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Diagnostic and Prognostic Biomarkers for Chronic Fibrosing Interstitial Lung Diseases with a Progressive Phenotype

Yoshikazu Inoue, MD, PhD, Robert J. Kaner, MD, Julien Guiot, MD, PhD, Toby M. Maher, MD, PhD, Sara Tomassetti, MD, Sergey Moiseev, MD, Masataka Kuwana, MD, PhD, Kevin K. Brown, MD

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Running header: Biomarkers in Interstitial Lung Diseases

Yoshikazu Inoue, MD, PhD; Robert J. Kaner, MD; Julien Guiot, MD, PhD; Toby M. Maher, MD, PhD; Sara Tomassetti, MD; Sergey Moiseev, MD; Masataka Kuwana, MD, PhD; and Kevin K. Brown, MD

AFFILIATIONS: From the Clinical Research Center (Dr Inoue), National Hospital Organization Kinki-Chuo Chest Medical Center, Osaka, Japan; Pulmonary and Critical Care Medicine, Genetic Medicine (Dr Kaner), Weill Cornell Medicine, New York, NY, USA; Department of Respiratory Medicine & Critical Care Medicine (Dr Guiot), CHU Liege, GIGA I^3 Research Group, University of Liege, Liege, Belgium; NIHR Respiratory Clinical Research Facility (Dr Maher), Royal Brompton Hospital, London, UK, and Fibrosis Research Group, National Heart and Lung Institute, Imperial College, London, UK; Department of Diseases of the Thorax (Dr Tomassetti), Ospedale GB Morgagni, Forlì, Italy; Tareev Clinic of Internal Diseases (Dr Moiseev), Sechenov First Moscow State Medical University, Moscow, Russia; Department of Allergy and Rheumatology (Dr Kuwana), Nippon Medical School, Tokyo, Japan; Department of Medicine (Dr Brown), National Jewish Health, Denver, Colorado, USA.

CORRESPONDENCE TO: Yoshikazu Inoue, MD, PhD, Executive Director, Clinical Research Center, National Hospital Organization Kinki-Chuo Chest Medical Center, 1180 Nagasone-cho, Kita-ku, Sakai, Osaka, 591-8555, Japan; e-mail: giichiyi@me.com

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ABSTRACT

Biomarkers have the potential to become central to the clinical evaluation and monitoring of patients with chronic fibrosing interstitial lung diseases with a progressive phenotype. Here we summarize the current understanding of putative serum, bronchoalveolar lavage fluid and genetic biomarkers in this setting, according to their hypothesized pathobiologic mechanisms: evidence of epithelial cell dysfunction (eg, Krebs von den Lungen-6 antigen), fibroblast proliferation and extracellular matrix production/turnover (eg, matrix metalloproteinase-1), or immune dysregulation (eg, CC chemokine ligand 18). While most of the available data comes from idiopathic pulmonary fibrosis, the prototypic progressive fibrosing interstitial lung disease, there are data available in the broader patient population of chronic fibrosing interstitial lung diseases. While a number of these biomarkers show promise, none have been validated. In this review article, we assess both the status of proposed biomarkers for chronic fibrosing lung diseases with a progressive phenotype in predicting disease risk or predisposition, diagnosis, prognosis and treatment response, and provide a direct comparison between idiopathic pulmonary fibrosis and other chronic fibrotic interstitial lung diseases. We also reflect on the current clinical usefulness and future direction of research for biomarkers in the setting of chronic fibrosing interstitial lung diseases with a progressive phenotype.

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Introduction

Over 200 distinct pulmonary disorders fall under the heading of interstitial lung disease (ILD), with idiopathic pulmonary fibrosis (IPF) being the most recognized.[1] Characterized by the development of progressive pulmonary fibrosis, IPF results in lung function and quality of life deterioration and worsening respiratory symptoms.[2] A similar chronic progressive fibrosing phenotype occurs in varying proportions of patients with other fibrotic ILDs, eg, idiopathic non-specific interstitial pneumonia (iNSIP), hypersensitivity pneumonitis (HP), systemic sclerosis-associated ILD (SSc-ILD), rheumatoid arthritis-associated ILD (RA-ILD), and sarcoidosis. Although no formal definition of 'progressive' exists, Cottin et al. suggest that patients meeting any of the following criteria within a 24-month period have experienced disease progression: a relative decline of ≥10% in forced vital capacity (FVC); a relative decline of ≥15% in diffusing capacity of the lung for carbon monoxide (DLCO); or worsening symptoms or radiological appearance accompanied by a ≥5% relative decrease in FVC.[4] All patients with ILD with a chronic progressive fibrosing phenotype share some clinical, radiological and pathological characteristics.[5-9] Prognosis is broadly consistent across cohorts of individuals with chronic fibrosing ILDs with a progressive phenotype, strengthening the rationale for grouping these diseases. However, determining an individual patient's risk of progression, long-term prognosis and likelihood of treatment response is challenging, due to the intrinsic variability seen among patients even with the same diagnosis.

