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Peripheral Blood Biomarkers in Idiopathic Pulmonary Fibrosis

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Abstract

In this article, we review the evidence for peripheral blood biomarkers in idiopathic pulmonary fibrosis (IPF), a life-threatening fibrotic lung disease of unknown etiology. We focus on selected biomarkers present in peripheral blood, as they are easy to obtain, can be measured longitudinally, and have the greatest likelihood of achieving clinical utility. This article concentrates on biomarkers with mechanistic plausibility that may be directly involved in the development of IPF, including KL-6, surfactant proteins A and D, matrix metalloproteases (MMP) 1 and 7, CCL18, VEGF, YKL-40, osteopontin, circulating fibrocytes, and T cells. After reviewing the evidence base for each, we designate the biomarkers that may have utility as: 1) diagnostic biomarkers to distinguish IPF from other interstitial lung diseases, 2) prognostic biomarkers that are correlated with disease progression or mortality, or 3) biomarkers that can be used as tools for serial monitoring of disease severity. Although there are no validated biomarkers that are currently available, the need for surrogates of diagnosis, prognosis, and monitoring of disease course with emerging therapies is great.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a life-threatening fibrotic lung disease of unknown etiology that affects up to 185,000 individuals in the United States(1,2). There are no effective therapies for IPF, leading to significant morbidity and mortality. The pathogenesis of IPF is not fully understood. Although IPF was initially thought to result from generalized pulmonary inflammation leading to fibrosis, the current paradigm has shifted towards alveolar epithelial cell dysfunction and disordered fibroproliferation(3). Current approaches looking for new biomarkers may provide new insights of underlying mechanisms of disease.

Although the median survival is 2–3 years, there is a wide spectrum of disease courses that can be generalized into three categories: stability or very slow decline, rapid deterioration, and periods of stability interspersed with periods of decline(3). Currently, it is difficult for clinicians to predict the disease course for an individual patient, as there are no accepted surrogates of these clinical courses.

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IPF Diagnostic Criteria

The most recent ATS/ERS/JRS/ALAT consensus statement recognizes IPF as a type of chronic, progressive interstitial lung disease with specific findings on high resolution CT scan and/or surgical lung biopsy(4). The characteristic CT finding for patients with IPF is a usual interstitial pneumonia (UIP) pattern, consisting of reticular abnormalities in a subpleural/basal distribution, with rows of subpleural cysts known as “honeycombing.” The histopathologic criteria for IPF are also described as a UIP pattern, characterized by temporally heterogeneous areas of fibrosis and subepithelial fibroblastic foci, which are aggregates of actively proliferating myofibroblasts and fibroblasts(5). The diagnosis of IPF also requires *exclusion* of other types of interstitial lung diseases. Some IPF patients may undergo a bronchoscopy with sampling of bronchoalveolar (BALF) fluid to evaluate for other etiologies at the discretion of their physician, but bronchoscopy is not required for diagnosis.

The need for biomarkers in IPF

Biomarkers act as surrogates for clinically meaningful outcomes and may or may not reflect the pathogenesis underlying a disease. Examples of clinical utility include diagnosis, the prediction of disease progression or regression, and prognostication of mortality. A biomarker should be easily acquired, reliably measured, and available for serial monitoring. Ideally, it would also provide an advantage over currently used clinical measures in ease, timeframe or expense.

The identification of biomarkers for IPF is attractive for several reasons. First, the diagnostic criteria rely on radiographic imaging and/or surgical lung biopsies interpreted by physicians with expertise in interstitial lung diseases. This expertise is often found at tertiary care centers, which may be geographically distant from patients and their primary physicians. The identification of serum biomarkers that are diagnostic for IPF would be helpful for both clinicians and patients, particularly in cases where a surgical lung biopsy cannot be obtained or access to dedicated interstitial lung disease physicians is limited. Secondly, the discovery of peripheral blood biomarkers that reflect disease activity would allow for serial monitoring as well as an objective marker to assess treatment efficacy. Lastly, a biomarker that provides prognostic information about disease course and/or mortality would be valuable for both clinical care and research study design.

