Serum biomarkers for lung disease

Biomarkers can play an important role in the management of idiopathic pulmonary fibrosis and other interstitial lung diseases

By David Torrens, MA, MSc, and Ryoichi Iwata

Idiopathic pulmonary fibrosis (IPF) is the most common and the most severe manifestation of a group of diseases known as idiopathic interstitial pneumonias (IIPs).[1,2] IIPs are a heterogeneous group of noninfectious, non-neoplastic disorders resulting from damage to the pulmonary parenchyma by varying patterns of inflammation and fibrosis. As distinct from bacterial (or alveolar) pneumonia, IIPs affect the interstitium, the space between epithelial and endothelial basement membrane, and often also the alveolar epithelium, small airways, and vessels.[3] The IIP classification includes nonspecific interstitial pneumonia (NSIP), desquamative interstitial pneumonia, respiratory bronchiolitis-associated interstitial lung disease (ILD), acute interstitial pneumonia, cryptogenic organizing pneumonia, and lymphocytic interstitial pneumonia.[2] Some forms of ILDs occur secondarily to a variety of occupational or environmental exposures or as complications associated with multiple rheumatic or connective tissue diseases, or granulomatous lung disorders.[2,4,5]

IPF commonly presents with cough, bibasilar inspiratory crackles, and finger clubbing, and should be considered in the differential diagnosis of all adult patients with unexplained chronic exertional dyspnea. Onset of IPF is usually in middle to older age, more commonly in males, with prevalence peaking between 65 and 79 years of age.[6] The incidence has been variously estimated within range of 10 cases per 100,000 per year.[1] The disease is usually sporadic, and the main known risk factors include smoking, environmental exposures (for example, metal dusts and farming-related dusts and animal hair), microbial agents (chronic viral infection as with Epstein-Barr virus or hepatitis C), gastroesophageal reflux, genetic predisposition (familial pulmonary fibrosis accounting for less than 5% of total IPF patients), and factors (gene polymorphisms) affecting sporadic cases.[1]

The disease course of IPF is variable, with some patients remaining stable for long periods, some demonstrating slow progression, some demonstrating rapid progression, and others (possibly 5% to 10%) experiencing acute exacerbations leading to respiratory failure and death (**Figure 1**). The acute declines are often idiopathic—without clinically apparent infection, left heart failure, pulmonary embolism, or other identifiable cause.[7] The means employed for staging and prognosis of IPF include various metrics applied to baseline dyspnea, baseline pulmonary function testing, the six-minute-walk test, histopathology, determination of pulmonary hypertension, assessment of emphysema status, and bronchoalveolar lavage and serum biomarkers. Median survival is estimated at 2 to 3 years from the time of diagnosis, with survival to 5 years in at best 20% of cases.[1,4]

The multidisciplinary diagnostic process for IPF begins by excluding known causes of ILD (such as drug toxicities, environmental exposures, and associations with connective tissue diseases) and differentiating the pathology at hand from other IIPs, especially NSIP. The definitive diagnosis—by high-resolution computed tomography (HRCT) and/or surgical lung biopsy—depends on the finding of what is referred to as a usual interstitial pneumonia (UIP) pattern, as distinct from an NSIP pattern.[1,4] A UIP pattern on HRCT, which may be sufficient for the diagnosis, relies on four criteria: subpleural basal predominance; reticular abnormality; honeycombing, with or without traction bronchiectasis; and absence of features known to be inconsistent with the UIP pattern.[1]

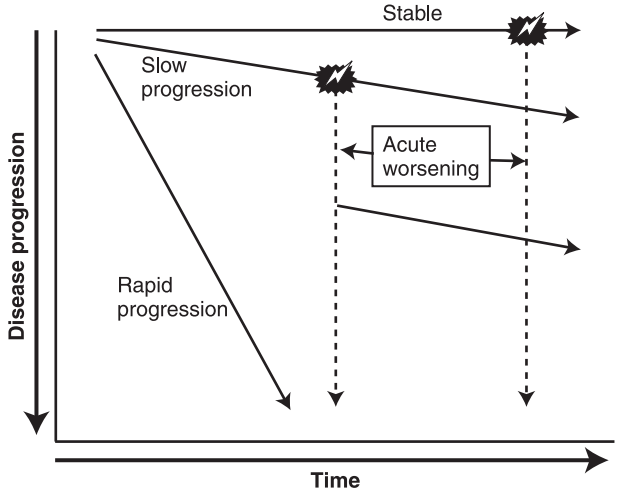

Figure 1. Natural histories for patients with idiopathic pulmonary fibrosis. While the majority of patients experience slow but steady worsening (Slow progression), some patients remain stable (Stable), others have an accelerated decline (Rapid progression), and still others experience unpredictable "Acute worsening" (lightning bolt), due to secondary complications or unrecognized reasons, which may be fatal or at least result in substantially worsened disease. Adapted from Raghu G et al.[1]

