Potential biomarkers for diagnosis and disease evaluation of idiopathic pulmonary fibrosis

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Abstract

Idiopathic pulmonary fibrosis (IPF) is a chronic progressive lung disease characterized by progressive lung fibrogenesis and histological features of usual interstitial pneumonia. IPF has a poor prognosis and presents a spectrum of disease courses ranging from slow evolving disease to rapid deterioration; thus, a differential diagnosis remains challenging. Several biomarkers have been identified to achieve a differential diagnosis; however, comprehensive reviews are lacking. This review summarizes over 100 biomarkers which can be divided into six categories according to their functions: differentially expressed biomarkers in the IPF compared to healthy controls; biomarkers distinguishing IPF from other types of interstitial lung disease; biomarkers differentiating acute exacerbation of IPF from stable disease; biomarkers predicting disease progression; biomarkers related to disease severity; and biomarkers related to treatment. Specimen used for the diagnosis of IPF included serum, bronchoalveolar lavage fluid, lung tissue, and sputum. IPF-specific biomarkers are of great clinical value for the differential diagnosis of IPF. Currently, the physiological measurements used to evaluate the occurrence of acute exacerbation, disease progression, and disease severity have limitations. Combining physiological measurements with biomarkers may increase the accuracy and sensitivity of diagnosis and disease evaluation of IPF. Most biomarkers described in this review are not routinely used in clinical practice. Future large-scale multicenter studies are required to design and validate suitable biomarker panels that have diagnostic utility for IPF.

Keywords: Alveolar epithelial cell dysfunction; Biomarker; Diagnosis; Fibrogenesis; Extracellular matrix remodeling; Idiopathic pulmonary fibrosis; Immune dysfunction

Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic progressive lung disease characterized by progressive lung fibrogenesis and the histological features of usual interstitial pneumonia (UIP). Symptoms include increased cough and dyspnea, which reduces the quality of life. The median survival of patients with IPF is 3–5 years from diagnosis.[1] The diagnosis of IPF requires the exclusion of interstitial lung disease (ILD) with known causes and the identification of a pattern of UIP assessed either using high-resolution computed tomography (HRCT) or based on histology. However, HRCT is not always useful for diagnosis because other chronic fibrotic lung disorders, such as chronic hypersensitivity pneumonitis (cHP) and ILD associated with connective tissue diseases (CTD-ILD), can exhibit a UIP-like pattern.[2,3] Additionally, many patients are unable to tolerate lung biopsy.[4] Therefore, biomarkers that are easy to implement, yet sensitive and specific, are needed for the diagnosis of IPF.

We searched the PubMed database for articles published from 2005 to 2020 using the keywords "marker" or "biomarkers" or "signature" and "idiopathic pulmonary fibrosis." In the present study, we summarize over 100 biomarkers identified in the serum, bronchoalveolar lavage fluid (BALF), lung tissue, and sputum. The biomarkers related to IPF reported in the literature can be divided into the following six categories according to their functions.

Differentially Expressed Biomarkers in the IPF Group Compared With Healthy Controls (HCs)

Our previous study schematically summarized the pathogenesis of IPF.[1] According to the different roles played in

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the pathogenesis of IPF, the differentially expressed biomarkers in IPF can be further divided into nine different categories, as summarized in Figure 1 and Supplementary Table 1, http://links.lww.com/CM9/B165.

Alveolar epithelial cells (AECs) dysfunction markers

Krebs von den Lungen (KL)-6: Baseline serum[5-8] and sputum[9] levels of KL-6 are significantly higher in patients with IPF than in HCs. The optimal cutoff values (Youden index) of serum KL-6 for discriminating IPF patients from HCs have been reported as 476 U/mL[9] or 398 U/mL.[5] KL-6 exerts chemotactic and antiapoptotic effects on fibroblasts *in vitro*,[10,11] suggesting a role in the pathogenesis of IPF.

Surfactant protein (SP)-A and SP-D: Baseline serum levels of SP-A and SP-D in patients with IPF are significantly higher than those in HCs,[6,8,12] a finding that was supported by the results of a meta-analysis including 21 studies and 1289 IPF patients.[13] IPF patients exhibit a two-fold higher BALF level of SP-A than HCs,[14] and lung tissue samples of patients with IPF show higher SP-A immunoreactivity in hyperplastic AECs.[14] In contrast, levels of SP-D expression were downregulated in explanted lung samples of patients with IPF compared to HCs.[15] The cutoff values of SP-A and SP-D for the detection of IPF were reported as 45 ng/mL and 110 ng/mL, respectively.[12] Another study reported similar cutoff values (44 ng/mL for SP-A and 107 ng/mL for SP-D) for distinguishing IPF patients from HCs.[6] Altered levels of SP-A and SP-D can arise from abnormal function or proliferation of type II AECs caused by injury.[16]

Receptor for advanced glycation end-products (RAGE): Patients with IPF were shown to have significantly higher RAGE levels in the serum[17] and lung tissue[18] than in HCs. Serum RAGE level correlated with levels in BALF specimens[17] and was shown to be a marker of type I AECs injury and/or proliferation.[19]

Haptoglobin: The serum level of haptoglobin was lower in patients with IPF than in HCs.[19,20] Haptoglobin functions as a scavenger of hemoglobin circulating in serum, and is released by hemolysis or through normal red blood cell turnover, thereby protecting against the toxic effects of free heme and exerting antioxidant and immunomodulatory effects.[21]

Transferrin: Transferrin receptor 1, also known as cluster of differentiation (CD) 71, is an integral membrane protein that mediates the uptake of diferric transferrin