We have chosen to focus on biomarkers that are present in peripheral blood, as they are easy to obtain, can be measured longitudinally, and have the greatest likelihood of achieving clinical utility. Currently there are no validated biomarkers that are routinely used in the clinical care of patients with IPF. For clarity, we have categorized them as markers of extra-pulmonary or pulmonary disease activity.

Biomarkers of extra-pulmonary manifestations

Brain natriuretic peptide (BNP)—Brain natriuretic peptide (BNP) is secreted by cardiac myocytes in response to volume or pressure overload(6). For patients with acute decompensated heart failure, elevated serum BNP levels are independently associated with mortality(7). BNP levels have also been studied as markers of pulmonary hypertension and right ventricular dysfunction(8).

Some IPF patients with advanced disease can develop pulmonary hypertension and right ventricular dysfunction. Song and colleagues evaluated the relationship between BNP and survival in 131 patients with IPF who had a serum BNP level and transthoracic echocardiogram performed within three days of one another(9). Echocardiographic evidence

of pulmonary hypertension was present in 25% of subjects, though only 14.5% of study subjects had elevated BNP levels. The mean survival of IPF patients with an elevated BNP was 11 months, significantly shorter than the 22.5 month mean survival of IPF patients with a normal BNP ($p<0.001$). Multivariate analysis demonstrated that elevated BNP levels were independent predictors of mortality (hazard ratio (HR) 1.118, 95% CI 1.047–1.194, $p=0.001$), and superior to elevated systolic pulmonary arterial pressures estimated by echocardiography. The 1-year mortality rate for IPF patients with elevated serum BNP levels was 70.5%, compared to 23.7% for patients with normal levels ($p<0.001$).

Serial measurements of BNP were available in a subset of 62 patients with IPF. Of the 51 patients who had a normal serum BNP initially, 13 (25.5%) developed elevated serum BNP levels on subsequent testing and demonstrated survival comparable to patients with elevated BNP at the initial measurement.

The relationship between elevated BNP and mortality has been validated in studies of interstitial lung disease patients, some of whom met criteria for IPF(10,11). BNP is not mechanistically related to the pathogenesis of IPF, but these results suggest that the development of an elevated BNP, initially or serially, may have value as a prognostic biomarker.

Albumin—The strong association between hypoalbuminemia and decreased survival has been demonstrated in several conditions, including cancers(12,13) and end stage renal disease(14,15). Zisman and colleagues studied the association between hypoalbuminemia and survival in 1269 IPF patients with severe disease who were listed for lung transplantation(16). The authors note that their multicenter cohort may have included patients with other types of interstitial lung diseases. In this study, lower serum albumin concentrations were strongly and independently associated with increased mortality. Adjusted for demographics, pulmonary function, and comorbidities, for each 0.5g/dL decrease in serum albumin, the hazard ratio for mortality was 1.54 (95% CI 1.32–1.79, $p<0.0001$). Although albumin is not directly involved in the development of IPF, these results suggest that low albumin levels may have utility as prognostic biomarkers.

Biomarkers that directly reflect pulmonary disease activity—Many of the potential biomarkers listed below were initially studied in BALF from patients with IPF. Although we will review data for each biomarker measured in BALF as well as peripheral blood, Table 1 summarizes available data regarding serum and peripheral blood biomarkers in IPF. If there is evidence that a biomarker can distinguish IPF from other interstitial lung diseases, we have noted that it may be a diagnostic biomarker. If the biomarker is correlated with disease progression or mortality, we have noted that it may be a prognostic biomarker. Lastly, if the biomarker is correlated with pulmonary function testing, we will note that it may have utility in serial monitoring as a reflection of the underlying disease process. Validation studies are needed before any of the following biomarkers can be applied for clinical use.

Krebs von den Lungen-6 Antigen (KL-6)—KL-6 is a mucin-like glycoprotein expressed at the extracellular surface of alveolar type II cells and bronchiolar epithelial cells(17). It acts as a chemotactic factor that promotes migration(18), proliferation, and survival(19) of lung fibroblasts. It has been studied in many interstitial lung diseases as a nonspecific marker of fibrosis.