A biomarker may be defined as 'any substance, structure, or process that can be measured in the body or its products and influences or predicts the incidence of outcome or disease'.[10] For the purposes of this article, we have considered molecular (protein and RNA) markers that can be quantified in biological tissue or fluids (eg, whole blood, serum, bronchoalveolar lavage fluid [BALF], induced sputum), that reflect physiologic or pathologic processes, or that reflect pharmacological responses to a therapeutic intervention.[11] Non-protein biomarkers (eg, mitochondrial markers, micro-ribonucleic acid, quantitative imaging, cell counts in BALF, lung microbiome analyses, lung physiology) are beyond the scope of this article. Biomarker development has been identified as a key step towards establishing personalized medicine.[12] In the field of chronic fibrosing ILDs with a progressive phenotype, biomarker development aims to establish easily measurable variables that allow improved clinical classification of

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different ILDs, predict prognosis or likelihood of response to therapy or which monitor treatment response.[13]

This review describes the typical classification of currently proposed biomarkers according to their hypothesized pathobiologic mechanisms: alveolar epithelial cell injury, inflammation and fibrosis, tissue remodeling and repair, and immunological changes.[11,14] We subsequently examine the most promising serum and BALF biomarkers and propose to classify them by whether they are associated with disease predisposition, diagnosis, disease progression and prognosis, or response to treatment. We elaborate this classification further by directly comparing biomarker profiles of IPF and non-IPF chronic fibrosing ILDs with a progressive phenotype.

Finally, we provide an analysis of the current clinical usefulness of biomarkers in fibrosing ILDs, and offer recommendations for future biomarker development and research directions.

Candidate Biomarkers by Mechanistic Pathway

Some of the most promising biomarkers of chronic fibrosing ILDs with a progressive phenotype are markers of proposed mechanisms involved in disease pathogenesis.

Epithelial cell dysfunction: A number of molecules are markers of alveolar epithelial cell injury/regeneration.[15] Krebs von den lugen-6 (KL-6), a submolecule of mucin 1,[16] is a glycoprotein expressed in type II pneumocytes and bronchiolar epithelial cells that may be involved in promoting the migration, proliferation and survival of lung fibroblasts.[17] The mucin gene *MUC5B* encodes mucin 5B, a major gel-forming protein in human airway secretions that has been linked to the maintenance of airway health.[18] Surfactant proteins A and D (SP-A and SP-D) are lipoprotein complexes secreted by type II pneumocytes and airway cells; they are involved in stabilizing alveolar surface tension at the air–liquid interface and supporting lung host innate-immunity.[14] YKL-40 is a chitinase-like protein, involved in innate immune system and cell processes in relation to extracellular matrix (ECM) remodeling.[14]

Extracellular matrix turnover: The development of pulmonary fibrosis is characterized by increased turnover of ECM. Transforming growth factor-β (TGF-β) plays an important role, but has not been widely explored as a pulmonary fibrosis biomarker due to its ubiquity and the

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difficulty of accurately quantifying it. Other markers of ECM production and turnover, however, appear more promising. Matrix metalloproteinases (MMPs) are zinc-dependent protease enzymes regulating the ECM remodelling.[14,19] Lysyl oxidase-like 2 (LOXL-2) catalyzes the cross-linking of collagen and has been identified as a key mediator of fibrosis.[20] It is highly expressed in fibrosing lungs, and is believed to play key roles in ECM remodeling and fibrogenesis.[11,14,21] Insulin-like growth factor (IGF) has been shown to stimulate the production of ECM by fibroblasts, and to encourage epithelial cells to proliferate. IGF binding proteins (IGFBPs) contribute to IGF activity by facilitating transportation and receptor binding of IGF.[22,23] Vascular endothelial growth factor (VEGF) is a growth factor with proangiogenic activity and is believed to help protect the epithelium from injury and to encourage tissue repair.[24] In response to lung injury, airway club cells produce and release a low-molecular-weight protein, the 16 kDa Clara cell secretory protein (CC16).[14] Periostin is an ECM protein which belongs to the fasciclin family; serum levels of periostin increase in IPF and other fibrotic idiopathic interstitial pneumonias, and are associated with declines in DLCO and vital capacity.[25]

Immune dysregulation: These biomarkers include chemokines, cytokines and their receptors, of which the most promising are C-C motif chemokine ligand 18 (CCL18) and interleukin 6 (IL-6).[14] CCL18 is derived from alveolar macrophages and appears to have numerous functions beyond its role as a chemoattractant, including the regulation of fibrosis.[26] Levels of the proinflammatory and profibrotic cytokine IL-6 are increased in serum and BALF in many pulmonary diseases.[14]

Biomarker Applications in Chronic Fibrosing ILDs with a Progressive Phenotype

Risk or predisposition biomarkers (table 1)

Data in IPF: Some genetic variants have been associated with an increased likelihood of developing IPF. Studies suggest that the single nucleotide polymorphism (SNP) rs35705950 in the promoter region of the *MUC5B* gene increases the risk of developing IPF although it is associated with a less progressive form of the disease.[27] *TOLLIP* encodes the toll-interacting protein, which regulates immune responses mediated through toll-like receptors, including the

