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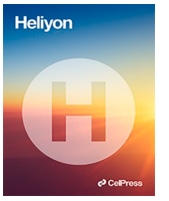

Review article

Predictive biomarkers of disease progression in idiopathic pulmonary fibrosis

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ABSTRACT

Idiopathic pulmonary fibrosis (IPF) is a chronic interstitial disease that cannot be cured, and treatment options for IPF are very limited. Early diagnosis, close monitoring of disease progression, and timely treatment are therefore the best options for patients due to the irreversibility of IPF. Effective markers help doctors judge the development and prognosis of disease. Recent research on traditional biomarkers (KL-6, SP-D, MMP-7, TIMPs, CCL18) has provided novel ideas for predicting disease progression and prognosis. Some emerging biomarkers (HE4, GDF15, PRDX4, inflammatory cells, G-CSF) also provide more possibilities for disease prediction. In addition to markers in serum and bronchoalveolar lavage fluid (BALF), some improvements related to the GAP model and chest HRCT also show good predictive ability for disease prognosis.

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic interstitial lung disease with unknown causes and progressive characteristics [1]. The annual incidence of IPF is estimated at 4.6–16.3 cases per 100,000 people and the prevalence is 13–20 per 100,000 people; the incidence of IPF is increasing. IPF has marked gender characteristics, with a higher prevalence rate in males than in females (1.5–1.7:1). The median survival of IPF patients is only 2–3 years, and the 5-year survival is only 30 %, which is lower than the rate of some cancers [2]. Treatment of IPF is currently limited to nintedanib and pirfenidone, which only slow the development of the disease. Acute exacerbation is closely related to patient mortality [3]. The annual incidence of acute exacerbation of IPF (AE-IPF) ranges from 13 % to 20 %; patients with AE-IPF have a very poor prognosis and a median survival of 3–4 months [4]. Delaying the development of IPF and reducing the frequency of acute exacerbations are challenges that need to be overcame.

Crurrently, specificbiomarkers for the diagnosis of IPF that reflect the progression of IPF are lacking. The ATS/ERS/JRS/ALAT Clinical Practice Guideline on Idiopathic Pulmonary Fibrosis does not recommend any serum markers as markers for monitoring disease progression [5] While pulmonary function testing can reflect disease progression in IPF patients, it cannot be used to evaluate condition and prognosis in patients who cannot undergo testing [6]. Blood and BALF are easy to obtain with low risk, and biomarkers in these samples have important clinical significance for IPF. These biomarkers can be categorized into disease susceptibility

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Table 1
Summary of study information in the review.

First author, year [ref.]	Biomarker	Study type	Study groups	Observation (months)	Main findings
New research on traditional biomarkers in IPF					
d'Alessandro M, 2021 [10]	KL-6	Single-center, case-control study	ILD: n = 142 Control subject: n = 12	24	KL-6 may be a prognostic biomarker for monitoring the response of IPF patients to nintedanib. The concentration of KL-6 in IPF-LC patients is higher than that in IPF and fHP groups
Raghu G, 2018 [11]	KL-6	Multicenter, prospective, case-control study	IPF: n = 154 Control subject: n = 57	13	KL-6 does not predict disease progression. HE4 and prostasin deserve further research.
Maher TM, 2017 [12]	SP-D CA19-9 CA-125	Multicenter, prospective, cohort study	Discovery cohort: IPF n = 106 Validation cohort: IPF n = 206	12	The baseline values of SP-D and CA19-9 in patients with progressive disease are significantly higher than those in stable patients. Elevated CA-125 concentrations within 3 months are associated with an increased risk of mortality.
Khan FA, 2022 [13]	MMP-7	Meta-analysis	9 studies; n = 1664	12	Untreated IPF patients with high baseline MMP-7 levels have an increased risk of adverse outcomes, while short-term changes in MMP-7 levels do not reflect disease progression
Clynick B, 2021 [6]	PI (osteopontin, MMP-7, ICAM1, periostin)	Multicenter, prospective, cohort study	AIPFR cohort: n = 189 TLF cohort: n = 205 PROFILE cohort: n = 122	12	The progression index (PI) model predicts the risk of progression, mortality, and progression-free survival of the disease.
Majewski S, 2022 [14]	YKL-40	Single-center, case-control study	IPF: n = 25 Control subject: n = 20	24	Serum YKL-40 concentration may be related to the response to anti-fibrosis therapy of IPF patients.
Caliskan C, 2020 [15]	CCL18	Multicenter, prospective, cohort study	Cohort A: n = 61 Cohort B: n = 126	Cohort A: 48 Cohort B: 36	Serum CCL18 level is associated with the CCL18 rs2015086 genotype and predicts mortality and disease progression of IPF patients.
Akiyama N, 2020 [16]	S100A4	Single-center, case-control study	IPF: n = 95 Control subject: n = 50	24	High serum S100A4 level is independently associated with high disease progression and mortality.
Molyneaux PL, 2022 [17]	CYFRA 21-1	A prospective longitudinal analysis of the PROFILE cohort	Discovery cohort: IPF n = 132 and healthy controls n = 50 Validation cohort: IPF n = 359 and healthy controls n = 50	36	CYFRA 21-1 can reflect the prognosis and treatment effect of IPF patients.
Novel biomarkers reflecting IPF progression					
Tian M, 2022 [18]	HE4	Single-center, cohort study	AE-IPF: n = 27 S-IPF: n = 32 Control subjects: n = 29	48	Serum HE4 can be used as a biomarker to predict disease severity and poor prognosis in patients with IPF.
Allen RJ, 2022 [19]	PKN2	Genome-wide association study	FVC analysis: n = 1329 DLCO analysis: n = 975	–	One variant (rs115982800) located in an antisense RNA gene for PKN2 is associated with the decline of FVC.
Kreuter M, 2021 [20]	Monocyte count	Retrospective, pooled analysis	ASCEND, n = 555; CAPACITY, n = 692; INSPIRE, n = 820	12	Elevated monocyte count is associated with increased risks of progression, hospitalization, and mortality in IPF patients.
Mikolasch TA, 2023 [21]	NLR	Multicenter, retrospective study	Derivation cohort: n = 71 Internal validation cohort: n = 134 External additional cohort: n = 794	40	NLR is a widely available indicator that is significantly related to lung function and can predict the outcome of IPF patients.
Cao M, 2022 [22]	GDF-15	Single-center, case-control study	AE-IPF: n = 47 S-IPF: n = 61 Control subjects: n = 31	–	Significantly elevated level of GDF-15 in AE-IPF patients may predict the progression and survival rate of IPF patients.
Forte G, 2021 [23]	Trace elements (Cd, Cr, Cu, Pb)	Single-center, case-control study	IPF: n = 31 Control subjects: n = 30	–	The concentration of certain trace elements in the sputum of IPF patients significantly increases.

