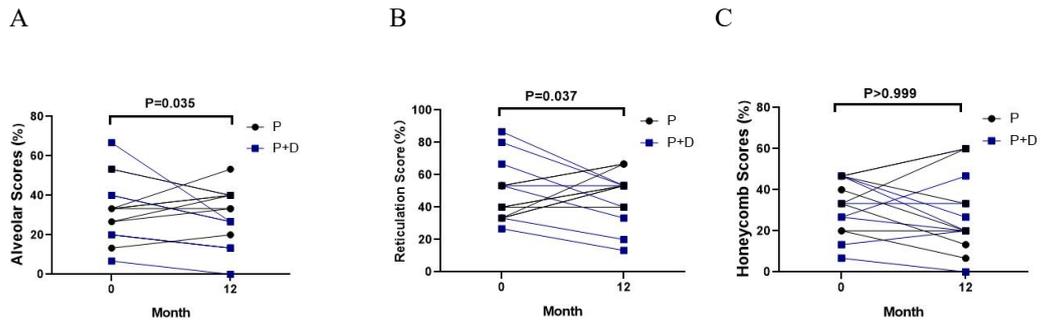


Figure 3. Alveolar score, interstitial score, and honeycomb score of patients treated with pirfenidone (P) or pirfenidone plus dextromethorphan (P+D). Comparison of Alveolar score, Interstitial score and Honeycomb score of HRCT between two groups of patients before and after treatment. Two-way ANOVA followed by Bonferroni *post hoc* multiple comparison test was performed.

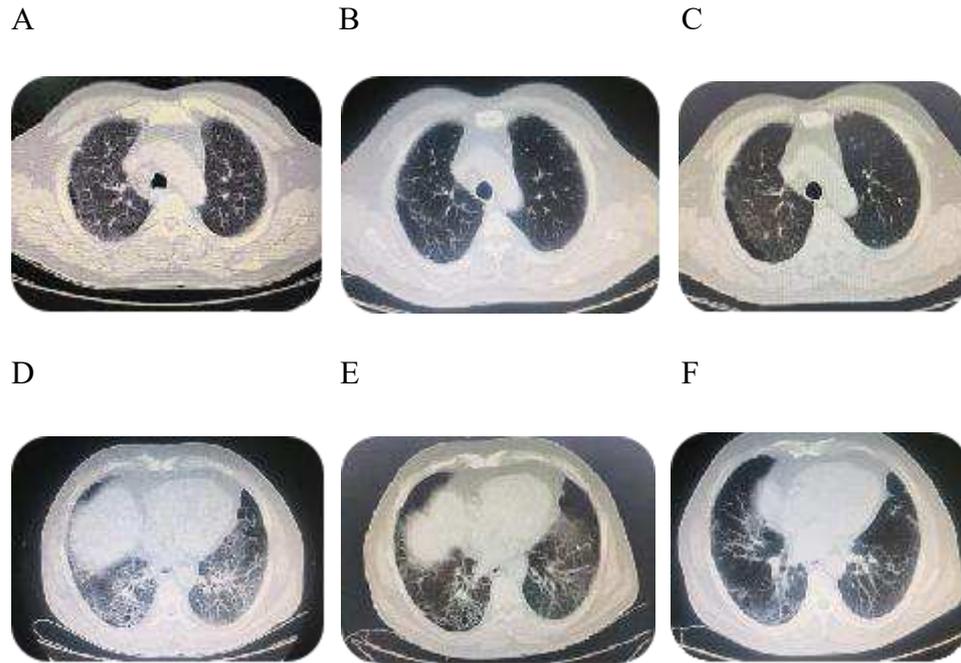


Figure 4. Comparisons of HRCT images of a patient received pirfenidone and DM before and after treatment. Male, 61y, with a history of pulmonary fibrosis for 4 years and a history of smoking. Initial HRCT (A and D), 6 months later HRCT(B and E), and one year later HRCT (C and F) displayed apparently less ground glass opacities, reticulation and honeycombing after treatment.