Serum KL-6 levels are significantly elevated in patients with IPF compared to healthy volunteers(20,22) and patients with other interstitial lung diseases(20). KL-6 levels in serum have been shown to correlate to KL-6 levels in BALF ($r=0.451$, $p=0.0003$)(18) reflecting

increased production of KL-6 by the lungs(21). Compared with SP-A and SP-D, serum KL-6 had the highest accuracy for the diagnosis of interstitial lung diseases(22).

Serum KL-6 has been linked to survival. In a prospective study of 152 patients with idiopathic interstitial pneumonias and 67 patients with connective tissue disease-associated interstitial lung disease, patients with a serum KL-6 level >1000U/mL had worsened survival compared to those with lower levels(23). Elevated serum KL-6 levels were independently associated with survival by multivariate analysis (HR 2.95, 95% CI 1.71–5.08, $p=0.0001$).

In a small study, Yokoyama and colleagues noted that serial increases in serum KL-6 levels marked worsened survival. In contrast, decreasing serum KL-6 levels were associated with improved survival(24). However, there is an important limitation in the application of these results: at the time of this study, IPF patients were routinely treated with high dose corticosteroid therapy, which is no longer recommended as standard of care. It would be beneficial to reexamine serial KL-6 in IPF patients who are not treated with immunosuppressive medications to verify that these results remain valid.

KL-6 has mechanistic plausibility in the development of pulmonary fibrosis. These studies suggest that KL-6 may have utility as a diagnostic and prognostic biomarker.

Surfactant Proteins A&D

Pulmonary surfactants are lipoprotein complexes synthesized by airway Clara cells and alveolar epithelial cells, and secreted into a liquid layer that lines the epithelium(25). Surfactant decreases surface tension at the air-liquid interface in the lung, allowing the lung to expand more easily during inhalation and preventing collapse during exhalation. In addition, surfactant plays an important role in the host defense against pathogens. Although surfactant is mainly composed of lipids, proteins comprise 5–10% of these complexes(26). Surfactant proteins A (SP-A) and D (SP-D) are members of the collectin subgroup of the C-type lectin family.

Several studies have examined the diagnostic and prognostic ability of SP-A. BALF SPA levels are significantly lower in IPF patients compared to healthy controls ($p=0.0001$), but not significantly different from patients with other interstitial lung diseases(20). In contrast, serum SP-A levels are also significantly elevated in patients with IPF compared to healthy volunteers(20,22,27) and patients with other interstitial lung diseases(20).

Several investigators have examined the association between SP-A and mortality. SP-A expression in lung tissue was significantly decreased in IPF patients who deteriorated and died, compared to IPF patients with clinical stability(28). In contrast, increased serum SP-A levels obtained at the time of diagnosis in IPF patients were independently associated with death in three studies (27,29,30). Kinder et al. found that increased serum SP-A levels were independently associated with death or lung transplantation within one year (HR 3.27 for each standard deviation increase, 95% CI 1.49–7.17, $p=0.003$)(29). These results suggest that decreased lung SP-A and increased serum SP-A are both associated with increased mortality.

Unlike SP-A, BALF SP-D levels were not significantly different from healthy controls, but were significantly lower in IPF patients compared to other interstitial lung diseases(20). Serum SP-D levels were significantly elevated in patients with IPF compared to healthy volunteers and patients with other interstitial lung diseases(20,22,27). Two studies have demonstrated that increased serum SP-D levels were associated with increased mortality(27,30).

Surfactant proteins have mechanistic plausibility in the development of fibrosis, as supported by data showing that mutations in surfactant protein C (SP-C) have been associated with familial forms of pulmonary fibrosis(31–34). Because multiple studies have demonstrated that SP-A and SP-D can distinguish IPF patients from those with other interstitial lung diseases, and are correlated with disease progression or mortality, both SP-A and SP-D have potential to be diagnostic and prognostic biomarkers.

Matrix Metalloproteases 1 & 7 (MMP1, MMP7)

Matrix metalloproteases are a structurally and functionally related family of zinc-dependent proteases involved in the breakdown of extracellular matrix components(35). Members of this family have various target substrates and expression patterns. MMP1 is the most highly expressed interstitial collagenase and degrades fibrillar collagens(36). MMP7 is the smallest member of the MMP family, and is capable of degrading multiple components of the extracellular matrix.