(*continued on next page*)

Table 1 (*continued*)

First author, year [ref.]	Biomarker	Study type	Study groups	Observation (months)	Main findings
Seeliger B, 2022 [24]	Lipidic metabolite	Single-center, cohort study	Healthy control: n = 20 Discovery cohort: IPF n = 122 Validation cohort: IPF n = 21	24	Changes in specific lipidic metabolites can be used as a signal to reflect disease progression or the efficacy of nintedanib.
Chandel A, 2023 [25]	DO-GAP	Multicenter, retrospective study	Discovery cohort: IPF n = 281 Validation cohort: IPF n = 281	36	DO-GAP can effectively predict mortality in IPF patients.
Torrisi SE, 2019 [26]	TORFAN prediction model	Multicenter, retrospective design	Derivation cohort: IPF n = 476 Validation cohort: IPF n = 461	12–60	TORFAN models have better predictive ability than GAP for the risk of death in IPF patients.
Hosein KS, 2020 [27]	CALIPER-revised CPI	Multicenter, prospective cohort study	IPF n = 185	18	Compared to the original version of CPI, CALIPER-revised CPI can better predict the survival rate of IPF.

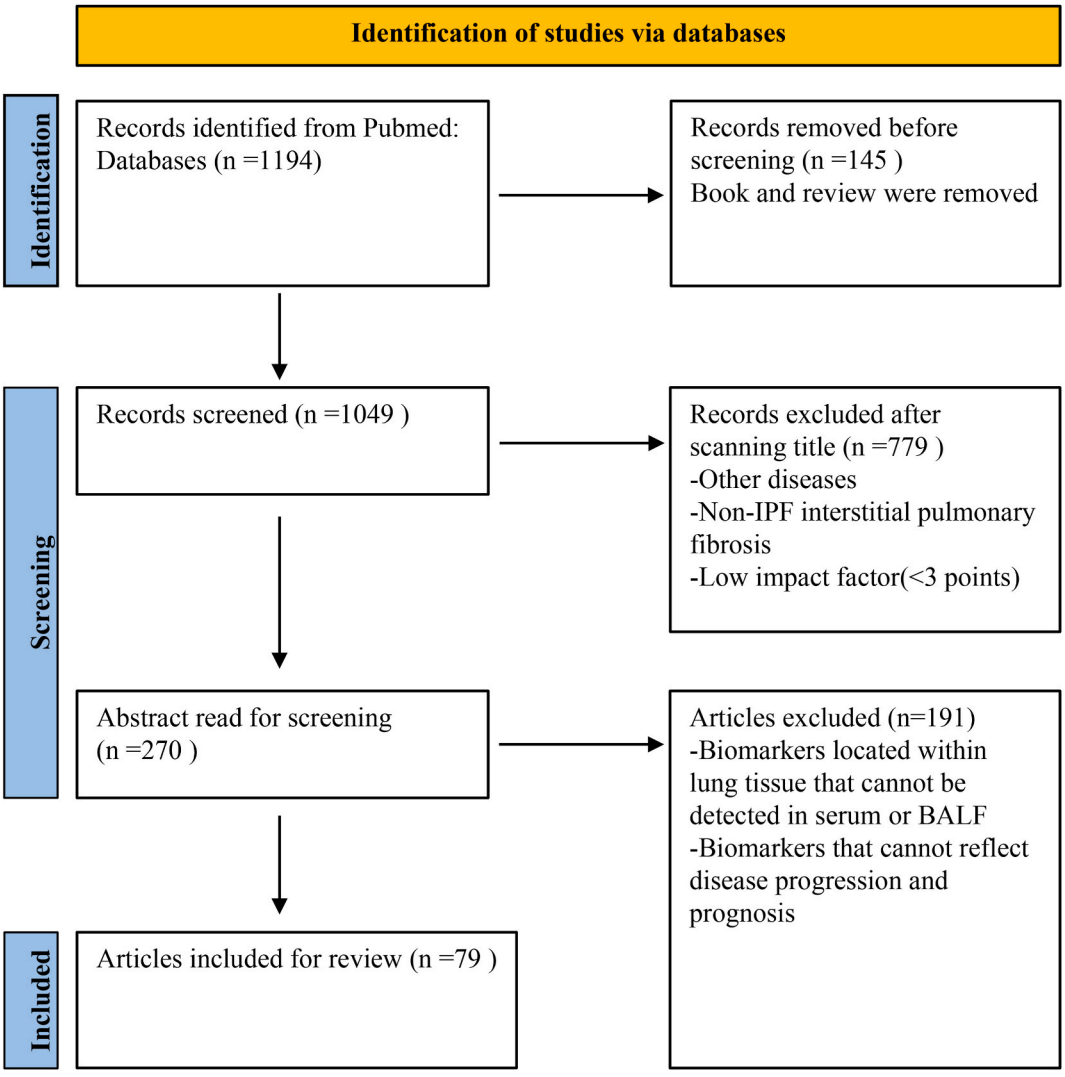

Fig. 1. Strategies of literature retrieval and screening.

biomarkers, diagnostic biomarkers, disease activity biomarkers, drug efficacy biomarkers, and prognostic biomarkers. However, there is no guideline-recognized indicator for disease susceptibility and diagnostic markers. Because of the lack of an effective marker that can independently predict the progression of IPF, researchers have begun to explore potential biomarkers that can reflect the prognosis or therapeutic efficacy of treatments in IPF patients [7,8]. The combination of several biomarkers may improve the accuracy of pulmonary fibrosis diagnosis, disease evaluation, and prognosis judgment, and many studies have attempted to use combinations