Figures

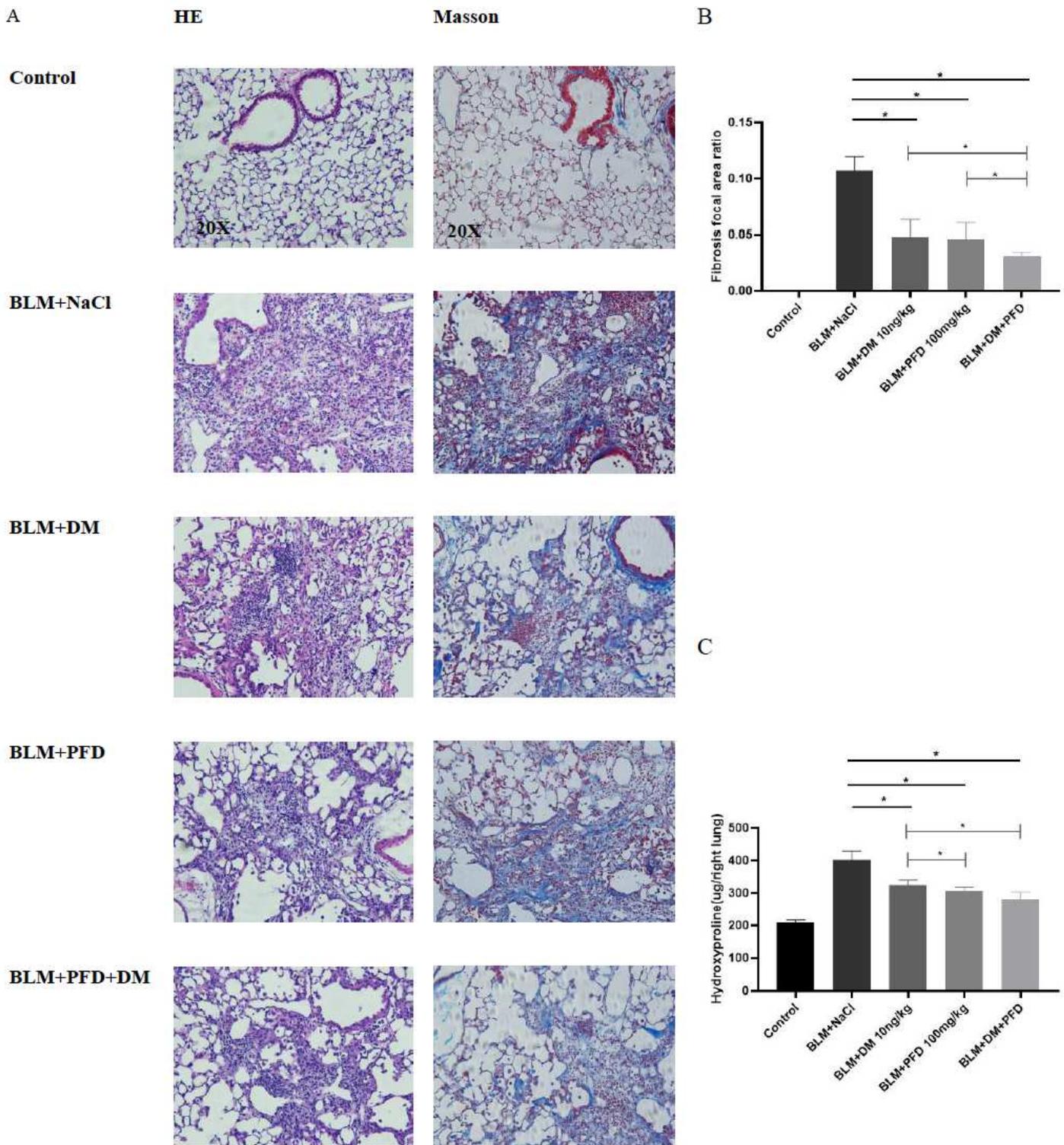
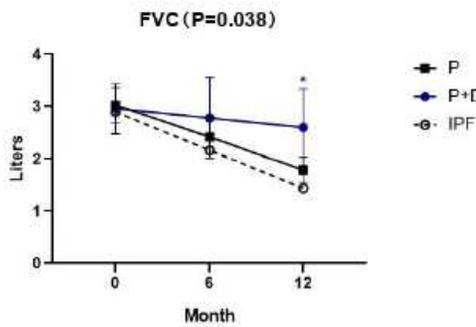


