Idiopathic Pulmonary Fibrosis: From Epithelial Injury to Biomarkers – Insights from the Bench Side

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Abstract

Idiopathic pulmonary fibrosis (IPF) is the most frequent fibrotic diffuse parenchymal lung disease. Its prognosis is devastating: \(>50\%\) of the patients die within 3 years after diagnosis. Options for the treatment of IPF are limited and lung transplantation is the only ‘curative’ therapy. Currently, in the absence of validated indicators of disease progression/activity and diagnostic tools, the clinical management of IPF remains a major challenge. A better understanding of the pathogenesis of IPF is critical for the identification of new therapeutic targets as well as molecules that may serve as surrogate markers for clinically significant endpoints. The current paradigm on the mechanisms leading from a normal to a fibrotic lung postulates that chronic epithelial lesion leads to aberrant wound healing activation, which is characterized by deregulated fibroblast proliferation and activation together with an uncontrolled extracellular matrix synthesis. In this review, we shed light on the role of epithelial cell damage in the pathogenesis of fibrosis. Finally, we examine the markers of epithelial damage and their potential use as biomarkers and the future of this continuously expanding field.

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Introduction

Pulmonary fibrosis constitutes the end stage of a broad range of heterogeneous interstitial lung diseases (ILDs), which are characterized by the destruction of pulmonary parenchyma together with deposition of extracellular matrix (ECM) in the interstitial and alveolar spaces. Idiopathic pulmonary fibrosis (IPF) is the most frequent fi...
brotic diffuse parenchymal lung disease. Its prognosis is devastating: >50% of the patients die within 3 years after diagnosis. As implicit by its name, IPF is characterized by the absence of an identified cause and a distinct histopathological pattern of usual interstitial pneumonia. Other salient histological features of IPF include honeycombing, sparse cellular inflammation and areas of fibroblast and myofibroblast accumulation and proliferation, known as fibroblastic foci [1]. Options for the treatment of IPF are limited and lung transplantation is the only ‘curative’ therapy. To date, only pirfenidone, an orally administered pyridine derivative, has been approved in the EU for the treatment of mild-to-moderate IPF, although more data on overall survival and quality of life on treatment are still needed to fully appreciate its potential clinical benefits [2].

Diagnosing and managing IPF remains a challenge in daily practice [3]. The ATS/ERS (American Thoracic Society/European Respiratory Society) international consensus statement on IPF in 2000 [4], updated in 2011 [5], and the ATS/ERS reclassification of ILDs in 2002 [6], updated in 2013 [7], constitute a real progress in the clinical understanding of IPF. These documents provided clinicians with powerful tools and led during the last decade to a better characterization of the IPF disease phenotype, resulting in diagnostic improvements and an unprecedented number of clinical trials [8]. However, in the absence of a gold standard, the diagnosis of IPF often requires a multidisciplinary approach between clinician, radiologist and pathologist [5]. The establishment of a confident or a consensus diagnosis of IPF is not the only challenge. The clinical course of individual patients with IPF is highly variable and unpredictable. IPF is not a uniform clinical dynamic disease: there is a wide spectrum of disease courses, including stability/slow progression over a period of years, rapid deterioration or even periods of disease courses, including instability/slow progression over a period of years, rapid deterioration or even periods of relative stability punctuated by events of rapid decline [9]. Currently, in the absence of validated indicators of disease progression/activity and diagnostic tools, the clinical management of IPF remains a major challenge, although some useful clinical scores, such as the GAP score (based on gender, age and physiological data), which relate to prognosis evaluation, are emerging [10].

A better understanding of the pathogenesis of IPF is critical for the identification of new therapeutic targets as well as molecules that may serve as surrogate markers for clinically significant endpoints. Although significant advances in the understanding of the pathogenesis of IPF have been made during the past decades, the exact mechanisms underlying the development of IPF remain largely unknown [1]. It has long been believed that lung fibrosis was preceded and provoked by a chronic inflammatory process that injures the lung and modulates fibrogenesis, leading to end-stage fibrotic scarring. This model provided a rational basis for the use of anti-inflammatory agents such as corticosteroids and immunosuppressive agents. However, inflammation is never a prominent histopathological finding in usual interstitial pneumonia, and there is little evidence of prominent inflammation in early disease. Recently, the PANTHER-IPF clinical trial was conducted to assess the safety and efficacy of the triple anti-inflammatory regimen of prednisone, azathioprine and N-acetylcysteine. However, the results of this randomized, double-blind, placebo-controlled trial revealed increased risks of death and hospitalization in treated patients compared to the placebo arm of the study [11]. Therefore, these results support the concept that inflammation is not the leading cause of fibrogenesis during IPF.