There is animal data to support biologic plausibility for MMP7. MMP7 knockout mice treated with bleomycin do not develop pulmonary fibrosis(37), suggesting that MMP7 may play a critical role in the development of fibrosis. In humans, MMP7 expression has been localized to the cell surfaces of epithelial cells and alveolar macrophages from lung tissue of IPF patients(38). MMP7 is expressed in lung tissue from IPF patients, but is not detected in healthy lung tissue(37–39). Furthermore, BALF MMP7 levels in IPF patients are higher compared to healthy controls(38–40), suggesting that BALF levels may correlate with lung activity. However, MMP7 may not be specific for IPF, as MMP7 expression in BALF and lung tissue were not significantly different in IPF patients compared to those with cryptogenic organizing pneumonia(40) or other interstitial lung diseases(39).

The relationship between pulmonary function testing and BALF and serum MMP7 levels has been studied. BALF MMP7 levels were negatively correlated with forced vital capacity ($r=-0.348$, $p=0.02$)(39). This relationship was also demonstrated for serum MMP7: for each 1ng/ml increase in serum MMP7 levels, forced vital capacity (FVC) declined by 4% ($r=-0.51$, $p=0.002$) and diffusing capacity for carbon monoxide (DLCO) declined by 4.1% ($r=-0.53$, $p=0.002$)(41).

Serum and BALF MMP1 & MMP7 levels are significantly elevated in IPF patients compared to healthy controls(41). Although elevated levels of either serum MMP1 or MMP7 alone could not adequately distinguish IPF patients from healthy controls, a combined approach (MMP1 ≥ 2.15 ng/ml and MMP7 ≥ 1.99 ng/ml) was able to do so. Furthermore, combining serum MMP1 & MMP7 levels could also distinguish between IPF and hypersensitivity pneumonitis.

In a recently published large study, Richards et al. examined the concentration of 92 proteins in 241 subjects with IPF(42). Five of these proteins (MMP7, ICAM1, IL8, VCAM1, and S100A12) were significantly associated with mortality and/or disease progression. Plasma MMP7 levels >4.3 ng/mL were independently associated with increased mortality (adjusted HR 2.9, $p=0.0013$) in the derivation cohort of 140 IPF patients, and trended towards an association (adjusted HR 2.2, $p=0.071$) in the validation cohort of 101 IPF patients. The investigators also developed an index incorporating gender, FVC, DLCO, and plasma MMP7, which predicted mortality in their validation cohort.

MMP1 and MMP7 have mechanistic plausibility in the development of fibrosis, and used together have potential to be used as diagnostic biomarkers. MMP7 is elevated in other interstitial lung diseases; therefore it likely has limited potential as a diagnostic biomarker

used independently. Evidence suggests that MMP7 may also have utility as a prognostic biomarker, and could be used for serial monitoring of disease.

CC Chemokine Ligand 18

CC Chemokine Ligand 18 (CCL18) is a small protein that acts as a chemoattractant to guide cell migration. CCL18 is derived from alveolar macrophages in a wide variety of fibrotic lung diseases, including IPF, sarcoidosis, and systemic sclerosis, that stimulates collagen production in pulmonary fibroblasts(43,44). CCL18 carries additional names in the literature including pulmonary and activation-regulated chemokine (PARC)(45).

In patients with IPF, CCL18 measured in BALF and serum is significantly increased compared to healthy controls. Similar to systemic sclerosis patients, CCL18 negatively correlated with total lung capacity (TLC) & DLCO(46). When examined serially over a six-month period, serum CCL18 levels were inversely correlated to pulmonary function testing changes. Serum CCL18 may have prognostic value as well. In a study of 72 IPF patients, baseline serum CCL18 was correlated to changes in TLC & FVC over a 6 month period(47). Despite having comparable demographic characteristics, baseline disease severity, and treatment strategies, IPF patients whose serum CCL18 was <150ng/ml had significantly improved survival compared to those with a CCL18 >150 ng/ml. Multivariate analysis revealed that a serum CCL18 >150ng/ml was independently associated with death in IPF patients (Hazard ratio 7.98, 95% CI 2.49–25.51, $p=0.005$).