Figure 1

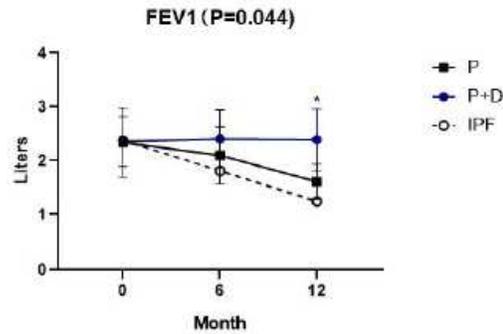
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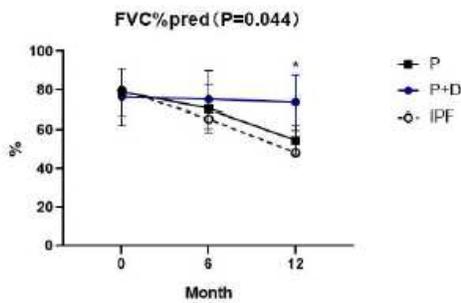
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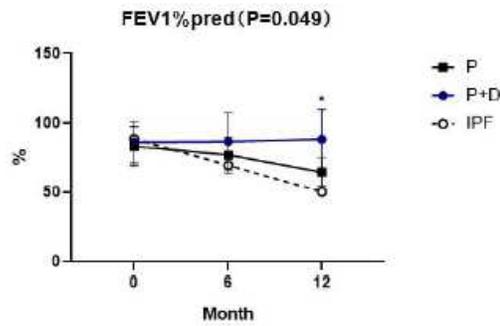
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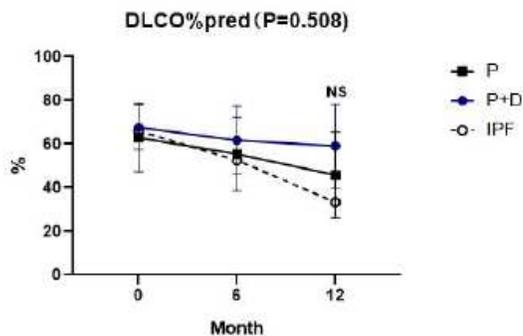


Figure 2

PFT indexes of two groups of patients. Except DLCO%pred, the comparison of FVC, FEV1, FVC%pred, FEV1%pred showed statistical differences between the two groups (PFVC=0.038, PFEV1=0.044, PFVC%pred=0.044, and PFEV1%pred=0.049). Reference baseline for lung function in IPF patients comes from other studies[20]. *p<0.05 and NS (no significance) compared to pirfenidone group. Two-way ANOVA followed by Bonferroni post hoc multiple comparison test was performed.