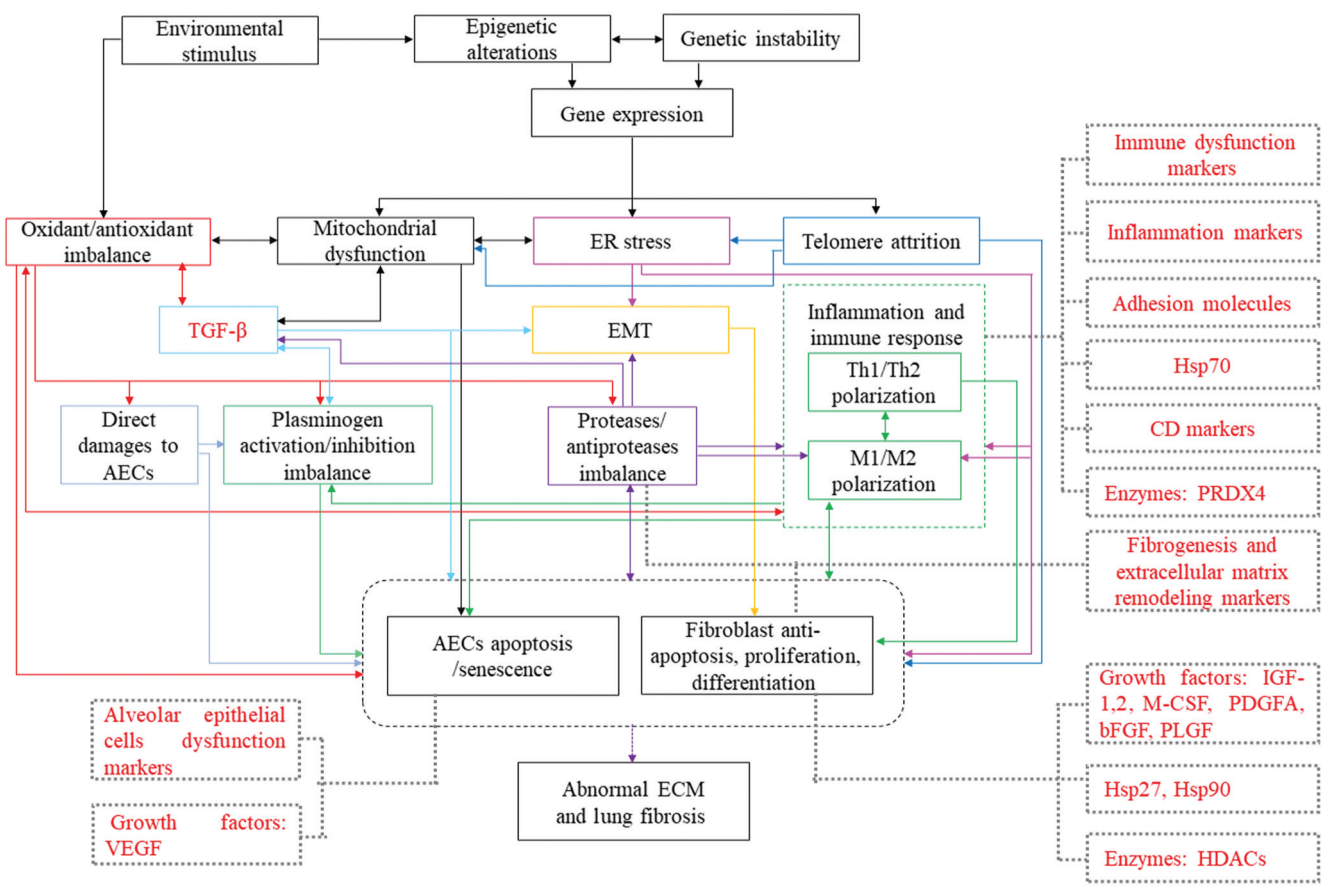

Figure 1: Relationships between markers for diagnosis of IPF and the pathogenesis of IPF. AECs: Alveolar epithelial cells; bFGF: Basic fibroblast growth factor; CD: Cluster of differentiation; ECM: Extracellular matrix; EMT: Epithelial-mesenchymal transition; ER: Endoplasmic reticulum; HDACs: Histone deacetylases; Hsp: Heat shock protein; IGF: Insulin-like growth factor; IPF: Idiopathic pulmonary fibrosis; M-CSF: Macrophage colony stimulating factor; PDGF: Platelet-derived growth factor; PLGF: Placental growth factor; PRDX: Peroxiredoxin; TGF-⊠: Transforming growth factor-⊠; Th: T helper; VEGF: Vascular endothelial growth factor.

complexes through receptor-mediated endocytosis.[22] The proportion of airway macrophages (AMs) lacking CD71 was shown to increase in patients with IPF compared to HC, and $CD71^{-/-}$ AMs showed an impaired ability to sequester transferrin, resulting in increased BALF concentrations of transferrin in patients with IPF.[23]

Mucin (MUC) 5B: To date, 21 human *MUC* genes encoding mucins have been identified, of which 16 are expressed in the lung.[24] MUC5AC and MUC5B together account for approximately 90% of the mucin content of sputum.[25] *MUC5B* is upregulated in the lungs of patients with IPF compared to HCs,[15] and immunohistochemical analysis revealed that the proportion of MUC5B-positive cells in the distal airways but not in the honeycomb cysts is more than two-fold higher than HCs, whereas the proportions of MUC5AC-positive epithelial cells of distal airways were similar between the two groups.[26] The overexpression of MUC5B in distal airways but not honeycomb cysts in the lungs of IPF patients may be driven by a single nucleotide polymorphism in the *MUC5B* gene.[27]