These observations led Selman et al. [12] to challenge the model of inflammation-driven fibrogenesis and to shift to another pathogenic paradigm (fig. 1). In 2001, they proposed that IPF is the result of aberrant wound healing responses following repetitive epithelial injury. This model was built on histological observations. Adherent to fibroblastic foci, there are prominent alterations in the alveolar epithelium, including hyperplasia and denudation [13–15]. Several animal models demonstrated similar defects [16–18]. Fibroblast differentiation and collagen production in vitro is enhanced in epithelial cell/fibroblast cocultures by injury to the epithelial cell component [19]. The potential importance of the alveolar epithelium in IPF pathogenesis is further highlighted by the observation that epithelial cell growth factors are decreased in patients with IPF [20, 21] and that those factors protect against scarring in animal models [22–24]. Finally, targeted injury of alveolar epithelial cells (AEC) consistently induces pulmonary fibrosis in experimental models [25]. Based on these observations, the corollary of this new paradigm is that IPF is an ‘epithelial-fibroblastic disease’, i.e. a fibroproliferative disorder preceded by alveolar epithelial injury and activation, with fibroblastic foci representing the primary sites of injury and aberrant repair. However, one should be aware that this is the prevailing concept and that it does not necessarily reflect the whole picture. New data and evidence may emerge and shift the thinking to a new concept in the future. Myofibroblasts in turn provoke basement membrane disruption and promote AEC apoptosis, perpetuating the damage and preventing subsequent re-epithelialization. The
In this review, we will highlight the potential contribution of AEC to IPF pathogenesis. We will first briefly discuss the role of normal epithelium in normal lung repair and homeostasis, and the mechanisms leading to epithelial injury and their consequences. Next, we will present how markers of epithelial damage can be used to monitor disease activity. Finally, we will discuss the future of this continuously expanding field.

**AEC in Physiological Lung Repair and Homeostasis**

Understanding the mechanisms of lung repair and homeostasis after injury represents one of the major mysteries of pulmonary biology. The lung is extremely complex, and both its development and its repair require interaction of >40 different cell types [for an excellent review, see ref. 27]. A functioning, intact alveolar epithelium is involved in ion transport and production of surfactant, and serves as a physical barrier, and all of them are necessary to maintain pulmonary homeostasis and fluid balance.

The alveolar epithelium consists of alveolar type I (AECI) and type II cells (AECII). The flat AECI cover >90% of the alveolar surface area. The attenuated cytoplasm provides for close approximation of the alveolar lumen and the bloodstream, optimizing respiratory gas exchange. The cuboidal AECII are multifunctional cells and play a crucial role in lung homeostasis. These cells are important for active alveolar liquid clearance. They are also involved in the metabolism of surfactant, which allows breathing at normal transpulmonary pressures by reducing surface tension. The surfactant proteins (SP)-B and SP-C are key components of surfactant. SP-A and SP-D are compounds of the innate immune defense system which are able to bind to the surface of pathogens, thereby facilitating their removal by alveolar macrophages [28–30]. AECII act as facultative progenitors, with the ability to replace themselves and to differentiate into AECI after injury [31]. In line, in the model of bleomycin-induced pulmonary fibrosis, intratracheal instillation of a purified population of syngeneic AECII cells was suffi-
cient to block fibrotic lung remodeling [32, 33]. It is likely that it is this central role of AECII in lung homeostasis which makes them vulnerable to injury, with the risk of promoting undesirable responses, such as fibrosis or apoptosis. Pulmonary fibrosis may be a disease resulting from exhaustion of the pool of alveolar epithelial stem cells resulting in failure of repair. This general scheme nicely integrates the notion of genetic predisposition to the disease resulting from telomerase mutations and the evidence that familial and sporadic IPF is associated with shorter telomeres in blood cells and AEC [34–36].