of biomarkers to predict disease progression [9]. CT is the main diagnostic method for IPF but is relatively lagging in predicting disease progression and prognosis. Researchers are also searching for ways to improve the ability of CT for predicting disease progression. This article summarizes the recent studies on the progressive and prognostic markers for IPF patients (Table 1, Fig. 2).

Clinical outcomes	Source of markers		
	Serum	BALF	Clinical scoring/HRCT
Progression	KL-6 SP-D YKL-40 MMP7 TIMP1/TIMP4 CCL18 S100A4 Periostin Calprotectin CA19-9 HE4 PKN2 Monocyte count S100A12 GDF15 PRDX4	S100A12 Monocyte/Macrophage Trace elements (Cd, Cr, Cu, Pb) esRAGE	GAP+PI FAPI PET ([68 Ga]Ga-FAPI-46)
Mortality/Survival	KL-6 MMP7 S100A4 Periostin CYFRA 21-1 CA125 HE4 GDF-15 soluble ST2	Neutrophils G-CSF Fatty acid metabolism-related genes esRAGE	GAP+NLR DO-GAP TORVAN CALIPER CALIPER-revised CPI
Drug efficacy	CCL18 CA-125 CXCL13 MMP7 YKL-40 OPN	VEGF-A bFGF PIGF IL-10 IL-4	CMS risk score (CA-125, MMP7, YKL-40, OPN, age and FVC%)
Genetic biomarkers			
Susceptibility gene	MUC5B rs35705950 minor allele (T) MUC5AC p.Ala5353Lys		
Progression/Survival	TOLLIP rs5743890 minor allele (C)		
Combination of biomarkers			
Diagnosis	MMP8+MMP9+TIMP1 SP-D+MMP7+Osteopontin (PI)		
Survival	Autophagy-related gene (MET, SH3BP4)		

Fig. 2. Summary of biomarkers in the review.

2. Methods

We searched the PubMed database from 2017 to 2023 using the following search terms: idiopathic pulmonary fibrosis (MeSH Terms), pulmonary fibrosis, idiopathic (Title/Abstract), IPF (Title/Abstract), biomarker (MeSH Terms), serial marker (Title/Abstract), GAP (MeSH Terms), CPI (MeSH Terms), and CT (MeSH Terms) We chose articles published in highly influential journals. Review, books, and documents were excluded (Fig. 1).