Serum amyloid A (SAA): SAA levels are much higher in patients with IPF than in HCs; the cutoff value of SSA for differentiating the two groups has been reported to be 6067 ng/mL.[28] SAA is produced by lung fibroblasts[29,30] and can induce the overproduction of matrix metalloproteinases (MMPs) including MMP1, MMP5, and MMP7.[31]

Caspase-cleaved cytokeratin-18 (cCK-18): The level of cCK-18, a marker of AEC apoptosis, was found to be significantly elevated in the serum of IPF patients compared to HCs. cCK-18 was also shown to be present in the alveolar epithelium of lungs of IPF patients.[32]

Serum Mac-2-binding protein (M2BP): M2BP levels were shown to be significantly higher in patients with IPF than in HCs.[33] M2BP, which is expressed in alveolar macrophages and AECs,[33] is a ligand of galectin (Gal)-3, also known as Mac-2, which mediates cell adhesion and promotes fibrosis through the receptor-ligand interaction, and may play a role in the host's response against infection and cancer.[34-36]

Club cell protein 16 (CC16): CC16 is a putative anti-inflammatory protein produced by club cells as well as bronchiolar epithelial cells and AECs.[37] CC16 levels in the serum and BALF are increased in IPF patients; the optimal cutoff value of serum CC16 for differential diagnosis of IPF patients from HCs was determined to be 41 ng/mL.[37]

Oncomarkers: Fibroblasts in fibroblastic foci and cultured fibroblasts derived from the lungs of patients with IPF express high levels of carbohydrate antigen (CA) 153.[38] Proliferating AEC II from IPF lungs strongly expressed CK19.[38] Serum carcinoembryonic antigen (CEA) concentration was shown to be elevated in almost half of IPF patients,[39] while CEA levels in BALF increase in 45% of IPF patients who are non-smokers.[40,41] Immunohistochemical analysis revealed that CEA localizes to the metaplastic epithelium lining of honeycombed bronchioles[39]; CEA upregulation in honeycomb cysts and its subsequent release in blood may be responsible for the increased levels of CEA observed in the BALF of IPF patients.

Taken together, among the AEC markers, KL-6, SP-A, SP-D, RAGE, haptoglobin, and MUC5B were more well studied than other markers. KL-6 has antiapoptotic effects on fibroblast, SP-A and SP-D represent abnormal proliferation or injury of AECs II, RAGE represents abnormal proliferation or injury of AECs I, and haptoglobin is a protective factor with antioxidant and immunomodulatory effects. These five markers should be promoted in clinical practice, and their clinical value should be further evaluated. For other alveolar epithelial markers, more clinical studies are needed.

Fibrogenesis and extracellular matrix (ECM) remodeling markers

MMPs: MMP1 levels in the plasma, BALF[4,42] and lung tissue[15,42,43] of patients with IPF were significantly higher than those in HCs. MMP1 is an enzyme that cleaves fibrillar collagen, an ECM component that is enriched in IPF lungs. It is primarily localized in the reactive alveolar epithelium but is mostly absent in fibroblasts of the interstitial compartment.[44] MMP3 was upregulated in the serum[45] and lung[45-48] of IPF patients relative to HCs, and is thought to contribute to the pathogenesis of IPF by inducing epithelial-to-mesenchymal transition (EMT).[44] MMP7 levels were significantly higher in plasma or serum,[4,6,8,42,49,50] BALF,[4,42,51] and lung tissue[42] of patients with IPF as compared to HCs; and a serum cutoff value of 6 ng/mL could distinguish between these two groups.[6,52] Also, MMP7 may mediate the profibrotic effects of osteopontin (OPN)[53] and facilitate the release of transforming growth factor (TGF)-β from the extracellular proteoglycan decorin, thereby promoting TGF-β activation.[54] MMP8 levels in plasma, BALF, and lung tissue homogenates were reported to be significantly higher in IPF patients compared to HCs.[42,55-57] In an experimental model, MMP8 enhanced inflammation and consequent fibrosis, and $MMP8^{-/-}$ mice were protected against bleomycin-induced lung fibrosis through mechanisms that have yet to be elucidated.[58] MMP9 levels are higher in the serum,[59] lungs,[60] and sputum[9] of IPF patients compared to HCs. MMP9 is expressed by AECs, macrophages, neutrophils, and fibroblasts in fibroblastic foci.[60] Baseline serum levels of MMP10,[50] MMP28,[61] and tissue inhibitor of metalloproteinase (TIMP) 3[59] were also elevated in IPF patients, and MMP28-deficient mice were protected from bleomycin-induced lung fibrosis, suggesting a profibrotic role for MMP28.[62] Moreover, MMP28 may contribute to EMT, which has been implicated in the pathogenesis of IPF, by inducing TGF-β activation.[62]

Periostin (POSTN): Serum POSTN levels have been reported to be upregulated in IPF patients compared to HCs[15] and can be used to distinguish between these two populations.[5,63] A semiquantitative analysis of POSTN expression in lung tissue showed that it was upregulated in

IPF.[64] The cutoff value of POSTN for distinguishing IPF patients from HCs was determined to be 77 ng/mL, which had a sensitivity of 73.3% and specificity of 79.6%.[5] POSTN plays an important role in the pathogenesis of pulmonary fibrosis by stimulating the production of inflammatory cytokines such as tumor necrosis factor (TNF)-α or interleukin (IL)-1α and activating nuclear factor (NF)-κB, leading to the production of various inflammatory cytokines and chemokines and lung fibrosis.[64,65] POSTN also promotes connective tissue growth factor (CTGF) production by fibrocytes, myofibroblast differentiation of fibroblasts, and collagen deposition, resulting in pulmonary fibrosis.[66,67] Finally, POSTN may contribute to fibrosis by promoting EMT of lung fibroblasts in IPF[68] via activation of the TGF-β signaling pathway.