Contribution of Epithelial Cell Injury to IPF

An early and consistent feature of IPF is a change in the AEC phenotype. These changes include increased apoptosis associated with regenerative hyperplasia, differentiation of mucus cells in the distal airspaces during a process called bronchioaliozation and enhanced proliferation [12]. Apoptosis of AECs during IPF has been the subject of extensive research and is now well established. There are several triggers of AEC apoptosis, and most attention has been given to Fas activation, the role of reactive oxygen species and TGF-β (for an excellent review on this topic see [37]). Irrespective of the triggers, there is a plethora of mechanisms by which AEC injury can drive aberrant cell cross talk and fibrogenesis. It is possible that epithelial cells undergo transdifferentiation into fibroblast epithelial-mesenchymal transition (EMT). During EMT, epithelial cells lose their characteristic markers (for instance E-cadherin) and acquire mesenchymal markers such as α-smooth muscle actin [38, 39]. In murine models of bleomycin-induced pulmonary fibrosis, several elegant lineage-tracing studies have suggested that EMT is a potential source of myofibroblasts during fibrogenesis, with up to 30% of pulmonary fibroblasts arising from EMT [40]. In contrast, one lineage-tracing study suggested no evidence for EMT, although the authors speculated that differences in the experimental setup could explain these differences [41]. Another hypothesis is that damage of AECII can lead to the loss of control exerted by AECII on fibroblast proliferation and collagen synthesis. For instance, AECII are an important source of prostaglandin E₂, which has been shown to inhibit multiple aspects of the fibroproliferative response, including fibroblast chemotaxis, proliferation and collagen synthesis [42–44]. A loss of AECII could diminish intra-alveolar levels of this antifibrotic mediator. It has been shown recently that prostaglandin E₂ deficiency results in increased AEC but reduced fibroblast sensitivity to apoptosis in IPF [45]. Moreover, it has been shown that chronically injured AEC release a number of profibrotic compounds, such as tissue factor, factor VII and factor X, which are all able to activate mesenchymal cells [46, 47]. It is also possible that apoptotic AECII release factors such as CCL2 or CXCL12 and attract circulating fibrocytes, which may locally expand the fibroblast pool [48, 49]. The relative importance of each individual mechanism in pulmonary fibrogenesis remains to be defined. Importantly, these different mechanisms are not mutually exclusive, and all potentially may drive potent fibroproliferative responses [50].

Monitoring the Disease: Insights from the Bench Side

It seems straightforward that epithelial cell damage, as well as aberrant scar formation, will lead to the liberation and/or exposure of molecules in the lung tissue, bronchoalveolar lavage fluid (BALF) or even in peripheral blood, which can reflect the presence of the disease. Several of these proteins and cells can be considered as attractive biomarkers, at least if they are found in serum or BALF and can be obtained in a noninvasive manner. By definition, a biomarker indicates a change in the expression or state of a biologic measurement (for instance, the levels of a protein in the serum) at a given time point that correlates with the risk or progression of a disease, or with the susceptibility of the disease to a given treatment at a future time point [51–53]. A biomarker acts as surrogate (i.e. an endpoint expected to predict clinical benefit, lack of benefit or harm based on epidemiologic, therapeutic, pathophysiological or other scientific evidence [53]) for clinically meaningful outcomes, and may or may not reflect the pathogenesis underlying a disease. That is, it may be a crucial contributor to disease pathogenesis, and as such constitute both a biomarker and a target, or just be an epiphenomenon. In IPF, an ideal biomarker should be reliable, valid, responsive to changes in disease status, able to show a clinically meaningful difference, predictive of clinical outcome and responsive to the treatment effect of a given intervention. Such meaningful biomarkers could be used as (1) diagnostic biomarkers to establish a confident diagnosis of IPF and allow discriminating between IPF and other idiopathic or nonidiopathic ILDs; (2) prognostic biomarkers that are correlated with disease progression or mortality, and (3) biomarkers that can be used as tools for serial monitoring of disease severity in longitudinal studies. Finally, given the number of thera-
Table 1. Principal findings obtained from clinical studies conducted on the indicated targets as potential biomarkers