2.1. New research on traditional biomarkers in IPF

Krebs von den Lungen-6 (KL-6) is a high molecular weight glycoprotein. When alveolar epithelial type 2 (AT2) cells and bronchiolar epithelial cells are injured, the permeability of the alveolar-capillary barrier changes, and KL-6 is released from AT2 cells into the blood. Recent studies have investigated the prognostic ability of KL-6 for IPF patients. Some studies suggest that baseline serum KL-6 $\geq$ 1000 U/mL may be a useful biomarker for disease progression in IPF [28]. A meta-analysis showed that the risk of acute exacerbation was significantly increased in IPF patients with elevated KL-6 concentration; however, KL-6 is limited in its ability to detect acute exacerbation of IPF patients and it did not correlate with mortality [29]. Patients with elevated serum KL-6 levels had more significant force vital capacity (FVC) reduction compared with patients without KL-6 elevation. Patients with continuous KL-6 change >51.8 U/ml/year had a significantly worse prognosis compared with patients with continuous KL-6 change <51.8 U/ml/year [30].

Lung cancer is one of the most severe comorbidities in patients with IPF, and these two diseases share various genetic, molecular, and cellular processes [31]. KL-6 was initially recommended as a serum biomarker for lung cancer, but its diagnostic accuracy is lower than that of other tumor markers [32]. The KL-6 level in IPF associated with lung adenocarcinoma was significantly higher than that in patients with IPF, and the optimal critical value was 1370 U/mL. This previous study also suggested that KL-6 may be a useful marker for predicting the progression of IPF. KL-6 can also predict the response of IPF patients to nintedanib [30,10]. However, findings on KL-6 predicting the progress of IPF patients remain controversial. An international, multicenter, prospective study suggested that KL-6 could not predict the disease progression of IPF [11]. While KL-6 shows strong sensitivity and accuracy in the diagnosis of interstitial lung disease (ILD) [6], it cannot be used to differentiate different types of ILD or to differentiate ILD from other lung diseases [7].

Surfactant protein D (SP-D) is mainly produced by AT2 cells and secreted into alveolar spaces. In healthy conditions, SP-D is present in the serum at low concentrations. Recent studies have shown that the baseline concentration of SP-D can distinguish patients with progressive disease from patients in stable period (46.6 ng/mL vs 34.6 ng/mL) [12]. Serum SP-D may be the most effective single baseline biomarker for predicting the efficacy of pirfenidone [33]. Human cartilage glycoprotein of 39 kDa (YKL-40) is a chitinase-like protein that is increased in the circulation and lungs of IPF patients [34]. Currently, little is known about the biological functions of YKL-40. A recent study further reported a significant negative correlation between serum YKL-40 concentration and the percentage of predicted FVC (FVC% pred) in patients with stable IPF during anti-fibrosis treatment [14]. Therefore, the longitudinal stable serum YKL-40 concentration may be related to the anti-fibrosis treatment effect.

Research has demonstrated the potential contribution of extracellular matrix (ECM) or ECM-modified proteins to the pathogenesis of IPF [35]. Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases that collectively degrade all components of ECM. There are 24 MMP genes in humans, including two duplicate genes encoding MMP-23. MMP-3, MMP-7, MMP8-9, MMP12, and MMP28 promote pulmonary fibrosis, while MMP1, MMP2, MMP10, MMP13, and MMP19 have anti-fibrotic potential [36]. The cyclic levels of MMP7, MMP8, MMP12, and MMP13 are correlated with diffusing capacity of the lungs for carbon monoxide (DLco) and composite physiologic index (CPI). MMP9 is only related to CPI. MMP7 has the greatest value in assessing the severity of IPF [37]. A meta-analysis of individual participant data showed that baseline MMP-7 levels predicted all-cause mortality and disease progression of IPF and were associated with the percentage of FVC change within 12 months. For each standard deviation increase of baseline MMP-7 value, the risk of total mortality increased by 23 % and the risk of disease progression increased by 27 % [13]. The interaction between tissue inhibitors of MMPs (TIMPs) and MMPs plays an important role in the turnover of extracellular matrix in the lungs. The circulating concentrations of TIMP1 and TIMP4 in IPF patients were significantly increased compared with levels in health controls. There is a correlation between TIMP4 levels and low DLco and high CPI. The combination of MMP8, MMP9, and TIMP1 has good discriminative ability in distinguishing healthy individuals and IPF patients [37].

Osteopontin is a matricellular protein produced by macrophages that induces fibroblast migration, proliferation, and adhesion [38]. It is upregulated in bronchoalveolar lavage fluid of IPF patients and co-localizes with MMP-7 in alveolar epithelial cells in IPF lungs [39]. Previous studies have indicated that IPF patients and patients with alternative idiopathic ILDs can be distinguished by SP-D >31 ng/ml, MMP-7 >1.75 ng/ml, and osteopontin >6 ng/ml. When the three indicators are combined, the diagnostic accuracy was significantly improved compared with each indicator alone. However, these three markers cannot distinguish IPF from rheumatoid arthritis–associated interstitial lung disease (RA-ILD), which may be because the lung pathology of RA-ILD patients also shows usual interstitial pneumonia [40]. A multicenter cohort study aggregated four biomarkers, osteopontin, MMP-7, intercellular adhesion molecule-1 (ICAM1), and periostin, into a progress index model using circulating concentration. The results showed that the progress index predicted the risk of disease progression, mortality, and progression-free survival [6].