OPN and alpha-actin-2 (ACTA-2): OPN mRNA and protein levels were shown to be significantly higher in the serum, BALF, and lung tissue of patients with IPF compared to HCs.[15,51,52,69] OPN is a matricellular protein produced by macrophages that induces migration, proliferation, and adhesion of fibroblasts and is implicated in the pathogenesis of IPF.[70,71] ACTA-2 mRNA[15,72] and protein[73] levels were higher in the lungs of IPF patients compared to HCs, which may be related to myofibroblast differentiation and proliferation and matrix remodeling.

Follistatin (FST), fibulin-1 (FBLN-1), and syndecan 2 (SDC2): The mRNA expression of the activin inhibitor FST was upregulated in IPF lungs,[74] and serum concentrations of FST were significantly higher in patients with IPF compared to HC subjects.[75] FBLN-1 is produced by lung fibroblasts and is required for the formation of alveolar septum.[9] FBLN-1 levels in the serum and lung tissue were increased in patients with IPF compared to HC.[76] The increased production of FBLN-1 likely reflects in the activated fibrogenic state of fibroblasts derived from patients with IPF.[77] SDC2 levels were increased in the lung tissue of patients with IPF, and may contribute to fibrosis by enhancing the assembly and deposition of ECM.[78]

Among the fibrogenesis and ECM remodeling markers, MMPs, POSTN, OPN, and ACTA-2 were more well studied than other markers. They induce EMT, migration, proliferation, and adhesion of fibroblasts, myofibroblast differentiation of fibroblasts, and collagen deposition, resulting in pulmonary fibrosis.

Immune dysfunction/regulation markers

YKL-40: The levels of YKL-40 in the serum and BALF were significantly higher in patients with IPF than in HCs,[8,79-81] which may be related to lung tissue remodeling that occurs in patients with IPF.[82]

Programed cell death-1 (PD-1): PD-1 upregulation was shown to be profibrotic through its capacity to enhance the expression of signal transducer and activator of transcription (STAT) 3, leading to the production of profibrotic cytokines such as TGF-β and IL-17A.[83] Cell surface expression of PD-1 detected by immunohistochemistry in $CD4^+$ T cells was significantly higher in IPF patients than in HCs.[83]

S100A6, S100A9, and S100A12: The S100 family of calcium-binding proteins has over 20 members. S100A6[84] and S100A9[51,85,86] levels were higher in the BALF of patients with IPF than in HCs; and S100A12 transcript expression was higher in the lungs of patients with IPF than in HCs.[87] S100A9 induces neutrophil recruitment.[88,89] Serum and BALF levels of S100A9 significantly correlated with BALF neutrophil counts.[85,86] Sustained neutrophil accumulation in the alveolar space and neutrophil-mediated injury to the alveolar wall contribute to interstitial fibrosis.[90] S100A9 also stimulates the proliferation of fibroblasts, which plays an important role in the development of lung fibrosis.[91]

Toll-like receptor 9 (TLR9): The innate immune sensor TLR9 has been reported to be upregulated in the lung tissue of IPF patients.[92] The overexpression of TLR9 in pulmonary fibroblasts in lung biopsies of IPF patients was able to induce myofibroblast differentiation,[92] and TLR9 expressed in an AEC line promoted EMT,[93] contributing to the pathogenesis of IPF.

Gal-1 and Gal-3: BALF levels of Gal-1[94] and Gal-3[95] were higher in patients with IPF than in HCs. Gal-1 skews the helper T cell (Th)1/Th2 balance toward the Th2 phenotype in fibrotic diseases. It also induces macrophage polarization to the M2 phenotype,[96] resulting in the accumulation of profibrotic mediators that promote accumulation and differentiation of fibroblasts into myofibroblasts as well as collagen production and deposition.[1] Alveolar macrophages in IPF patients had higher Gal-3 expression than macrophages of HCs,[95] suggesting that the higher Gal-3 concentration in BALF in IPF was at least partially due to increased production by alveolar macrophages. Gal-3 contributes to the pathogenesis of IPF by activating macrophages and fibroblasts and promoting inflammation through increased TNF-α production by macrophages, leading to a positive feedback loop between TNF-α and Gal-3. Additionally, Gal-3 stimulated fibroblast migration and collagen synthesis *in vitro*, suggesting that it directly affects fibrogenesis.[95]

Defensin, heme oxygenase-1 (HO-1), cystatin C, TNF receptor superfamily member (TNFRSF) 1A and 1B: Serum levels of α-defensin (HAD),[97,98] β-defensin (HBD) 1, and HBD2[98] in patients with IPF were significantly higher than in HCs, although there were no differences in HBD levels in the BALF.[98] HBD2 was shown to be expressed in AEC II,[99] implying that it is important for lung function. HO-1 was shown to be expressed at a lower level in the lung tissue of IPF patients compared to HCs.[15] Cystatin C is highly expressed in myofibroblasts of IPF patients,[100] and may promote the development of lung fibrosis by impairing the collagenolytic activity of cysteine cathepsins. The protein level of cystatin C, the most potent circulating inhibitor of cathepsins, was higher in BALF samples from IPF patients than in those of HCs.[100] Serum protein levels of the soluble TNFRSF1A and TNFRSF1B were shown to be significantly higher in IPF patients than in HCs.[42]

αvβ6 integrin: αvβ6 integrin immunopositivity was higher in the lung tissues of patients with IPF than in those of HCs.[101] αvβ6 integrin is expressed in the alveolar epithelium[101] and activates TGF-β1, a key pro-fibrotic mediator.[102] αvβ6 integrin also regulates other basic cellular functions such as proliferation,[103] migration,[104] and ECM degradation.[105]