CCL18 may play a role in the development of fibrosis, but is likely non-specific. These data suggest that CCL18 may have potential as a prognostic biomarker that could also be used for serial monitoring of disease.

Vascular Endothelial Growth Factor

Vascular Endothelial Growth Factor (VEGF) is a glycoprotein expressed in alveolar epithelial cells(48) that induces vascular permeability and is a major regulator of angiogenesis(49). Although many interstitial lung diseases are associated with aberrant angiogenesis, its role in the pathogenesis of fibrosis has not yet been elucidated(50). In two separate studies, VEGF levels in BALF from IPF patients were significantly lower than those from healthy non-smoking individuals(51,52). BALF VEGF concentration was also correlated with DLCO for patients with IPF and sarcoidosis ($r=0.76$, $p<0.001$)(52). There was no correlation between BALF and serum VEGF levels in IPF patients; however this analysis was performed in only six patients with IPF.

Ando and colleagues further analyzed the significance of serum VEGF in IPF patients grouped by alveolar-arterial difference of oxygen (AaDO₂)(49). In this cohort of 41 IPF patients, the median AaDO₂ was 18.4 torr. There were significant differences in the median VEGF levels between IPF patients with low and high AaDO₂, 141.4 pg/mL and 241.0 pg/ml respectively ($p=0.030$), as well as between IPF patients with a high AaDO₂ and healthy volunteers (162.2pg/mL, $p=0.007$). Although serum VEGF levels did not correlate with baseline pulmonary function testing, there was an inverse correlation between baseline VEGF and change in vital capacity per month ($r=-0.38$, $p=0.044$). These investigators also identified a trend whereby IPF patients with serum VEGF levels above the median tended to have worse survival compared with patients with levels below the median (5 year survival 42.9% vs 80.0%, $p=0.075$). These data suggest that serum VEGF may reflect the severity of lung disease as well as predict declines in pulmonary function testing. The authors acknowledge that this was a single center retrospective study, and recommend that multicenter, larger studies are necessary to validate these findings.

The role of VEGF in the development of fibrosis remains unclear; however, VEGF may have potential as a prognostic biomarker.

YKL-40

YKL-40 is a chitinase-like protein that regulates cell proliferation and survival. It is not specific for IPF, as increased levels are also seen in fibrotic liver diseases(53), sarcoidosis(54), COPD(55), and asthma(56). Although its exact mechanism of action is unclear, YKL-40 appears to have mitogenic effects on lung fibroblasts(57), and induces alveolar macrophages to release profibrotic and proinflammatory cytokines(55). Immunohistochemical staining has demonstrated that YKL-40 localizes to bronchiolar epithelial cells and alveolar macrophages adjacent to fibrotic areas in IPF patients(58). Furuhashi and colleagues demonstrated that YKL-40's elevated activity in IPF patients is further reflected in BALF: YKL-40 levels are significantly elevated in IPF patients compared to healthy controls (17.8 ± 19.1 ng/mL vs. 0.3 ± 0.9 ng/mL, $p < 0.0001$)(58). Furthermore, these authors showed that mean serum levels of YKL-40 follow the same trend: serum YKL-40 is elevated in IPF patients compared to healthy controls, 245.8 ± 180.2 ng/mL vs. 116.0 ± 58.3 ng/mL, respectively ($p < 0.0001$). There was no correlation between serum and BALF levels of YKL-40, although only 7 IPF patients had matched samples available for analysis. Serum YKL-40 is negatively correlated with DLCO ($r = -0.41$, $p = 0.03$) and PaO₂ ($r = -0.42$, $p = 0.01$), and is positively correlated with AaDO₂ ($r = 0.39$, $p = 0.01$).

Korthagen and colleagues also examined the ability of YKL-40 to distinguish between IPF and healthy volunteers, and predict survival(59). Their findings corroborate that BALF and serum levels of YKL-40 are significantly elevated compared to controls without lung diseases (p values < 0.0001)(59). These authors analyzed serum and BALF samples from 60 IPF patients, and did not identify a correlation ($r^2 = 0.09$, $p < 0.05$). Additionally, there were no significant correlations between BALF or serum YKL-40 and pulmonary function tests. Serum YKL-40 levels > 79 ng/ml were associated with worsened survival ($p < 0.01$), as were BALF levels > 17 ng/ml ($p < 0.01$). After adjusting for demographic characteristics and disease severity, the hazard ratio for serum YKL-40 was 10.9 (95% CI 1.9–63.8, $p < 0.01$).