CC-chemokine ligand 18 (CCL18) is mainly expressed by alveolar macrophages and shows relatively high expression level in lung tissue. Alveolar macrophages stimulate the production of collagen by producing CCL18 in lung fibroblasts. Collagen stimulates alveolar macrophages to produce CCL18, thus forming a positive feedback circuit of fibrosis [41]. The ASCEND and CAPACITY trials showed that CCL18 can predict the absolute change of FVC% pred in IPF patients and is the most consistent predictor of disease progression. The efficacy of pirfenidone is not affected by the baseline CCL18 concentration [7]. Another study further explained the role of single nucleotide polymorphisms (SNPs) rs2015086 of the CCL18 gene in gene regulation. Before anti-fibrosis treatment,

heterozygous carriers of the C allele (CT genotype) had higher serum CCL18 levels, higher CCL18 mRNA expression, and lower survival rate compared with homozygous carriers of the common T allele. In the anti-fibrosis treatment cohort, increased CCL18 was associated with poor prognosis. The genotype of the CCL18 gene promoter SNP has no predictive value; furthermore, CCL18 level increased no matter the effect of anti-fibrosis (nintedanib and pirfenidone) treatment [42,15]. Another study showed that CCL18 cannot predict the progression of IPF [11]. Thus, the predictive ability of CCL8 for the progression of IPF disease remains controversial. There are few biomarkers that reflect the effectiveness of anti-fibrosis therapy. In addition to CCL18 mentioned above, studies have suggested that increased concentrations of CA-125, CXCL13, MMP7, YKL-40, and OPN predict the transplant free survival (TFS) of patients treated with anti-fibrotic (AF). Compared with patients who did not receive anti-fibrotic treatment, AF-treated patients had a higher threshold for the aforementioned markers. This study also proposed a clinical-molecular signature (CMS) risk score composed of CA-125, MMP7, YKL-40, OPN, age and FVC%, and found that AF patients with high-risk CMS had a higher risk of death than those with low-risk CMS [43]. IPF patients treated with nintedanib can help restore their own immune response [44]. This discovery suggests that researchers can search for immune related biomarkers that can predict anti-fibrotic efficacy. In addition to biomarkers in serum, biomarkers that can reflect the efficacy of pirfenidone have also been found in BALF, such as angiogenesis cytokines (Placental Growth Factor, Vascular Endothelial Growth Factor-A, and basic Fibroblast Growth Factor), anti-inflammatory cytokines (Interleukin-10 and Interleukin-4) [45].

S100 calcium binding protein A4 (S100A4) is a marker of fibroblasts and is highly expressed in mesenchymal progenitor cells (MPCs), which are the precursors of IPF fibroblasts. S100A4 interacts with protein-L-isoaspartyl methyltransferase to promote p53 degradation and MPC proliferation. MPCs can transform self-limited bleomycin-induced pulmonary fibrosis into S100A4-dependent persistent fibrosis [46]. High serum S100A4 level is associated with a high rate of disease progression and high mortality in IPF [16]. Moreover, extracellular S100A4 and S100A4-expressing macrophages were also increased in theBALF of IPF patients [47].

Periostin is an ECM protein that is strongly expressed in the lungs of patients with IPF. Serum periostin was significantly increased in patients with IPF and negatively correlated with pulmonary function [48]. Serum periostin level is not only related to the clinical progress of IPF but it also predicts the long-term survival outcome of IPF patients [49]. A recent study detected periostin in exhaled breath condensate (EBC) and found that the concentration of EBC periostin can distinguish between healthy controls and IPF patients; however, it had no relation with patient survival [50].Calprotectin is a heterodimer complex and a damage-related molecular model protein; it is an important proteome in the innate immune system that promotes inflammatory response [43]. Calprotectin produced by neutrophils has also been proven to be related to DLco and CPI [51]. Cytokeratin-18 is a cytoskeletal protein that is activated by unfolded protein response (UPR) when epithelial cells undergo apoptosis. Cytokeratin-18 is cleaved twice by caspase, producing an 18-kD fragment called caspase cleavage of cytokeratin-18 (cCK-18). cCK-18 may be a marker for cell apoptosis or UPR in IPF patients. Serum cCK18 is not associated with the severity or progression of IPF. However, it can distinguish IPF from chronic HP and NSIP [52]. Currently, research on cCK18 is mostly focused on liver diseases, with relatively little research on IPF.