Recent guidelines for managing IPF state clearly the insufficiency of evidence supporting any specific pharmacologic therapy.[1] Targeting of the inflammatory response in IPF may involve immunosuppressive/anti-inflammatory/antifibrotic agents including (alone or in combination) corticosteroids, colchicine, cyclophosphamide, azathioprine, mycophenolate mofetil, interferon, and pirfenidone.[1,5] For IPF patients who fail to respond to pharmacologic therapy, lung transplantation is a recommended option, with 5-year survival estimated at somewhat more than 50%.[1] Patients with rapid progression or at risk of rapid progression of IPF, when they can be identified, may be supported with intravenous high-dose corticosteroid pulse therapy.[8,9]

Accurate identification

In general with the IIPs, and especially with IPF, there is a need for more timely and accurate identification—in diagnosis, in disease staging and prognosis, and in monitoring of the safety and effectiveness of treatment. Although early diagnosis of IPF offers benefits such as enrollment for lung transplantation, avoidance of inappropriate drugs, and access to clinical trials and new treatments, it is too often the case that identification of the disease is delayed due to lateness of suspicion and lack of differentiating multidisciplinary review of examinations/tests/histopathology, with that unfortunate situation then compounded by the nonavailability of validated prognostic indicators.[3,4] There is a serious need for more reliable and rapidly detectable markers that would facilitate identification of patients at risk for disease progression, of those who might benefit from specific therapies, and of those who are unaffected or adversely affected by the treatments under trial.

A range of molecules involved in epithelial damage and repair, inflammation, myofibroblast accumulation and matrix deposition, angiogenesis, coagulation, and oxidative stress have been investigated for their potential in fulfilling this role, as biomarkers in tissue, bronchoalveolar lavage fluid, and serum, with a broadening of understanding of the events underlying the scarring of the lung (**Table 1**).[3,4] The serum biomarkers have the appeal of being measurable noninvasively and being easily suitable for follow-up. Much of the research on the serum biomarkers, dating back to the 1980s, has been performed in Japan.

Biomarker KL-6

As an example of the benefits potentially associated with biomarkers for ILD, Krebs von den Lungen-6 (KL-6) is a high-molecular-weight glycoprotein antigen (classified as a MUC1 mucin), first described by Kohno et al in 1985, that in normal lungs is expressed mainly on type II pneumocytes and bronchiolar epithelial cells, while only weakly in basal cells of the terminal bronchiolar epithelium, a small number of middle layer cells of the bronchial epithelium, and serous cells of the bronchial gland.[10] In patients with ILD, KL-6 is strongly expressed on alveolar macrophages and the type II pneumocytes that are regenerated over the alveolar basement membrane after the death of type I pneumocytes during the first stage of fibrosing lung injury. The soluble form of KL-6 is evaluable in bronchoalveolar lavage fluid and also noninvasively in serum, based on its leakage from the alveolar space into the blood due to an enhanced permeability or destruction of the air-blood barrier in the diseased lungs.[11,12]

The assay for KL-6 employs a murine monoclonal antibody that recognizes a sialylated sugar chain on the molecule.[10] In Japan, a KL-6 enzyme immunoassay (EIA) kit has been available since 1999, and it was the world's first ILD marker kit. A latex turbimetic immunoassay (LTIA) for KL-6 was introduced in Japan in 2009. KL-6 has been employed in clinical practice for several years in Japan.[11] The LTIA kit for KL-6 recently received Conformité Européenne (CE) marking but does not yet have U.S. Food and Drug Administration (FDA) approval.

KL-6	A high-molecular-weight glycoprotein highly expressed in tissue sections from patients with ILDs. Elevated levels of serum KL-6 have been found in patients with clinically confirmed progression and have been associated with increased mortality risk.
SP-A and SP-D	C-type lectins produced mainly by alveolar epithelial type II cells. Serum levels of SP-A and SP-D are increased in IPF (but also other pulmonary diseases) and are strongly predictive of mortality.
CD28	A co-stimulatory molecule normally expressed on most CD4+ T-cells. Down-regulation of CD28 on peripheral CD4+ T-cells has been associated with increased risk of lung transplantation within 1 year.
Circulating fibrocytes	Thought to be progenitors for fibroblasts participating in the pathogenesis of lung fibrosis. Circulating fibrocytes are increased in IPF, with significant further increases during acute exacerbations. A proportion of >5% of peripheral blood leukocytes is associated with increased mortality in these patients.
Angiogenic factors	Thought to play a role in the pathogenesis of IIPs. Elevated levels of the potent angiogenic factors VEGF and IL-8 have been associated with IPF and progressive disease.
MMPs	Matrix degrading enzymes thought to be critically involved in the pathology of pulmonary fibrosis. Plasma MMP1 and MMP7 levels are significantly elevated in IPF patients. MMP7 levels are consistently elevated in asymptomatic versus symptomatic IPF, indicating that it may be a marker for early disease.
Oxidative stress	May be implicated in the epithelial dysfunction underlying pulmonary fibrosis. Oxidant burden has been shown to be elevated in the serum of IPF patients.