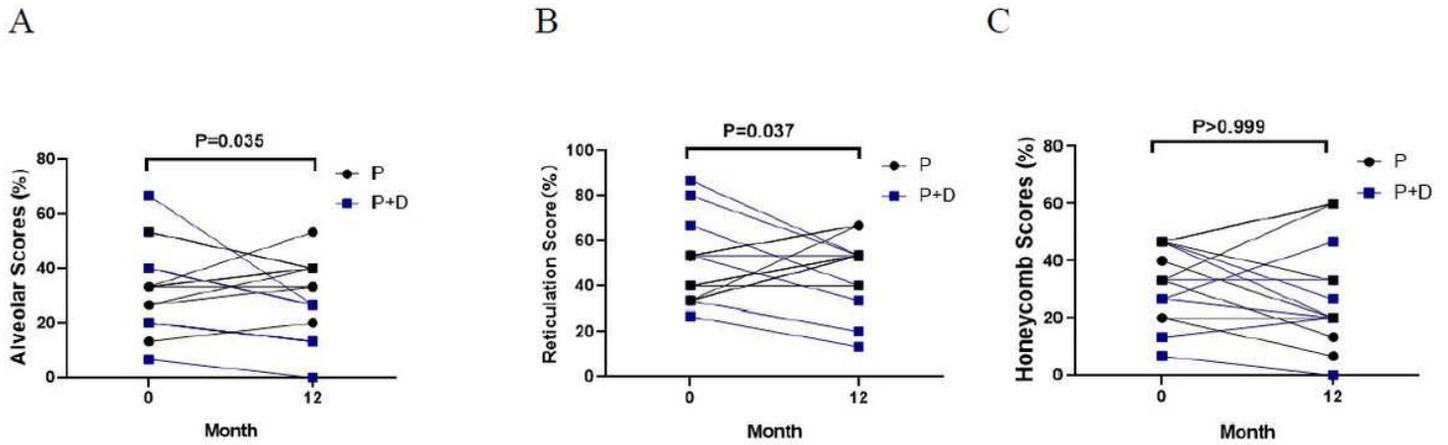


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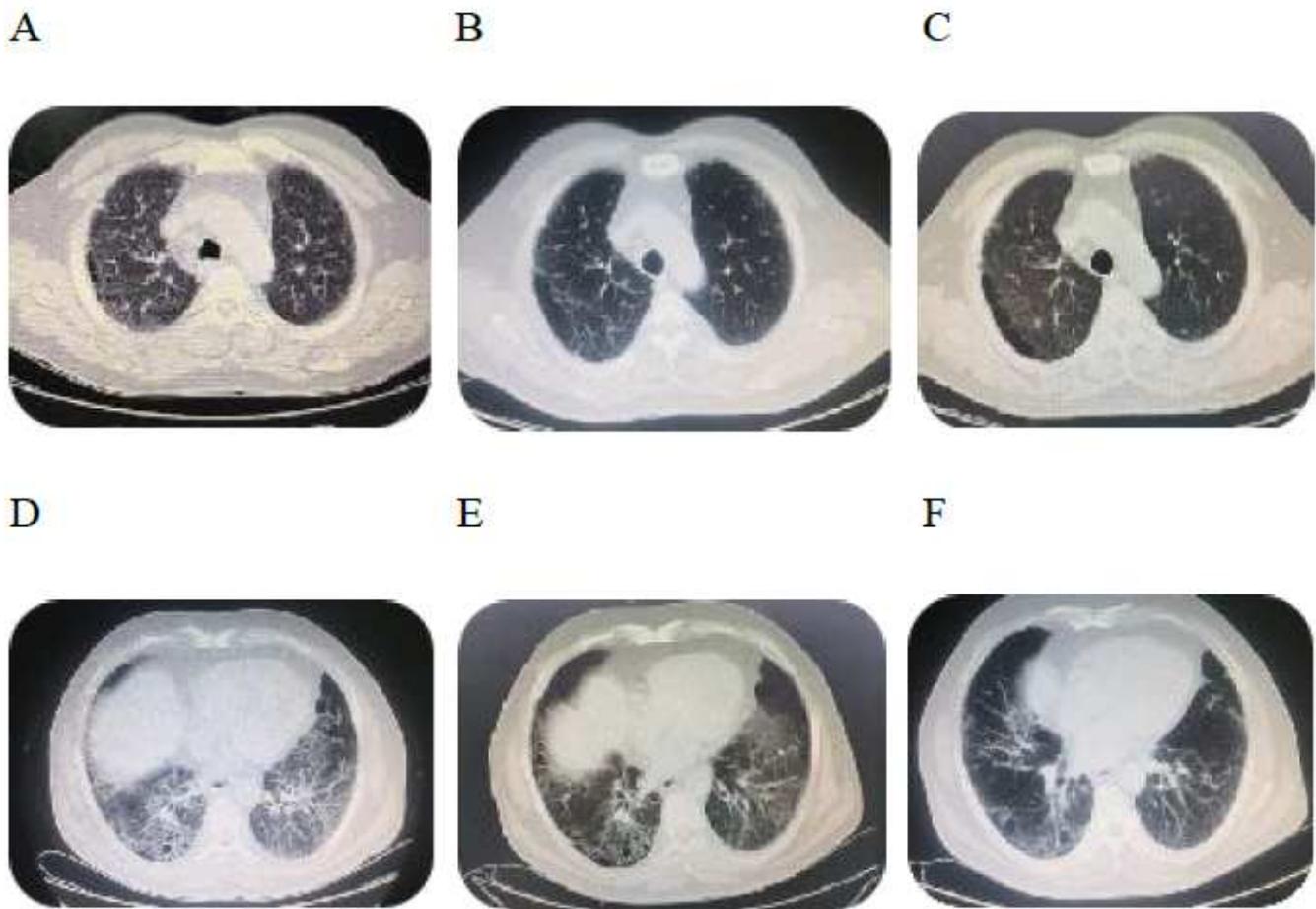


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