CYFRA21-1, a cleaved fragment of cytokeratin 19, is more abundant in the lung tissue of IPF patients than in healthy individuals, and it is mainly located in hyperplastic and bronchiole epithelial cells. The baseline level of CYFRA 21-1 predicts the disease progression of IPF patients over 12 months and also predicts the overall survival rate. Furthermore, an increase in CYFRA 21-1 was associated with overall survival within 3 months, and this association was strengthened after adjusting for baseline disease severity [17]. The baseline concentration of CA19-9 can distinguish IPF patients with progressive disease from patients with stable disease. Increased CA-125 concentration is associated with an increased risk of death [12]. Serum CA19-9 level in progressive pulmonary fibrosis showed a dynamic increase in IPF patients treated with anti-fibrosis drugs [53].

MUC5B encodes the precursor protein of mucin 5B, secreted by proximal submucosal glands and distal airway secretory cells, involved in the production of lung mucus, homeostasis, and immune regulation of bronchoalveolar epithelial [54]. *MUC5B* rs35705950 minor allele T is a susceptibility gene for IPF [55]. MUC5AC is another glycoprotein that is also secreted in respiratory inflammation, in addition to MUC5B. *MUC5AC* p.Ala5353Lys mutation is also involved in susceptibility to IPF [56]. Toll interacting protein (TOLLIP) is mainly produced by alveolar type II cells, pulmonary macrophages, and basal cells and can inhibit reactive oxygen species (ROS) damage caused by mitochondrial damage. The TOLLIP and MUC5B genes are both located on chromosome 11. TOLLIP reduces mitochondrial ROS levels and upregulates autophagy, preventing bronchial epithelial cells from apoptosis induced by bleomycin. The downregulation of TOLLIP in healthy lung epithelial cells may make them susceptible to injury-induced apoptosis and promote the development of IPF [57]. Secondary allele C at TOLLIP rs5743890 is associated with an increased risk of death and disease progression [55]. The increased survival rate of IPF patients treated with pirfenidone may be related to the *TOLLIP* rs5743890 genotype CC and CT [58].

2.2. Novel biomarkers reflecting IPF progression

2.2.1. Serum biomarkers

Human epididymis protein-4 (HE4) was initially found to play an important role in tumor cell migration, invasion, and apoptosis [59]. HE4 is strongly expressed by myofibroblasts. HE4 is a putative serine protease inhibitor that inhibits the degradation of type I collagen induced by MMP2 or MMP9 and promotes the formation of fibrosis [60]. In lung specimens of patients with IPF, HE4 is mainly localized in bronchiolar epithelial cells [61]. High expression of HE4 has been found in progressive fibrosing ILDs [62]. Serum HE4 level was significantly increased in patients with IPF, and it was significantly correlated with indicators reflecting the severity of the disease (DLCO%, FVC%, oxygen index, GAP). Furthermore, HE4 is an independent predictor of decreased survival in IPF patients [11, 18].

Protein kinase N2 (PKN2) is a Rho and Rac effector protein that regulates the proliferation, growth, and motility of fibroblasts [63,

64]. rs115982800 is a variant in the PKN2 antisense RNA gene that is significantly associated with FVC decline [19]. PKN2 inhibitors are currently being developed for cancer treatment [65]. The PKN2 inhibitor fostamatinib has also been used as a candidate drug for the treatment of acute respiratory distress syndrome in severe COVID-19 patients [66]. At present, research on PKN2 is mainly focused on cancer and there is little research on the relationship between PKN2 and IPF [67]. A study on pancreatic tumors suggested that PKN2 deficiency reduces the growth of fibroblasts and the differentiation of myofibroblasts [63]. Fibroblasts are also involved in the disease progression of IPF; there is no research on whether PKN2 can affect the function of fibroblasts in IPF. PKN2 may be a new target to prevent the progression of IPF.

Elevated monocyte count is associated with an increased risk of disease progression, hospitalization, and death in IPF patients [20]. An analysis from the Australian IPF registry also showed that the increase in monocyte count is an important predictor of poor survival and an increase in the total number of neutrophils and white blood cells also indicates poor outcome in IPF [68]. S100 calcium-binding protein A12 (S100A12) is a member of the calcium-binding protein S100 family and is highly expressed in monocyte clusters [69]. It plays an important role in the adhesion and migration of white blood cells, as well as the production of cytokines and chemokines [70]. Research has shown that S100A12 is upregulated in both blood and BALF of IPF patients. It is significantly negatively correlated with lung function in IPF patients and positively correlated with the St. George's Respiratory Questionnaire. S100A12 may be an effective biomarker for disease severity and prognosis in patients with IPF [69].