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modulation of TGF-β signaling.[28] Three SNPs in the *TOLLIP* gene have been linked with susceptibility to IPF.[29] Telomerase complex genes include *TERT* and *TERC*, which encode telomerase reverse transcriptase and ribonucleic acid component, respectively. Heterozygous mutations in either the *TERT* or *TERC* genes, or shortened telomeres, may be associated with an increased risk of IPF.[30] In addition to these variants in *MUC5B*, *TOLLIP* or *TERT/TERC*, a recent resequencing study of 3,624 IPF and 4,442 control samples highlighted that rare variants in the *FAM13A* and *RTEL1* genes were also contributing to the genetic risk of developing IPF.[31] Human leukocyte antigen (HLA) class I and II histocompatibility genes encode HLAs, which regulate the immune response to presenting antigens. The HLA-A*02-DRB1*04 haplotype has been associated with genetic susceptibility for IPF.[32]

Data in other chronic fibrosing ILDs with a progressive phenotype: As described above, there is evidence that individuals with the SNP rs35705950 in the *MUC5B* gene have an increased risk of developing IPF; this SNP is also associated with an increased risk of RA-ILD.[33] However, rs35705950 does not appear to be associated with pulmonary fibrosis in SSc, sarcoidosis or myositis-associated ILD.[34]

Certain HLA haplotypes are also associated with the development of non-IPF ILDs. The HLA-DRB1*1501/HLA-DQB1*0602 haplotype, for example, has been associated with chronic sarcoidosis.[35] The rare *HLADRB5*01:05* allele may predict the development of ILD in patients with SSc.[36] There is also evidence that specific HLA alleles, including *HLA-DRB1*1502*, are associated with an increased risk of ILD in patients with RA.[37,38]

Diagnostic biomarkers (table 2)

Data in IPF: Elevations in levels of MMP-1 (serum), MMP-7 (serum, BALF and induced sputum) and other MMPs are recognized in IPF.[14,39,40] In addition, serum and sputum from patients with IPF display significantly higher levels of IGFBP-2 ($P < .001$) compared with healthy subjects.[41,42]

Increased circulating levels of C-X-C motif chemokine 13 (CXCL13) have been observed in patients with IPF vs healthy controls.[43] Elevated levels of the proinflammatory monocyte/macrophage-derived calcium-binding proteins, S100A8 (also known as calgranulin A

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– levels increased in plasma) and S100A9 (calgranulin B – levels increased in BALF), have been found in IPF.[14,44]

While the numbers of circulating fibrocytes (precursors of fibroblasts) have been suggested to be increased in patients with IPF,[14,45] there are contradictory data regarding VEGF and its role. Serum levels of VEGF have been shown to be elevated in patients with IPF,[46] whereas BALF levels appear to be reduced in patients with IPF vs healthy controls.[24,47]

Data in other chronic fibrosing ILDs with a progressive phenotype: Elevated KL-6 concentrations have been reported in the sera and BALF of patients with idiopathic interstitial pneumonia, HP, pulmonary sarcoidosis, asbestosis, and connective tissue disease-associated ILDs (CTD-ILDs).[14,37,48-52] Recently, BALF levels of both KL-6 and S100A9 were also found to be significantly higher in patients with fibrotic iNSIP than in healthy subjects, and were similar to those in patients with IPF.[53] Serum levels of SP-A and/or SP-D are increased in fibrosing ILDs (eg, HP, iNSIP, SSc-ILD); high levels of these lipoproteins have been associated with a progressive phenotype in patients with iNSIP, and with decreases in DLCO and FVC.[14,37,54] Increased concentrations of the chemokine CC16 have also been observed in the sera and BALF of patients with pulmonary sarcoidosis, asbestosis and SSc-ILD.[55-57]

Increased levels of MMP-1 and MMP-7 have been reported in sarcoidosis, RA-ILD and SSc-ILD.[58-60] Serum levels of MMP-7 are significantly higher in patients with RA-ILD vs RA patients without ILD.[61] Increased serum and/or BALF levels of other MMPs and tissue inhibitors of MMPs (TIMPs) have been reported in various ILDs.[14,37] For example, BALF levels of MMP-2 are higher in patients with NSIP than in those with IPF.[62] It was reported that, among patients with elevated MMP-7, the absence of concurrent increases in SP-D and osteopontin suggests the presence of a non-IPF/non-usual interstitial pneumonia pattern ILD.[63] Patients with sarcoidosis show increased expression of MMP-12 versus controls, with correlation between expression levels and disease severity.[64] An increased number of circulating fibrocytes has been observed in patients with RA-ILDs.[65]

Just as for IPF, serum levels of S100A8 and S100A9 were reported to be elevated in SSc, with the highest levels in patients with lung fibrosis.[14,66]