Although the role of YKL-40 in the development of fibrosis is unknown, YKL-40 has potential to be a prognostic biomarker that could also be used for serial monitoring of disease.

Osteopontin

Osteopontin is a phosphorylated acidic glycoprotein that functions as a key proinflammatory cytokine involved in immune response and tissue repair(60,61). Osteopontin promotes migration, adhesion, and proliferation of fibroblasts in bleomycin-induced mouse models of pulmonary fibrosis(62), as well as lung fibroblasts and alveolar epithelial cell lines(63). mRNA expression of osteopontin is significantly higher in the lung tissue of patients with IPF compared to healthy controls(64). Immunohistochemical staining demonstrates that osteopontin is localized to alveolar epithelial cells and macrophages(63). In experimental models, osteopontin induces the growth rate and migration of fibroblasts and epithelial cells, and promotes extracellular matrix deposition, providing a mechanistically plausible role for osteopontin in the development of fibrosis(63).

BALF osteopontin levels are significantly higher in IPF patients than in healthy controls (148.8 ± 83 ng/mL vs 3.8 ± 2.0 ng/mL, respectively, $p < 0.01$)(63). In a study of 17 patients with interstitial lung disease, 8 of whom had IPF, Kadota et al. demonstrated that plasma osteopontin concentrations were higher in patients with interstitial lung disease

(945.5±256.0 mg/ml) than in healthy controls (156.7±52.2 ng/mL) and subjects with sarcoidosis (321.2± 146.8 ng/mL) ($p<0.001$)(65). Osteopontin levels of 300–380 ng/mL could distinguish between patients with interstitial lung disease and healthy controls with 100% sensitivity and specificity. There were no significant differences in plasma osteopontin between subjects with IPF and other interstitial lung diseases. Plasma osteopontin was inversely correlated with PaO₂ ($r=-0.734$, $p=0.008$), but not vital capacity or DLCO.

Osteopontin has mechanistic plausibility in the development of pulmonary fibrosis, and may have utility as a biomarker that can be used for serial monitoring.

Periostin

Periostin is an extracellular matrix protein that contributes to fibrosis in the lungs, heart, and bone marrow(66), and can be excreted by bronchial epithelial cells in response to interleukin-13 (IL-13)(67). Immunohistochemical analyses suggest that the percentage of lung tissue expressing periostin is significantly higher in IPF than in other interstitial lung diseases including non-specific interstitial pneumonia and cryptogenic organizing pneumonia, as well as controls without interstitial lung disease. Elevated serum periostin levels may be correlated with larger declines in vital capacity over a six month period(66).

Several studies have shown that IL-13 is involved fibroblast proliferation(68–71) and may contribute to the development of IPF. As a marker of IL-13 expression in patients with asthma, elevated periostin levels were associated with favorable responses to treatment with a monoclonal antibody to IL-13(72). This raises the possibility that periostin might be used similarly to predict treatment response for patients with IPF. Periostin likely plays a role in the pathogenesis of IPF, and future studies may confirm its use as a diagnostic and prognostic biomarker.

Circulating Fibrocytes

Circulating fibrocytes are circulating bone-marrow derived mesenchymal progenitor cells that produce extracellular matrix components and have the ability to differentiate into fibroblasts and myofibroblasts during wound healing(73). Impaired recruitment of fibrocytes has been shown to protect against fibrosis in murine models(74), supporting a mechanistic role for fibrocytes in the development of pulmonary fibrosis. Fibrocytes have been detected in lung tissue from patients with IPF, and may be recruited to the lungs via CXCR4 and CXCL12(75).