Growth differentiation factor 15 (GDF15) is a member of the TGF-β superfamily and also known as macrophage inhibitory cytokine 1. Both mouse telomere dysfunction and bleomycin stimulation can induce GDF15 expression. After administration of bleomycin to mice, GDF15 protein expression is detected in peripheral blood and bronchoalveolar lavage. In IPF patients, GDF15 is produced by epithelial cells, and its circulating concentration is significantly increased; high GDF15 expression is related to the severity of the disease and patient survival [71]. In a cohort study of IPF patients, the serum level of GDF15 was positively correlated with multiple parameters reflecting disease severity. Serum GDF15 is an independent risk factor for predicting the survival of patients with IPF. The survival rate of patients with GDF15 levels higher than 1075.76 pg/ml is significantly worse than that of patients with levels below this threshold [22]. Regarding the mechanism of GDF15 in IPF, research suggests that GDF15 promotes fibroblast differentiation into myofibroblasts, leading to extracellular matrix deposition. After administering anti-GDF15 monoclonal antibody to mice, bleomycin-induced pulmonary fibrosis was weakened [71].

Suppression of thrombogenicity-2 (ST2) is a member of the interleukin-1 (IL-1) receptor family, and soluble ST2 in serum is an independent predictor of event-free survival of IPF patients. While soluble ST2 cannot directly predict the all-cause mortality of IPF patients, a higher level of serum soluble ST2 is associated with a worse outcome of IPF patients [72]. To determine the impact of soluble ST2 on the prognosis of IPF, additional multicenter, large sample quantity, and quantitative studies are needed.

Peroxiredoxins (PRDX) is a recently discovered antioxidant family that includes six members (PRDX1–6). PRDX1 is an important endogenous anti-inflammatory molecule produced by pulmonary macrophages that can prevent the amplification of pro-inflammatory reactions in macrophages. Mice lacking PRDX1 are sensitive to bleomycin-induced lung injury and pulmonary fibrosis. PRDX1 may play an important role in reducing lung inflammation and fibrosis [73]. PRDX2 oxidation is not associated with the onset of IPF/UIP [74]. PRDX6 is highly expressed in alveolar epithelial type II cells and plays a role in antioxidant stress [75]. However, no studies have examined if it participates in IPF. PRDX4 is the only PRDX family member that can be detected both intracellularly and extracellularly. The serum level of PRDX4 protein in IPF patients with acute exacerbation was shown to be significantly higher than that in stable IPF patients. The best critical value for PRDX4 to predict AE-IPF was 5.84 ng/ml [76].

Aberrant metabolic and lipid pathways have also been reported to be involved in IPF pathophysiology. The metabolic changes of the amino acids glycine, glutamine, and arginine, as well as the dysregulation of glycolysis, promote the formation of a TGF-β-dependent fibrotic phenotype [77,78]. The levels of long chain and medium chain fatty acids in the lungs of IPF also increased [79]. Studies found that IPF patients have elevated triglyceride and ceramide levels. Moreover, compared with patients without lipidic metabolite changes after treatment with nintedanib, patients with lipidic metabolite changes have slower disease progression. These findings indicate that specific lipid metabolite signatures, especially triglycerides, can be used as biomarkers for IPF progression or beneficial therapeutic response to nintedanib [24].

2.3. BALF biomarkers predicting prognosis in IPF

Inflammatory cells in BALF of IPF patients have also been studied. Neutrophils in BALF of IPF patients can predict long-term mortality. Baseline BALF neutrophilia predicts early mortality in IPF [80]. Granulocyte colony-stimulating factor (G-CSF) is a neutrophil-activated glycoprotein. The level of G-CSF in the BALF of IPF patients is higher than that of healthy controls, but the increase in G-CSF level is related to the number of neutrophils in BALF. Therefore, high level of G-CSF in the lung is not specific to IPF. However, G-CSF concentration can predict the survival rate of IPF patients. G-CSF concentration is an independent risk factor for IPF regardless of clinical or physiological factors [81]. Some studies have examined the immune cells in BALF of IPF patients and suggested that the increase of monocytes and macrophages may be related to the progress of IPF [82]. A relationship between IPF and autophagy has also been reported. The receptor tyrosine kinase MET, which was activated by transforming growth factor-β1 (TGF-β1), is upregulated in IPF lung tissue and human lung fibroblasts. In contrast, SRC homology 3 domain binding protein 4, which was activated by TGF-β1, was decreased in IPF lung tissue and human lung fibroblasts [82]. A risk-scoring prognosis model based on these two autophagy-related genes was shown to effectively predict the prognosis of IPF patients [82].

Several reports analyzed the fatty acid metabolism–related genes in BALF cells of IPF patients and the results indicated that gene expression markers related to fatty acid metabolism can be used as potential biomarkers to predict clinical outcomes and immune cell infiltration levels [83]. As environmental and occupational exposure is one of the potential risk factors for IPF, studies have tested trace

elements in the BALF of IPF patients. The levels of Cd, Cr, Cu, and Pb in BALF of IPF patients were significantly increased compared with levels in the healthy control group. IPF patients also have higher lead levels in BALF compared with patients with other lung diseases. Moreover, Zn levels are positively correlated with the severity of IPF disease [23]. Monitoring the changes of trace elements in BALF is of great significance for patients with occupational exposure to trace elements.