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A number of chemokines have shown correlations with the presence of ILD. Elevated levels of CCL18 have been found in the sera, BALF and lung tissue of patients with SSc-ILD and other chronic fibrosing ILDs with a progressive phenotype.[67] In sarcoidosis, levels of CCL18 appear to correlate with the extent of disease activity.[68] Increased BALF and sera levels of CCL2 were seen in patients with SSc, and have been shown to correlate with the presence of ILD.[69]High levels of CCL15 have been associated with progressive sarcoidosis.[35] CXCL10 and CXCL11 have also been identified as possible diagnostic markers for this disease, with the possibility of CXCL10 enabling differentiation between active and inactive forms.[35] RA patients with ILD have significantly higher serum CXCL10 levels than those without ILD.[61] The cytokine IL-6, known to be elevated in a variety of inflammatory diseases (such as sepsis),[70] was found to be increased in BALF of patients with SSc-ILD, alongside IL-4, IL-7 and IL-8.[60,69] Increased levels of interleukins (e.g. IL-12, IL-18) have been associated with sarcoidosis.[35,71] Several other biomarkers related to immune function – soluble IL-2 receptor (sIL-2R), C-reactive protein (CRP) and serum amyloid A (SAA) – have shown high sensitivity for sarcoidosis.[71]

Serum autoantibodies and immunological proteins associated with pulmonary fibrosis in SSc-ILD include anti-topoisomerase I antibodies, autoantibodies to small nuclear ribonucleoproteins (RNPs; eg, anti-U1, -U3 and -U11/U12 RNPs), and anti-endothelial cell antibodies.[37] Serum autoantibodies against myxovirus resistance protein 1 (MX1) have been identified as a possible diagnostic biomarker for iNSIP.[72] Other potential serum and BALF biomarkers that have been investigated include circulating cells and molecules associated with macrophage/monocyte activation or endothelial cell injury.[14,37,73]

Chitotriosidase is an enzyme produced by alveolar macrophages in patients with sarcoidosis. Detected in BALF, chitotriosidase levels have been reported to correlate with the severity of sarcoidosis.[35]

Prognostic biomarkers (table 3)

Data in IPF: Threshold serum levels of KL-6, or sequential changes in KL-6 levels, have been shown to predict lung function decline or outcome in patients with IPF.[74] In addition, increased serum and BALF YKL-40 levels have been shown to predict lower survival rates,[75] and high

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levels of circulating fibrocytes have been associated with increased risk of mortality.[14,45] In patients with IPF, elevated MMP-7 was strongly associated with reduced survival.[40,76,77] High serum levels of SP-A and/or SP-D have been associated with decreases in DLCO and FVC;[76,78,79] these increases may also be predictive of mortality.[76,80]

The potential for markers of ECM turnover to serve as biomarkers was investigated in the Prospective Observation of Fibrosis in the Lung Clinical Endpoints (PROFILE) study.[81] Six collagen-derived neoepitopes showed increased serum levels in patients with IPF compared with healthy volunteers, and higher levels in patients with progressive vs stable disease. Patients with IPF showing faster rates of increase in these biomarkers over 3 months showed more rapid disease progression and reduced survival. Serum LOXL-2 levels are also increased, and linked to an increased risk of disease progression (hazard ration [HR] 5.41, 95% CI 1.65–17.73).[20,43] However, a recent therapeutic trial of an anti-LOXL2-targeted therapy (simtuzumab) failed to demonstrate an effect on IPF progression.[82]

There is some evidence that CCL18 levels can be predictive of disease progression (eg, reduction in FVC) and mortality in IPF.[79,83] Furthermore, serum IL-6 levels have been shown to be predictive of DLCO decline.[84] CA19-9 and CA-125, which are markers of epithelial damage and secreted from the metaplastic epithelium, are also higher in patients with a progressive disease. Rising concentrations of CA-125 over 3 months were shown to be associated with increased risk of mortality.[85] The hexameric ECM glycoprotein tenascin-C, expressed during tissue injury, was shown to be highly upregulated in fibrotic lungs compared with normal lung tissue.[86] A correlation between lung levels of tenascin-C and the progression of lung fibrosis (%FVC decline over 6 months) has been demonstrated.[86] The same study reports tenascin C presence within fibroblastic foci, which represent active sites of altered wound healing in usual interstitial pneumonia.[86]

Importantly, genetic variants of *MUC5B* and *TOLLIP* have been shown to have prognostic significance in IPF.[87,88] Shortened telomere length has been associated with decreased survival.[89] Additionally, IPF patients with anti-heat shock protein 70 autoantibodies in their plasma appear to have increased risk of lung function deterioration and mortality.[90]

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Combined assessment of multiple biomarkers appears promising on the basis that it may enable the detection of multiple aspects of disease progression (eg, epithelial cell injury and repair, alveolar macrophage activation, neutrophil recruitment/activation or oxidative stress in the lung).[88] Investigation of the relationship between gene expression in peripheral blood mononuclear cells and survival among patients with IPF highlighted that survival was lower in patients with decreased expression of *CD28*, *ICOS*, *LCK* and *ITK.*[91] These four genes were later confirmed, as part of a 52-gene expression signature, to be predictive of prognosis in patients with IPF.[92] Prior to that, a panel of five plasma biomarkers: MMP-7, S100 calcium-binding protein A12 (S100A12), IL-8, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 (VCAM-1) also showed potential as predictors of IPF prognosis.[77]