Anti-heat shock protein (Hsp) 70: Anti-Hsp70 immunoglobulin G (IgG) autoantibodies were more frequently detected in IPF patients than in HCs.[106] IgG autoantibodies can cause cytotoxicity and promote neutrophil recruitment via formation of antibody–antigen complexes and complement activation in target organs.[107] Neutrophilia is a prominent feature of IPF.[4,108]

Among these markers, YKL-40, PD-1, TLR9, S100A9, Gal-1, Gal-3, αvβ6 integrin, and anti-Hsp70 were more well studied. They may lead to lung tissue remodeling, collagen synthesis, and the production of profibrotic cytokines, and induce myofibroblast differentiation, proliferation of fibroblasts, EMT, and inflammation, contributing to pulmonary fibrosis. Although studies have reported that the expressions of defensin, HO-1, cystatin C, TNFRSF1A, and TNFRSF1B are up-regulated in IPF patients, their pathogenic mechanism is not completely clear, and there is still a long way to go before their clinical application.

Inflammation markers

Chemokines: The levels of C-X-C motif chemokine ligand (CXCL) 7 in the BALF[51]; CXCL8 (or IL-8) mRNA and protein levels in sputum[9]; and serum levels of CXCL8,[75] CXCL10,[109] CXCL13,[110] and CXCL14[111] were shown to be elevated in IPF patients compared to HCs. The CXCL10 is a cytokine that is induced in monocytes and macrophages by interferon (IFN)-γ and mediates the recruitment of activated lymphocytes to the lungs.[112] Cells of the macrophage lineage express high levels of CXCL13 in patients with IPF.[110] CXCL13 binds to the C-X-C motif chemokine receptor (CXCR) 5 expressed on the B cell surface to mediate B cell trafficking. CXCL14 expression was induced by Sonic hedgehog (Hh) overexpression in the mouse lung.[111] Hh signaling contributes to fibrogenesis and cross-talk with other signaling pathway components in IPF such as TGF-β, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and IL-13 to promote myofibroblast differentiation, ECM production, and cell motility and survival.[113,114] Thus, CXCL14 is a peripheral biomarker of Hh signaling activation.

BALF levels of C–C motif chemokine ligand (CCL) 2,[115,116] CCL3,[115] CCL4,[115] CCL16,[37] CCL18,[51] and CCL24[51] and serum levels of CCL2,[116] CCL16,[37] and CCL18[8,59] were significantly higher in IPF patients than in HCs. CCL2 and CCL3 are produced by a variety of cells including alveolar macrophages, monocytes, epidermal cells, lymphocytes, fibroblasts, and endothelial cells.[116] CCL2 and CCL3 are both proinflammatory chemokines responsible for homing and migration of lymphocytes and for the recruitment of mononuclear macrophages.[116] Alveolar macrophages play an important role in the pathogenesis of lung fibrosis.[117,118] After binding to the CCR2 receptor, CCL2 induces the expression of MMP2, MMP9, and TGF-β1 or stimulates the proliferation of fibroblasts by interacting with IL-13, leading to fibrosis.[119] CCL16 is expressed by bronchioles and AECs,[37] while CCL18 is mainly produced by macrophages.[120] CCL18 upregulates collagen production by lung fibroblasts.[120]

Interleukins: Levels of IL-4 level in the BALF[121] and of IL-6 mRNA in the sputum[9] were significantly higher, whereas serum concentrations of IL-10 and IL-15 were lower[69] in patients with IPF than in HCs, although another study found that IL-10 levels in BALF increased in IPF.[121] IL-6 contributes to both inflammation and fibrosis.[122]

Adhesion molecules

Intercellular adhesion molecules (ICAMs) 1, 2, 5, and E-selectin: Serum levels of ICAM1,[8] ICAM2,[123] and ICAM5[59,124] and ICAM1 levels induced in the sputum[123] were higher in patients with IPF than in HCs. They participate in lymphocyte recruitment to the interstitium and the areas of honeycombing.[125] Soluble E-selectin was present at a higher level in the serum of IPF patients compared to HC subjects.[126]

Insulin-like growth factor-binding protein (IGFBP): Serum levels of IGFBP-1[7,42] as well as serum[7] and sputum[9] levels of IGFBP-2 were significantly higher in IPF patients than in HC subjects. IGFBP-2 was also observed in the BALF of patients with ILD.[127] IGFBP-3, IGFBP-5, and IGFBP-6 levels were increased in lung tissue of IPF patients and in cultured fibroblasts isolated from IPF patient lungs.[15,128] IGFBPs are thought to induce the initiation and/or progression of fibrosis by stimulating the production of components of the ECM such as collagen type I and fibronectin in lung fibroblasts.[128] IGFBP-5 is also involved in EMT; collagen deposition; and peripheral blood mononuclear cell proliferation, infiltration, and senescence.[15]

Platelet endothelial cellular adhesion molecule (PECAM), latent TGF-β2-binding protein (LTBP), and histidine-rich glycoprotein (HRG): Serum PECAM-1 levels were significantly higher in IPF patients than in HCs.[129] PECAM-1 plays a role in leukocyte transmigration. LTBP2 is secreted by myofibroblasts[130] and the serum levels of LTBP2 were found to be higher in patients with IPF than in HCs; the optimal cutoff level to discriminate between these two populations was 15.1 ng/mL. HRG concentration was significantly diminished in plasma but increased in the BALF of IPF patients compared to HC subjects.[131] HRG was shown to suppress EMT *in vivo*[132]; this effect may be compromised in IPF patients due to their low levels of plasma HRG.