Table 1. Candidate biomarkers for idiopathic pulmonary fibrosis (IPF) KL-6: Krebs von den Lungen-6; SP: surfactant protein; MMP: matrix metalloproteinase; ILD: interstitial lung disease; IIP: idiopathic interstitial pneumonia; VEGF: vascular endothelial growth factor; IL: interleukin. Adapted from Valeyre D.[4]

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The usefulness of the KL-6 biomarker has been investigated in several Japanese studies, with consistent findings of increased serum levels in various ILDs including radiation pneumonitis, collagen vascular disease with ILD, and drug-induced lung disease.[3] In a retrospective study of 27 IPF patients published in 2006, multivariate analysis revealed that only the KL-6 (not lactate dehydrogenase [LDH] or C-reactive protein [CRP]) was a predictor of survival at the time of diagnosis.[13] In terms of the ongoing evaluation of disease activity and gauging the effectiveness of treatment, it has also been reported that the serum level of KL-6 in patients with active IIP is significantly higher than it is in those with inactive IIP.[14] An *in vitro* study of purified KL-6 concluded that the biomarker is one of the chemotactic factors for most fibroblasts and that increased KL-6 in the epithelial lining fluid in small airways could actually be one cause of the intra-alveolar fibrosis in ILDs.[15]

In one Japanese study of 57 patients with rheumatoid arthritis, 47 with systemic sclerosis, 21 with polymyositis/dermatomyositis, and 18 with systemic lupus erythematosus, KL-6 was found to be a useful marker for diagnosis and evaluation of interstitial pneumonia activity associated with collagen diseases.[14] According to investigators in this study, the serum KL-6 level is basically determined by two factors—extent of fibrosis and degree of inflammation of interstitial pneumonia—and in their estimation, determination of the serum KL-6 level has at least three advantages over conventional chest radiographs, HRCT, and spirometry: KL-6 level is expressed as a quantitative value; KL-6 can be measured repeatedly; and KL-6 can be measured in seriously ill patients, such as those under mechanical ventilation.[14] They found that its mean serum level was significantly higher ($p < 0.0001$) in patients with active interstitial pneumonia than in those with inactive interstitial pneumonia, and that the serum KL-6 levels increased with the deterioration of interstitial pneumonia while significantly decreasing when interstitial pneumonia treatment was successful (**Figure 2,** page 42).[14] Similarly, a study of 14 Japanese patients

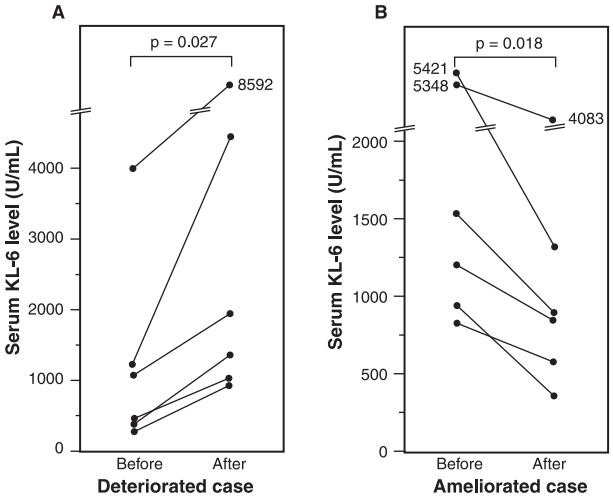

Figure 2. Serum KL-6 levels measured in patients before and after deterioration (A) or improvement (B) of interstitial pneumonia associated with collagen diseases. Levels of serum KL-6 increased with the deterioration of interstitial pneumonia, while successful treatment of interstitial pneumonia resulted in a significant decrease of serum KL-6 levels. P values were determined by Wilcoxon's rank sum test. Adapted from Nakajima H et al.[14]

with rapidly progressive IPF demonstrated that diminishing levels in response to steroid pulse therapy were associated with increased survival six months after the start of therapy.[8]

Looking ahead

Much remains unsettled with regard to the management of ILD, from diagnosis to treatment. The investigative work that has been performed with serum biomarkers represents but one hopeful sign for clinical decision making. The KL-6 biomarker has been investigated extensively over time in Japan, and research has been conducted as well on other potential biomarkers. For all of the candidate biomarkers, some questions remain regarding their correlation with clinical findings, the uniqueness of the data they provide, and the possibility of augmenting their value through composite testing. □

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