Data in other chronic fibrosing ILDs with a progressive phenotype: At different cut-offs, serum KL-6 has been shown to predict lung function decline and/or outcome in patients with CTD-ILDs, iNSIP and HP.[54,93-96] In SSc-ILD, for example, serum KL-6 levels have been associated with the degree of inflammation and fibrosis, current impairment, as well as future decline in lung function (FVC or DLCO).[52,97-101] In iNSIP, patients with higher concentrations of both KL-6 and S100A9 BALF levels had more advanced disease with notably lower FVC, DLCO and 6-min walk test distance.[53] High serum levels of SP-A and/or SP-D have been associated with decreases in DLCO and FVC in patients with diseases such as HP, iNSIP, SSc-ILD.[14,37,95,102]

In patients with SSc-ILD, serum YKL-40 elevations appear to correlate with airway obstruction, low DLCO and mortality.[103] Among patients with HP, increases in serum YKL-40 have been correlated with DLCO and appear to predict poor prognosis.[104] In addition, there is some evidence that serum YKL-40 may be associated with disease activity and ongoing fibrosis in patients with pulmonary sarcoidosis and polymyositis/dermatomyositis-associated ILD.[105,106] High MMP-7 levels in HP have been linked with reduced survival.[107] In patients with SSc-ILD, increased serum levels of MMP-12 appear to correlate with decreased FVC, and raised levels of TIMP-1 are associated with decreased DLCO.[108] High levels of circulating fibrocytes have been associated with reduced lung function and an increased risk of ILD-associated mortality.[65]

Serum levels of CC16 correlate inversely with both lung function and disease activity in patients with SSc-ILD.[57] A randomized, placebo-controlled trial showed that IL-6 plays a mechanistic role in SSc-ILD, as anti-IL-6 treatment slowed the decline in FVC more than

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placebo.[109] A large cohort study of patients with SSc-ILD reported that IL-6 predicted declines in DLCO and FVC, and death.[84] There have been numerous reports that other biomarkers of immune dysregulation may be particularly associated with SSc-ILD. Elevated levels of CRP have been linked with increased risk of progression.[101] Several studies have demonstrated CCL18 potential as a biomarker of change in total lung capacity, disease progression and prognosis, though the data on its ability to predict physiologic change are mixed.[101,110,111] BALF concentrations of CCL2 have also been associated with lung function parameters and computed tomography fibrosis scores in SSc-ILD patients.[69] In line with the data in IPF, patients with CTD-ILD or SSc-ILD appear to have an increased risk of mortality if they also have high levels of CA-19-9 or CA-125.[107,112-114] Similarly, in CTD-ILD and HP, mortality risk has been shown to be raised in patients with high plasma concentrations of VCAM-1.[107] Analysis of specimens from a multicenter study suggested that the pro-angiogenic and profibrotic factor, CXCL4, may be a useful serum biomarker for SSc-ILD because of its correlation with pulmonary fibrosis and disease severity.[115] It may also be useful for monitoring response to immunosuppressive therapy.[116] Serum CXCL10 may have utility in identifying progressive disease in SSc-ILD and in predicting outcomes in pulmonary sarcoidosis.[35,117] SSc-ILD disease progression was also correlated with high serum levels of another chemokine, fractalkine (CX3CL1).[118] High levels of CXCL13 have been associated with decreased survival in patients with CTD-ILD or HP.[107]

Telomere shortening also correlates with worse outcome in chronic fibrosing ILDs with a progressive phenotype.[119]

Candidate biomarkers for predicting the development of ILD in patients with RA include anti-citrullinated protein antibodies.[120] Levels of interferon γ in BALF of patients with RA-ILD have also been suggested to predict the risk of disease progression.[61]

iNSIP patients with anti-MX1 autoantibodies have been reported to have improved prognosis compared to those without anti-MX1 autoantibodies.[72]In SSc, patients testing positive for anti-Scl-70 antibodies and negative for anti-centromere antibodies appear to have higher risk of progressive ILD.[101] Conversely, the presence of autoantibodies against heat shock protein 70 in patients with ILDs other than IPF does not seem to have any clinical significance.[90]

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There may be potential for using biomarkers to predict acute exacerbations of fibrosing lung disease with a progressive phenotype. Blood or serum levels of KL-6 or α-defensins appear promising in this regard, while levels of SP-D and leptin have also been reported to increase in patients with acute exacerbations.[11,121] However, relatively little research has been performed in this area and further data are required.