Growth factors

Insulin-like growth factor (IGF): Serum levels of IGF-1 and IGF-2 were decreased in patients with IPF compared

to HCs[7]; and in an animal model, IGF-1[133] and IGF-2[134] induced myofibroblast differentiation, which is a critical step in the development of pulmonary fibrosis. IGF-2 also exerts profibrotic effects by inhibiting protease production and degradation of ECM, and stimulating the expression of two TGF-β isoforms.[134]

Platelet-derived growth factor subunit A (PDGFA): Gene expression profiling showed that PDGFA was downregulated in the serum of IPF patients compared to that of HCs.[69]

Vascular endothelial growth factor (VEGF): VEGF mRNA and protein levels were lower in BALF of patients with IPF than in HCs.[15,135,136] This decrease can be attributed to the apoptosis of vascular endothelial cells or to damage to the AECs, which are the main sources of VEGF in the lung.[135,136]

Macrophage colony stimulating factor (M-CSF): M-CSF was shown to induce CCL2 production and secretion *in vitro* and *in vivo*, and in fibroblasts, CCL2 induced collagen production, as well as TGF-β,[137] which stimulated CTGF expression.[138] M-CSF-induced CCL2 secretion may promote mononuclear phagocyte recruitment, fibroblast proliferation, TGF-β-mediated CTGF expression, and fibrosis. Patients with IPF had higher levels of M-CSF in BALF but not in serum compared to HCs.[116]

Basic fibroblast growth factor (bFGF) and placental growth factor (PLGF): bFGF is a potent mitogenic factor for fibroblasts and myofibroblasts, and bFGF levels were shown to be correlated with ECM production.[139] bFGF was observed to be upregulated in the mast cells of the lung tissue from patients with IPF[139]; and the levels of bFGF and PLGF in BALF were significantly higher in patients with IPF than in HCs.[121]

TGF-β, activin-A and activin-B: TGF-β mRNA and protein levels in sputum were found to be higher in patients with IPF than in HCs.[9] Activins belong to the TGF-β superfamily of growth factors and mainly signal through serine/threonine kinase receptors that induce Smad signaling.[140] Activins are involved in lung fibroblast proliferation and differentiation.[141] The genes encoding activin-A and activin-B were upregulated in lung tissue of IPF patients compared to HCs[74]; and the immunoreactivity of activin subunits was increased in hyperplastic AECs of IPF lungs.[74]

Hsp

Hsp27,[73] Hsp70,[135] Hsp90-α, and Hsp90-β[73] were upregulated in the lung tissue of IPF patients compared to HC subjects, and Hsp72 was found to be elevated in the serum of IPF compared to HC.[142] Lung biopsies of IPF patients showed high expression of Hsp72 in hyperplastic AEC II near fibrotic foci.[142] It was reported that Hsp27 expression is upregulated in lung fibroblasts in pulmonary fibrosis and that Hsp27 contributes to the fibrotic process by modulating lung fibroblast differentiation via Smad3 and extracellular signal-regulated kinase (ERK) pathways.[143] Hsp70, which is primarily distributed in different pulmonary cells including airway and AECs, macrophages, and endothelial cells, is an autoantigen for $CD4^+$ T cells in IPF that can induce lymphocyte proliferation and IL-4 production.[135] Hsp90 is a positive regulator of fibroblast activation; inhibition of Hsp90 ATPase activity was shown to attenuate pulmonary fibrosis.[144] Taken together, Hsp may contribute to the fibrotic process by modulating lung fibroblast differentiation or activation, and by inducing lymphocyte proliferation or inflammation. The specimen types in these related studies are mainly lung tissue. In clinical practice, we can further observe the clinical value of HSP by performing immunohistochemistry on the lung tissue of IPF patients.

CD markers

CD11b (integrin subunit alpha M, ITGAM), CD16a (Fc gamma receptor [FCGR] 3A, FCGR3A), CD18 (integrin subunit beta 2, ITGB2), CD32a (FCGR2A), CD66d (CEA cell adhesion molecule 3, CEACAM3), and CD87 (urokinase-type plasminogen activator receptor [UPAR]) were up-regulated, while CD25/interleukin 2 receptor, alpha chain (IL-2RA) and D301/Clen10a were down-regulated in serum from IPF patients.[69] These results are mainly from gene expression analysis by real-time quantitative polymerase chain reaction (RT-qPCR) of whole blood from control and IPF subjects; future studies can further explore the clinical value of these CD molecules with BALF and lung tissue samples and further validate the assay at the protein level. CD133 (or prominin-1) was highly expressed in the lung of IPF patients compared to HCs and may be involved in EMT.[15] CD163 is a transmembrane protein expressed on the cell membrane of monocytes and macrophages,[145] while soluble CD163 (sCDl63) is present in serum and tissue fluid.[146] CD163 is a marker of activated M2 macrophages. IPF patients were shown to have high expression of sCD163, suggesting that it promotes pulmonary fibrosis by inducing M2 macrophage polarization.[109] The protein levels of CD248, also known as endosialin, were reported to be higher in lung fibroblasts derived from IPF patients than in those from normal lungs, and silencing of CD248 expression significantly reduced the proliferation of lung fibroblasts.[106]