The receptor for advanced glycation end-product (RAGE) is a transmembrane receptor that is highly expressed in normal lungs, especially in type 1 alveolar epithelial cells [84]. Endogenous secretory RAGE (esRAGE) is formed by selective splicing of the RAGE gene and has anti-inflammatory properties [85]. The BALF esRAGE levels in IPF patients were lower than those in the healthy control group. A decrease in BALF esRAGE levels is not only associated with a decrease in serum esRAGE levels but also with lower DLco and poorer prognosis [86].

2.4. Other biomarkers for IPF detection and progression

The GAP index identifies three stages (Phase I, Phase II, and Phase III) that can predict mortality in IPF patients, with 1-year mortality rates of 6 %, 16 %, and 39 %, respectively [87]. When the GAP index is combined with the aforementioned PI in a statistical model, it can better predict the disease progression of IPF patients at 12 month [6]. Both 6MWD and exertional hypoxia are closely related to overall survival of IPF patients and were merged into a new model, the distance agent GAP (DO GAP) index. Compared with GAP index, the DO GAP index can better predict mortality [25]. Another study proposed a predictive model called TORVAN. Compared with GAP, the TORVAN model incorporates comorbidities and shows improved predictive ability for the risk of death in IPF patients. When incorporating comorbidities in this model, patient gender becomes a less important indicator [26]. Determination of the neutrophil to lymphocyte ratio (NLR) is a simple, widely available, and inexpensive test; incorporating the NLR into the GAP index significantly improved the prognosis prediction ability of the GAP index [21].

A study has proposed a computer-based quantitative algorithm Computer-Aided Lung Informatics for Pathology Evaluation and Rating (CALIPER) with pulmonary vessel volume (PVV) as the core, which may become a new indicator for evaluating mortality in IPF [88]. To better apply the CALIPER algorithm to clinical work, Jacob et al. developed a revised version of CALIPER CPI ($66.0 - (0.47 \times \text{DLCO}) - (0.67 \times \text{FVC}) + (0.32 \times \text{FEV1})$, which better predicted the survival of IPF patient [89,27]. FVC percentage predicted and DLCO percentage predicted have been used to define the severity of IPF and predict disease prognosis [90]. However, in IPF patients receiving anti-fibrotic drug treatment, the decrease rate of FVC is often between 5.0% and 9.9 %. Evaluating the severity of traction bronchiectasis using the 5-point scale of CT can identify disease progression and address any uncertain FVC decline [91].

In addition to biomarkers in serum, biomarkers expressed in lung tissue have also been reported. Fibroblast activating protein-α (FAP) is a serine protease that is selectively expressed on activated stromal fibroblasts during tissue remodeling. FAP is related to pulmonary fibrosis [92]. Radiolabeled quinoline-based small molecule fiber activation protein inhibitors (FAPIs) have been used as radiotracers of positron emission tomography-CT (PET-CT) to evaluate FAP expression [93]. FAPI of PET-CT successfully detected pulmonary fibrosis injury and disease activity in the early stage in a bleomycin mouse model by detecting the expression of fibroblast activating protein. Thus, the FAPI of PET-CT may be a promising tool for evaluating early human pulmonary fibrosis [94]. In a clinical trial, a radioactive tracer was used for PET examination, and CCR2 cells were found to accumulate in the pulmonary fibrosis area of IPF patients. IL-1β blockade and pirfenidone significantly reduced the accumulation of CCR2 cells [95]. Some studies have found that cysteine (Cys) is only significantly expressed in the lungs of IPF mice compared with pneumonia mice. This suggests that Cys can be used as a biological indicator to distinguish pneumonia from IPF [96].

3. Conclusion

Identification of biomarkers that can reflect the disease progression and treatment effectiveness of IPF patients is critical to help doctors develop treatment plans more accurately and promptly. Research on the biomarkers related to IPF has begun to shift from examining their diagnostic value to determining their prognostic value. Compared with single biomarkers, a combination of biomarkers can more accurately predict disease progression. Several biomarkers mentioned in the guidelines (KL-6, MMP7, SP-D, CCL18) are not yet clinically applicable because of insufficient research evidence. Establishing an accurate and unified threshold is particularly important for these classic biomarkers. Increasing studies suggest that inflammatory cells and inflammatory factors are involved in the progression of IPF disease. Predicting the severity and outcome of IPF by detecting inflammatory cells (neutrophils) or inflammatory factors (G-CSF, GDF15) in blood and BALF is a simple and low-cost option. HRCT is a non-invasive and accurate tool for diagnosing IPF, and the emergence of various computer algorithms has also led to new areas and potential applications in research on disease progression and prognosis of IPF.

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Data availability statement

No data was used for the research described in the article.

CRediT authorship contribution statement

Weiwei Zhu: Writing - original draft. **Chunquan Liu:** Writing - original draft. **Chunting Tan:** Writing - review & editing, Writing - original draft. **Jie Zhang:** Writing - review & editing, Writing - original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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