Therapeutic biomarkers (table 4)

Data in IPF: KL-6 is a well-established biomarker in IPF. However, its utility as a potential therapeutic biomarker is complicated by conflicting data regarding the extent to which KL-6 levels are affected by anti-fibrotic (nintedanib or pirfenidone) treatment.[122]

Serum concentrations of IGFBP-2 in patients with IPF were reported to be higher than in healthy subjects.[41] The same study showed that IGFBP-2 levels in patients receiving anti-fibrotic therapy were significantly lower than in untreated patients, while remaining significantly higher than in healthy subjects.[41]

The same team later reported that levels of the cell-free nucleosomes 5mC and mH2A1.1 (epigenetic biomarkers) were significantly lowered ($P < .05$ and .01, respectively) in serum samples from untreated patients with IPF, compared to patients treated with anti-fibrotic therapy (pirfenidone or nintedanib).[123]

Interestingly, rs3750920 polymorphism of *TOLLIP* seems to influence the response of patients with IPF to N-acetylcysteine therapy. Evidence for potential interaction between N-acetylcysteine and rs35705950 within *MUC5B* was also observed but not significant.[87]

In the INMARK® trial (NCT02788474) of patients with IPF, treatment with nintedanib vs placebo for 12 weeks did not affect the rate of change in C-reactive protein degraded by MMP 1 and 8, suggesting that it is not a marker of response to nintedanib in patients with IPF.[124]

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New directions for biomarker development in chronic fibrosing ILDs with a progressive phenotype

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While numerous associations have been reported between fibrosing ILDs and serum, BALF and genetic biomarkers, very few are purposefully or routinely used in patient clinical evaluation. Most of these potential biomarkers were investigated in an observational and retrospective manner and, more importantly, without robust validation of assays or replication of findings in separate prospective cohorts. Lack of progress towards using biomarkers in clinical practice is frustrating given that significant numbers of studies have been published, for example on MMP7 and KL6. Strategies for moving the field forward in fibrosing lung disease are outlined in table 5. Potential biomarkers need to be thoroughly validated, according to standardized guidelines. Only then will biomarkers gain regulatory approval and insurance coverage, enabling their transition from 'reported associations' to clinical implementation. Biomarkers have the potential to help enable differential diagnosis (eg, between IPF and non-IPF fibrosing ILDs), more effective patient stratification (eg, determine the subtype of ILD or identify patients at risk of progression) and better up front selection of therapy. Most importantly, they may allow clinicians to monitor early treatment response, which remains a huge unmet need. Ongoing placebo-controlled clinical trials in patients with non-IPF fibrosing ILDs involve measurement of potential biomarkers as clinical endpoints. Chronic fibrosing ILDs with a progressive phenotype are an important emerging target for anti-fibrotic therapies, with two recently published clinical trials for non-IPF patients supporting the use of anti-fibrotic therapy in this patient group [7,8,84] and the FDA granting nintedanib breakthrough therapy designation in this setting.

In addition to assessing biomarkers in clinical studies, we emphasize the need to continue observing biomarkers in large cohorts of patients, such as the EUSTAR group.[125] We also believe that it is important to continue assessing biomarkers in large registries of patients, such as the EUSTAR group or in prospective cohort studies such as PROFILE and IPF-PRO.[126,127]

In light of the emerging new concept of chronic fibrosing ILD with a progressive phenotype,[7] effective treatment development will require the implementation of new specific and sensitive therapeutic biomarkers. In terms of future research directions, we believe there is the potential for combinations of blood biomarkers, or even combinations of blood biomarkers with demographic, clinical or imaging findings, to optimise diagnosis and disease management. Thought will need to be given to avoid complexity and to ensure that combinatorial biomarker

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signatures retain applicability in a routine clinical setting. Novel multiplex biomarker assays are in development;[128] such platforms should contribute to enhanced patient screening, prognostication and care. Analysis of biomarkers relating to a particular therapy could be useful for predicting the likelihood of a response to the treatment.[129] Finally, emergence of novel categories of biomarkers (eg, exosomes, mitochondrial DNA, micro-ribonucleic acid, quantitative imaging, transcriptomic, microbiome-related) offer new and thriving areas of research which should complement and strengthen existing biomarker strategies.[130]

Conclusions

In the challenging field of chronic fibrosing ILDs with a progressive phenotype, successful biomarker development should improve the diagnosis and prediction of longitudinal disease behaviour (eg, identify subgroups of patients mostly at high risk of disease progression), as well as monitoring enabling measurement of the outcomes of treatment.

In future, it is hoped that the ongoing implementation of multiple biomarker analyses in large international, prospective and adequately powered clinical studies, will deliver significant data that will convince clinicians of the value of using biomarkers at multiple stages of the diagnosis and management of chronic fibrosing ILDs with a progressive phenotype.

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Tables

TABLE 1 Risk/Predisposition Biomarkers

	Mechanistic Pathway	Biomarker	Disease Subcategory[a]
IPF	Epithelial cell dysfunction and ECM remodelling	*MUCB5*[27,43,131] *TERT; TERC*[30,132] *FAM13A; RTEL1*[31]	
	Immune dysregulation	*TOLLIP*[29] *HLA*[32]	
Chronic fibrosing ILDs with a progressive phenotype	Epithelial cell dysfunction and ECM remodelling	*MUCB5*	RA-ILD[33]
	Immune dysregulation	*HLA*	Sarcoidosis,[133,134] SSc-ILD,[36] RA-ILD[37,38]

HLA = human leukocyte antigen; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis; RA-ILD = rheumatoid arthritis-related ILD; SSc-ILD = systemic sclerosis-related ILD.

[a]For chronic fibrosing ILDs with a progressive phenotype.