Enzymes

Serum levels of lactate dehydrogenase (LDH),[5] napsin A,[147] peroxiredoxin (PRDX)4,[148] and a disintegrin and metalloproteinase (ADAM)17 and levels of ADAM17 in peripheral blood mononuclear cells[149] were higher in IPF patients than in HCs. PRDXs are a recently identified family of antioxidants consisting of six members in mammals (PRDX1–PRDX6). However, overexpression of PRDX4 in the lung may not exert a protective effect but may, instead, exacerbate pulmonary fibrosis by inducing inflammatory cytokine production.[148] In a murine model, PRDX4 was expressed in alveolar macrophages and AECs.[148] Class I and II histone deacetylases (HDACs) were shown to be overexpressed and upregulated in lung tissue from patients with IPF compared to control subjects.[150] Aberrant overexpression of HDACs in the lung of patients with IPF can contribute to alveolar bronchiolization and the generation of fibroblasts

resistant to apoptosis.[150] BALF levels of pepsin, a marker of gastric aspiration, was significantly elevated in patients with IPF compared to HCs.[151]

Other markers

The p16 protein encoded by the *CDKN2A* gene was shown to be upregulated in the lung of patients with IPF. Immunohistochemical analysis showed that the expression of p16–a marker of senescence–in lung fibroblasts is a potential diagnostic biomarker in IPF.[15] High mobility group box (HMGB) binds to membrane-bound receptors, such as RAGE and TLR. Their interactions lead to the activation of pro-inflammatory intracellular signaling, which is associated with the pathophysiology of IPF.[152] Nitric oxide (NO) is an intracellular signaling molecule involved in inflammation and nitrosative and oxidative stress, which play an important role in lung fibrogenesis.[153,154] Serum levels of HMGB1[154] and alveolar concentration of NO (CaNO)[155] were found to be higher in IPF patients than in subjects with HC.

As described in our previous review,[1] IPF is likely the result of complex interactions between environmental, genetic, and epigenetic factors. The pathogenesis of IPF involves various imbalances centered on AEC/fibroblast apoptosis imbalance, including endoplasmic reticulum (ER), telomere length homeostasis, mitochondrial dysfunction, oxidant/antioxidant imbalance, Th1/Th2 imbalance, M1–M2 polarization of macrophages, protease/antiprotease imbalance, and plasminogen activation/inhibition imbalance. Among them, AEC/fibroblast apoptosis imbalance is the core of the pathogenesis of IPF, because although other imbalances affect each other and promote each other, they eventually lead to dysregulated crosstalk between AECs and fibroblasts. As a result, the dysregulated crosstalk and abnormal mediators between them result in AECs apoptosis, fibroblast anti-apoptosis, and abnormal ECM.

AEC dysfunction markers and growth factors (VEGF) are produced by aberrantly activated AECs or are related to the damage, apoptosis, and senescence of AECs. Growth factors (IGF-1, 2′ M-CSF, PDGFA, bFGF, and PLGF), fibrogenesis and ECM remodeling markers, Hsp (Hsp27 and Hsp90), and enzymes (HDACs) are related to fibroblast anti-apoptosis, proliferation, and differentiation. Therefore, these types of biomarkers represent AEC/fibroblast apoptosis imbalance. Under normal conditions, there is a dynamic balance between AECs and fibroblasts. In the normal repair of AECs, activated fibroblasts produce ECM and provide a provisional scaffold for AECs migration, proliferation, and re-epithelization. After the AECs damage has been repaired, fibroblasts undergo apoptosis in order to restore normal cellular homeostasis and maintain tissue architecture and organ function. Fibroblast apoptosis is essential in normal wound healing but is dysregulated in IPF. In IPF patients, AECs undergo damage, aging, and apoptosis, while fibroblasts undergo proliferation, differentiation, and anti-apoptosis, which contributes to AEC/fibroblast apoptosis imbalance, leading to abnormal ECM and pulmonary fibrosis.

Immune dysfunction/regulation markers, inflammation markers, adhesion molecules, Hsp (Hsp70), CD markers, and enzymes (HDACs) are mainly related to inflammation and immune response (Th1/Th2 imbalance, M1–M2 polarization of macrophages). Th1 and Th2 cells take on opposite roles in fibrogenesis. Th1 cells and their secretory cytokines (IFNγ) are thought of as being antifibrotic. By contrast, Th2 and associated cytokines (IL-4, IL-5, and IL-13) exhibit a profibrotic property. An overzealous or prolonged M2 polarization results in excessive amounts of profibrotic mediators, which promote fibroblast accumulation and the differentiation of fibroblasts into myofibroblasts, and collagen production and deposition. Therefore, Th1/Th2 imbalance and M1–M2 polarization of macrophages are actually an imbalance between profibrosis and antifibrosis.

Fibrogenesis and ECM remodeling markers are also related to protease/antiprotease imbalance, which may cause fibrosis by promoting EMT, inflammation, promoting M1–M2 polarization, and activating TGF-β signaling.

Biomarkers That Are Specific for IPF

The gene expression of MMP-1, MMP-2, MMP-7, POSTN, OPN, ACTA-2, IGFBP-5, and Prominin-1 were increased in patients with IPF compared to NSIP.[15,63,156] Serum levels of SP-D >31 ng/mL, MMP-7 >1.75 ng/mL, and OPN >6 ng/mL each significantly distinguished patients with IPF compared with patients with alternative idiopathic ILDs (a-ILD).[156] A study identified a gene signature that was truly specific for IPF compared to nonspecific interstitial pneumonia (NSIP), which includes MUC5B, ACTA-2, and IGFBP-5.[15] MMP28 in serum is able to distinguish IPF from cHP and CTD-ILD.[61] BALF levels of HAD[98] and serum levels of PDGF-BB, granulocyte-colony stimulating factor (G-CSF), FST, and PECAM-1[75] in patients with IPF were significantly higher than in patients with sarcoidosis. BALF S100A9 levels were significantly higher in IPF patients than in patients with sarcoidosis or those with systemic sclerosis.[157] Serum levels of cCK-18 were significantly different in IPF patients compared with patients with HP or with NSIP patients.[32] Serum CC16 was found significantly higher in IPF compared with cHP and CTD-ILD.[37] CaNO has also been reported to have the best diagnostic accuracy to discriminate CTD-ILD from idiopathic ILDs.[158] CTD-ILD patients reported significantly higher values of CaNO than in patients with IPF.[158]