Moeller et al studied the quantity and significance of circulating fibrocytes expressing CD45 and collagen-1 in IPF patients with stable disease and acute exacerbations(76). The quantity of circulating CD45⁺Col-1⁺ fibrocytes in patients with IPF was significantly higher than healthy controls. Fibrocyte counts in 7 IPF subjects with an acute exacerbation were elevated compared to 51 subjects with stable disease. In 3 IPF subjects who recovered from an acute exacerbation, the fibrocyte counts returned to pre-exacerbation levels. A rigorous analysis demonstrated that fibrocyte counts did not correlate with clinical parameters of disease severity including TLC, FVC, DLCO, 6 minute walk test results, and HRCT severity score. The survival for IPF subjects with more than 5% circulating fibrocytes was worse than subjects with less fibrocytes, although the majority of this subset comprised patients with an acute exacerbation.

Circulating fibrocytes may play an integral role in the pathogenesis of pulmonary fibrosis, and may have utility as prognostic biomarkers.

T cells

Although the role of T lymphocytes in IPF is controversial, there is an emerging literature that supports their role in the development of IPF(77–83). T cells have been shown to be a predominant mononuclear cell type in the lungs of IPF patients, and CD3+ cell density was a significant marker for poor survival(79). CD8+ T cells in the lung parenchyma are significantly correlated with decreased lung function(80), and percentages of activated CD4+ T cells have been found to be significantly higher in BALF of subjects with asymptomatic ILD compared with related subjects without lung disease(81).

In a study of 89 subjects with IPF, the proportion of total circulating CD4 T-cells in peripheral blood that expressed CD28 (CD28%) was significantly higher in healthy controls compared to IPF subjects(83). This finding suggests that CD28 is down-regulated in IPF patients. In a subset of patients with pulmonary function testing performed concurrent with T-cell assays, CD28% was correlated with DLCO ($r=0.34$, $p=0.01$), but not with other measures of lung function. Twenty-seven subjects had serial CD28 assays. Longitudinal changes in CD28% were correlated with interval changes in absolute FVC ($r=0.49$, $p=0.012$) and rate of FVC change ($r=0.43$, $p=0.028$). In this cohort of IPF patients, a CD28% <82 was associated with significantly worse survival ($p=0.0004$).

Kotsianidis and colleagues examined CD4+CD25+FOXP3+ T regulatory cells (Tregs) in a large cohort that included 21 male IPF patients(84). Compared with healthy controls and patients with other interstitial lung diseases, the percentage of Tregs was reduced in BALF and in peripheral blood in subjects with IPF. Although there was no association between the number of Tregs and pulmonary function testing, there were significant correlations between Treg function and FVC ($r=-0.54$, $p=0.037$) and TLC ($r=-0.53$, $p=0.043$).

The role of T cells in the pathogenesis of IPF remains under investigation. Both the percentage of CD4:CD28 T cells and Tregs may have utility as prognostic biomarkers that also could be used for serial monitoring.

Limitations

There are several limitations to the candidate biomarkers reviewed above. First, the diagnostic criteria for IPF have been refined several times. As a result, it is possible that some of the IPF patients who participated in the studies listed above might not meet the most recently published diagnostic criteria(4). Secondly, many of the serum biomarkers that have been studied in IPF patients were only measured once. Serial measurements may provide additional insight into the course of this disease, and may prove useful in disease monitoring. Third, many of the serum biomarker levels are continuous variables. Additional measurements and statistical analyses are needed in order to determine the thresholds that correspond to significant abnormalities. Lastly, many of the candidate biomarkers have been studied in one cohort, but have not been validated in separate cohorts. This is a crucial step in the development of these IPF biomarkers.

Conclusion

While we have reviewed several biomarker candidates, it is likely that additional biomarkers will arise, as understanding of pathogenesis in IPF evolves. The need for surrogates of diagnosis, prognosis and monitoring of disease course with emerging therapies is great. Based on the current literature, it seems unlikely that a single biomarker will serve these multiple purposes; however, a panel of several biomarkers may accomplish these goals.

Diagnostic biomarkers will continue to evolve as larger cohorts are studied. Given the lack of therapies in IPF, the clinical utility of biomarkers as surrogates for monitoring disease is limited. The discovery of therapeutic interventions that impact disease outcomes will allow for the identification and testing of biomarkers that predict these outcomes. Which one of these biomarkers will be most successful as a clinical tool remains uncertain and should prompt continued efforts for validation studies.