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TABLE 2 Diagnostic Biomarkers

	Mechanistic Pathway	Biomarker	Disease Subcategory[a]
IPF	Epithelial cell dysfunction and ECM remodelling	MMP-1/-7 and others MMPs[39,40,135]	
		IGFBP-2[41,42]	
		VEGF[24,46,47]	
		Periostin[25]	
		PAI-1[136]	
	Immune dysregulation	CXCL13[43]	
		SA100A8 / A9[14,44]	
Chronic fibrosing ILDs with a progressive phenotype	Epithelial cell dysfunction and ECM remodelling	KL-6	IIP, HP, CTD-ILD, sarcoidosis, asbestosis, iNSIP[14,37,48-51]
		SD-A; SD-D	HP, iNSIP, SSc-ILD[14,37,54,95]
		CC16	SSc-ILD, sarcoidosis, asbestosis[55-57]
		MMP-1 /-7 /-12	Sarcoidosis, RA-ILD, SSc-ILD[58-64]
		TIMP-1	SSc-ILD[14,37]
		Periostin	NSIP, cryptogenic organizing pneumonia[25]
	Immune dysregulation	CCL18; CCL2	SSc-ILD[67,69,137-139]
		CCL15; CCL18	Sarcoidosis[35,68]
		S100A8 / A9	iNSIP,[53] SSc-ILD[14,66]
		CXCL10	RA-ILD,[61] sarcoidosis[35]
		IL-4/-6/-7/-8	SSc-ILD[60,69,70]
		IL-12/-18	Sarcoidosis[35,71]
		sIL-2R	Sarcoidosis[35,71]
		Anti-topoisomerase I, anti-U1 RNP, anti-U3, anti-11/12 RNP, anti-endothelial antibodies	SSc-ILD[37]

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		CRP	
		SAA	Sarcoidosis[35,71]
		Anti-MX1	Sarcoidosis[35,71]
			iNSIP[72]

CCL = C-C motif chemokine ligand; CRP = C-reactive protein; CTD-ILD = connective tissue disease-associated ILD; CXCL = C-X-C motif chemokine; IGFBP2 = insulin-like growth factor binding protein 2; IL = interleukin; ILD = interstitial lung disease; iNSIP = idiopathic non-specific interstitial pneumonia; IPF = idiopathic pulmonary fibrosis; KL-6 = Krebs von den lugen-6; MMP = matrix metalloproteinase; MX1 = myxovirus resistance protein 1; PAI-1 = plasminogen activator inhibitor-1; SAA = serum amyloid A; sIL-2R = soluble interleukin-2 receptor; SP-A = surfactant protein A; SP-D = surfactant protein D; SSc-ILD = systemic sclerosis-related ILD; TIMP-1 = tissue inhibitors of MMP-1; VEGF = vascular endothelial growth factor.

[a]For chronic fibrosing ILDs with a progressive phenotype

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TABLE 3 Prognostic Biomarkers

	Mechanistic Pathway	Biomarker	Disease Subcategory[a]
IPF	Epithelial cell dysfunction and ECM remodelling	KL-6[74,140,141]	
		MMP-7[42,76,77]	
		SP-A; SP-D[76,78-80]	
		YKL-40[75]	
		ICAM-1; VCAM-1[77]	
		MUCB5; TOLLIP[87,88]	
		TERT; TERC[89,142]	
		CA-19-9[85]	
		CA-125[85]	
		Tenascin C[86]	
	Immune dysregulation	CCL18[79,83,143]	
		IL-6/-8[77,84]	
		LOXL-2[20,43]	
		S100A12[58]	
Chronic fibrosing ILDs with a progressive phenotype	Epithelial cell dysfunction and ECM remodelling	KL-6	iNSIP, HP, CTD-ILD, SSc-ILD[53,54,93-101,144-147]
		SP-A; SP-D	iNSIP, HP, SSc-ILD[14,37,95,102]
		YKL-40	HP, SSc-ILD, sarcoidosis[103-106]
		MMP-7	HP[107]
		MMP-12; TIMP-1	SSc-ILD[108,148]
		CC16	SSc-ILD[57]
		Tenascin C	SSc-ILD, sarcoidosis, HP[86,149,150]
		CA-19-9	CTD-ILD, SSc-ILD[112-114]
		CA-125	CTD-ILD, SSc-ILD[107,112-114]

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		VCAM-1	CTD-ILD, HP[107]
	Immune dysregulation	SA100A9	iNSIP[53]
		CCL2; CCL18	SSc-ILD[69,110,111,138,139,151,152]
		IL-6/-2	SSc-ILD[84,109]
		CRP	SSc-ILD[101]
		IFN-γ	RA-ILD[61,153]
		CXCL4; CXCL10; CX3CL1	SSc-ILD[72]
		CXCL13	CTD-ILD, HP[107]
		Anti-MX1	iNSIP[72]
		Anti-citrullinated protein	RA-ILD
		Chitotriosidase	Sarcoidosis[35]

CCL = C-C motif chemokine ligand; CTD-ILD = connective tissue disease-associated ILD; CX3CL1 = fractalkine; CXCL = C-X-C motif chemokine; HP = hypersensitivity pneumonitis; ICAM-1 = intercellular adhesion molecule 1; IFN-γ = interferon gamma; IL = interleukin; ILD = interstitial lung disease; iNSIP = idiopathic non-specific interstitial pneumonia; IPF = idiopathic pulmonary fibrosis; KL-6 = Krebs von den lugen-6; LOXL-2 = lysyl oxidase-like 2; MMP = matrix metalloproteinase; MX1 = myxovirus resistance protein 1; RA-ILD = rheumatoid arthritis-related ILD; SP-A = surfactant protein A; SP-D = surfactant protein D; SSc-ILD = systemic sclerosis-related ILD; VCAM-1 = vascular cell adhesion molecule 1.