Biomarkers for Differentiating AE-IPF From Stable IPF

The diagnostic criteria for IPF AE include a clinical worsening within 30 days, and the presence of new radiologic abnormalities on HRCT characterized by a new bilateral opacification/consolidation of ground glass not fully explained by cardiac failure or fluid overload.[159] Among the differentially expressed biomarkers in the IPF group compared with HCs, the following markers may be helpful for differentiating AE-IPF from stable IPF: KL-6,[160,161] SP-A,[162] SP-D,[160,161] haptoglobin,[163] S100A9,[163] α-defensin,[164] CXCL8,[165,166] CXCL13,[10] CCL2,[166] CCL18,[166] CCL22,[166] IL-6,[165] LTBP2,[130] and HMGB1.[167]

Biomarkers Predicting Disease Progression

Disease progression of IPF is defined as a decrease in lung function (defined as either a ≥10% decline in the forced vital capacity [FVC]% predicted value [%FVC], or a ≥15% decline in the diffusion capacity of the CO [DLCO]% predicted value [%DLCO]), an increase of the honeycombing score on HRCT, respiratory hospitalizations, AE of IPF, lung transplantation, or mortality from any cause.[8,50,158] Among the biomarkers that are differentially expressed in the IPF group, the following can predict disease progression: KL-6,[6] SP-A,[12,13] SP-D,[12,168] RAGE,[17] M2BP,[33] CEA,[168] MMP3,[45] MMP7,[9,26,42,54] MMP10,[50] MMP28,[61] POSTN,[5,15,63] YKL-40,[81] S100A12,[87] anti-HSP70,[106] TLR9,[93] αvβ6 integrin,[101] CXCL8,[165] CXCL10,[109] CXCL13, CXCL14,[169] CCL2, CCL18,[169] IL-6,[165] ICAM 1,[87] E-selectin,[126] CD71,[23] VEGF,[170] LDH,[171] HMGB1,[172] and alveolar NO.[173]

Biomarkers Related to Disease Severity

The disease severity of IPF is often assessed by lung function parameters, such as FVC or %FVC, FEV1% predicted value (%FEV1), DLCO or %DLCO, and total lung capacity (TLC),[59,174] fibrosis score on HRCT,[93,175] the 6-min walking distance (6MWD),[59] and the composite physiological index (CPI).[59] Among the biomarkers differentially expressed in the IPF group, the following biomarkers are related to the severity of the disease: KL-6, RAGE,[17] M2BP,[33] SAA,[28] CEA,[39] MMP3,[45] MMP7,[9,42,50,54] MMP10,[50] MMP28,[61] YKL-40,[79] α-defensin,[97] CXCL8,[170] CXCL13,[45] CCL2,[115] ICAM 2,[123] LTBP2,[123,130] E-selectin, HRG,[131] CD248,[176] VEGF,[170] ADAM17,[1] napsin A,[177] HMGB1,[172] p16,[178] and alveolar NO.[173]

Treatment-related Biomarkers

Serum IGFBP-2 was significantly reduced in patients receiving specific antifibrotic therapy (pirfenidone and nintedanib) compared to untreated patients.[7] Angiogenesis cytokines (bFGF, PLGF, and VEGF-A), anti-inflammatory cytokines (IL-10 and IL-4), and SP-D levels in BALF increased significantly after 6 months of pirfenidone therapy,[121] while Gal-3 levels in BALF appeared to be lower in IPF patients receiving corticosteroid therapy.[95]

Conclusions

HRCT is not always useful for diagnosis due to the presence of other chronic fibrotic lung disorders such as cHP and CTD-ILD. In addition,other idiopathic ILDs may exhibit a UIP-like pattern. This study summarized over 100 differentially expressed biomarkers for patients with IPF compared with HCs, and among these markers, 19 were specific to IPF, which is of great clinical value for the differential diagnosis of IPF. The existing physiological and imaging measurements (pulmonary function tests and HRCT) used to evaluate the occurrence of AE, disease progression, and disease severity present limitations. Pulmonary function tests are influenced by several factors and repeated imaging examination may cause radiation damage to patients. Thus, the present review summarized biomarkers for identifying the onset of AE, for predicting the prognosis of patients, for assessing disease severity, and for predicting the patient's response to treatment to achieve better patient stratification. Combining physiological measurements and biomarkers may increase the accuracy and sensitivity of predictive analysis. With the exception of SP-A, SP-D, and KL-6 which are used in clinical practice in Japan, most other biomarkers are still being evaluated in clinical trials. The biomarkers mentioned in this article are mainly evaluated individually, and the research to evaluate the value of biomarker panels in clinical practice is very limited. The etiology of IPF is complex, and many factors and signal pathways are involved in the pathogenesis of IPF. Thus, a single biomarker is unlikely to have a transformative effect on clinical practice, and therefore, the combined effect of various biomarkers can be considered to improve the accuracy of diagnosis, disease assessment, and monitoring of response to treatment for IPF. Thus, large-scale multicenter studies are needed to design and validate suitable biomarker panels that have diagnostic utility for IPF. The combined application of biomarker panels and physiological and imaging parameters in the diagnosis and treatment of IPF may be a trend in the future.

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Conflicts of interest

None.

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