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Table 1

Peripheral Blood Biomarkers in IPF

Serum Biomarker	Differences between IPF and other ILDs	Correlates With				Study Population	
		Baseline Parameter of Disease Severity	Disease Progression/Longitudinal Change in Parameter	Mortality	Study author	Number of subjects	Number of IPF subjects
KL-6	N	--	--	Y	Sato et al. 2006 ²³	219	Not specified: 152 IPF
	N	--	--	--	Ohnishi et al. 2002 ²²	115	21
	Y	--	--	--	Ishii et al. 2003 ²⁰	66	19
Surfactant Proteins							
SP-A	N	--	--	--	Ohnishi et al. 2002 ²²	115	21
	Y	--	--	--	Ishii et al. 2003 ²⁰	66	19
	--	N	--	Y	Kinder et al. 2009 ²⁹	82	82
	--	N	*	Y	Greene et al. 2002 ²⁷	543	210
	--	N	N	Y	Takahashi et al. 2000 ³⁰	160	52
	Y	--	--	--	Ohnishi et al. 2002 ²²	115	21
SP-D	Y	--	--	--	Ishii et al. 2003 ²⁰	66	19
	--	--	--	--	Kinder et al. 2009 ²⁹	82	82
	--	N	*	Y	Greene et al. 2002 ²⁷	543	210
	--	N	$\Delta\% \text{ VC/yr, } \Delta\% \text{ TLC/yr}$	Y	Takahashi et al. 2000 ³⁰	160	52
	--	N					
Matrix Metalloproteases							
MMP1	Y	N	--	--	Rosas et al. 2008 ⁴¹	322 (D)	74 (D)
MMP7	Y	FVC, DLCO	--	--	Rosas et al. 2008 ⁴¹	33 (V)	9 (V)
	--	--	--	**	Richards et al. 2011 ⁴²	322 (D)	74 (D)
	--	--	--			33 (V)	9 (V)
	--	--	--			140 (D)	140 (D)
CCL18	--	TLC, DLCO	ΔTLC	--	Prasse et al. 2007 ⁴⁶	101 (V)	101 (V)
						78 (D)	16 (D)

Serum Biomarker	Differences between IPF and other ILDs	Correlates With				Study Population	
		Baseline Parameter of Disease Severity	Disease Progression/ Longitudinal Change in Parameter	Mortality	Study author	Number of subjects	Number of IPF subjects
	--	--	Δ TLC, Δ FVC	Y	Prasse et al. 2009 ⁴⁷	40 (V) 72	17 (V) 72
VEGF	--	N	Δ V (subset)	--	Ando et al. 2010 ⁴⁹	98	41
YKL-40	--	N	--	Y	Korthagen et al. 2011 ⁵⁹	211	85
	--	DLCO, AaDO ₂ , PaO ₂	--	--	Furuhashi et al. 2010 ⁵⁸	63	41
Osteopontin	N	PaO ₂	--	--	Kadota et al. 2005 ⁶⁵	46	Not specified: 17 ILD
Periostin	Y	Not reported	Δ TLC, Δ FVC	--	Okamoto et al. 2011 ⁶⁶	105	51
Fibrocytes	--	N	--	Y	Moeller et al. 2009 ⁷⁶	75	58
T cells							
CD4:CD28%	--	FVC	Δ FVC, Δ FVC rate	Y	Gilani et al. 2010 ⁸³	89	89
Tregs	Y	FVC, DLCO	TLC, FVC	--	Kotsianidis et al. 2009 ⁸⁴	84	21

Legend: Y = Yes, N = No, -- = Not studied.

Abbreviations: AaDO₂ = Alveolar-arterial oxygen difference, D = Derivation cohort, DLCO = Diffusing capacity for carbon monoxide, FVC = Forced vital capacity, IIP = Idiopathic Interstitial Pneumonia, ILD = Interstitial Lung Disease, IPF = Idiopathic Pulmonary Fibrosis, TLC = Total lung capacity, Tregs = Regulatory T cells, V = Validation cohort.

* Longitudinal data available for a subset of 19 subjects. No significant changes over time, although patients were relatively stable.

** MMP7 levels were associated with mortality in derivation cohort, but not validation cohort.