[a]For chronic fibrosing ILDs with a progressive phenotype

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TABLE 4 Therapeutic Biomarkers

	Mechanistic Pathway	Biomarker
IPF	Epithelial cell dysfunction and ECM remodelling	KL-6[122,154] IGFBP-2[41] CRPM-1/-8; C3M; C1M[87,124] 5mC and mH2A1[123] *TOLLIP*[87] *MUC5B*[87]

C3M = collagen 3 degraded by metalloproteinase-9; C1M = collagen 1 degraded by metalloproteinase-2/9/13; CRPM-1/8 = C-reactive protein degraded by metalloprotinease-1/8; IGFBP = insulin-like growth factor binding protein; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis; KL-6 = Krebs von den lugen-6.

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TABLE 5 Proposed Approaches to Biomarker Development

Research Method	Rationale
Validation of potential biomarkers according to FDA (US), EMA (Europe) and PMDA (Japan) guidelines	Validation includes confirmation of biomarker behaviour in multiple prospective cohorts. The handling characteristics of specific assays need to be defined and shown to conform to regulatory expectations to ensure clinic-readiness.
Clinical trial validation of putative therapeutic biomarkers	Therapeutic biomarkers need to be tested in appropriately designed randomised controlled trials. The relevance of change in biomarker levels needs to be assessed against current end-points (FVC, mortality) and in exploratory responder-analyses.
Assessment of biomarkers in large registries and prospective cohort studies	Results of these studies will complement those from clinical trials and provide further support for the clinical relevance of the biomarker. Such studies may also be beneficial in determining interactions based on genotype and in identifying specific disease endotypes.
Investigate combinations of biomarkers	Machine learning and artificial intelligence approaches afford the opportunity for identifying combinatorial biomarker signatures which may be more informative than single markers alone. Such strategies have the potential to integrate information from multiple pathogenic pathways (eg epithelial turnover, matrix synthesis/degradation and inflammatory cell activation). A danger of this approach is increased complexity, ultimately any multi-biomarker signature needs to retain clinical relevance to ensure use in practice.
Omics analysis of multiple biomarkers and clinical data including pulmonary function tests, radiological data and disease behaviour	May help identify the best biomarkers or combinations of biomarkers for diagnosis, treatment and prognosis, in relation to a progressive phenotype.
Unbiased biomarker discovery	The advent of novel, unbiased broad-scale proteomic assays affords the opportunity for identifying novel disease biomarkers. Any proteins identified in this way will need robust validation as outlined in the steps above.

EMA = European Medicines Agency; FDA = Food and Drug Administration; PMDA = Pharmaceutical and Medical Devices Agency.

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Abbreviations:

BALF = bronchoalveolar lavage fluid; C1M = collagen 1 degraded by metalloproteinase-2/9/13; C3M = collagen 3 degraded by metalloproteinase-9; CC16 = 16 kDa Clara cell secretory protein; CCL = C-C motif chemokine ligand; CRPM-1/8 = C-reactive protein degraded by metalloprotinease-1/8; CTD-ILD = connective tissue disease-associated ILD; CX3CL1 = fractalkine; CXCL = C-X-C motif chemokine; DLCO = diffusing capacity of the lungs for carbon monoxide; ECM = extracellular matrix; EMA = European Medicines Agency; FDA = Food and Drug Administration; FVC = forced vital capacity; HLA = human leukocyte antigen; HP = hypersensitivity pneumonitis; ICAM-1 = intercellular adhesion molecule 1; IFN-γ = interferon gamma; IGF = insulin-like growth factor; IGFBP = IGF binding protein; IL = interleukin; ILD = interstitial lung disease; iNSIP = idiopathic non-specific interstitial pneumonia; IPF = idiopathic pulmonary fibrosis; KL-6 = Krebs von den lugen-6; LOXL-2 = lysyl oxidase-like 2; MMP = matrix metalloproteinase; MX1 = myxovirus resistance protein 1; PAI-1 = plasminogen activator inhibitor-1; PMDA = Pharmaceutical and Medical Devices Agency; RA-ILD = rheumatoid arthritis-related ILD; SAA = serum amyloid A; sIL-2R = soluble interleukin-2 receptor; SNP = single nucleotide polymorphism; SP-A = surfactant protein A; SP-D = surfactant protein D; SSc-ILD = systemic sclerosis-related ILD; TIMPs = tissue inhibitors of MMPs; TGF-β = transforming growth factor-beta; VCAM-1 = vascular cell adhesion molecule 1; VEGF = vascular endothelial growth factor

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