<table>
<thead>
<tr>
<th>Target</th>
<th>Diagnosis</th>
<th>Correlates with</th>
<th>Reference</th>
<th>IPF patients, n</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>KL-6</td>
<td>No</td>
<td>N/A</td>
<td>54</td>
<td>19</td>
<td>Elevated in ILD compared to controls</td>
</tr>
<tr>
<td></td>
<td>N/A</td>
<td>Prognosis</td>
<td>61</td>
<td>14</td>
<td>Serial increases in serum KL-6 are associated with poor survival</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>Prognosis</td>
<td>62</td>
<td>ND</td>
<td>ILD patients with KL-6 &gt;1,000 U/ml have worse survival</td>
</tr>
<tr>
<td>SP-A</td>
<td>Yes for SP-A</td>
<td>Prognosis (SP-A/SP-D)</td>
<td>54</td>
<td>19</td>
<td>Serum SP-A is significantly higher in IPF than in NSIP, sarcoidosis, diffuse panbronchiolitis and chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>SP-D</td>
<td>No for SP-D</td>
<td>Prognosis (SP-A/SP-D)</td>
<td>68</td>
<td>78</td>
<td>Serum SP-A and SP-D levels highly predictive of survival in IPF patients</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>Prognosis (SP-A/SP-D)</td>
<td>69</td>
<td>52</td>
<td>Alveolitis (SP-A/SP-D) Extent of parenchymal collapse or deterioration per year in pulmonary function (SP-D)</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>Prognosis (SP-A but not SP-D)</td>
<td>70</td>
<td>82</td>
<td>Each increase of 49 ng/ml (1 SD) in baseline SP-A is associated with a 3.3-fold increased risk in mortality</td>
</tr>
<tr>
<td>VEGF</td>
<td>ND</td>
<td>Disease course</td>
<td>74</td>
<td>41</td>
<td>Prognosis not determined in this study Altered lung function (percent of predicted VC)</td>
</tr>
<tr>
<td>MMP1</td>
<td>No for MMP7</td>
<td>Disease course (MMP-7)</td>
<td>80</td>
<td>42</td>
<td>Negative correlation between MMP7 levels and FVC</td>
</tr>
<tr>
<td>MMP7</td>
<td>Not tested for MMP1</td>
<td>Disease course</td>
<td>75</td>
<td>140 (derivation)</td>
<td>Negative correlation of MMP7 levels with overall, transplant-free and progression-free survival</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>Prognosis not determined Disease course</td>
<td>82</td>
<td>74</td>
<td>Negative correlation of MMP7 levels with FVC and CO diffusing capacity</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>No</td>
<td>Disease course</td>
<td>86</td>
<td>17 IP patients</td>
<td>Osteopontin levels correlated with PaO2</td>
</tr>
<tr>
<td>Fibrocytes</td>
<td>ND</td>
<td>Prognosis</td>
<td>47</td>
<td>58</td>
<td>Presence of circulating fibrocytes predicts a poor outcome</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>Disease severity Not prognosis</td>
<td>90</td>
<td>26</td>
<td>Number of alveolar fibrocytes correlated with a less severe disease but not with a better outcome</td>
</tr>
<tr>
<td>Anti-periplakin autoantibody and related protein</td>
<td>ND</td>
<td>Disease severity Not prognosis</td>
<td>94</td>
<td>40</td>
<td>Presence of autoantibodies associated with more severe disease</td>
</tr>
<tr>
<td>Anti-HSP70 autoantibody and related protein</td>
<td>No</td>
<td>Prognosis Disease course</td>
<td>95</td>
<td>122</td>
<td>Autoantibodies associated with FVC reduction</td>
</tr>
<tr>
<td>Plasma BLyS</td>
<td>ND</td>
<td>Prognosis Disease course</td>
<td>96</td>
<td>110</td>
<td>BLyS correlated with pulmonary artery pressure</td>
</tr>
<tr>
<td>CD28</td>
<td>ND</td>
<td>Lung function Prognosis</td>
<td>97</td>
<td>89</td>
<td>Downregulation of CD28 correlates with decreased lung function and survival</td>
</tr>
<tr>
<td>Periostin</td>
<td>ND</td>
<td>Disease progression</td>
<td>98</td>
<td>54</td>
<td>Lung function (VC, DLCO) Not associated during acute exacerbations</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>Disease course</td>
<td>99</td>
<td>51</td>
<td></td>
</tr>
</tbody>
</table>

BLyS = B lymphocyte-stimulating factor; FVC = forced vital capacity; IP = interstitial pneumonia; ND = not determined; NSIP = nonspecific IP; VC = vital capacity.

The identification of specific and sensitive biomarkers would also be crucial to evaluate the efficacy of new treatment regimens [51–53]. Special attention has been given to the peripheral blood protein markers. They present advantages over the other compartment as they are minimally invasive and readily available in clinical settings, and can easily be measured longitudinally and during exacerbations (for excellent review on the different types of pulmonary biomarkers and existing limitations, see Doyle et al. [54]).
Over the last 2 decades, a plethora of serum markers was tested regarding their use in IPF. Among them, the most promising are a range of molecules involved in epithelial damage and repair, inflammation, myofibroblast accumulation and ECM deposition. Table 1 summarizes the findings obtained from clinical studies conducted on these molecules with respect to diagnosis, prognosis and monitoring of the disease course. Exhaustive analysis of biomarkers linked to other cell compartments and/or ECM can be found in a study by Vij and Noth [55]. Reviewing these biomarkers in their whole would fall beyond the scope of this article.

Krebs von den Lungen-6 Antigen

Krebs von den Lungen-6 antigen (KL-6), otherwise known as MUC1, is a mucin-like glycoprotein expressed at the extracellular surface of various epithelial cells, including regenerating AECII [56]. Upon epithelial damage, KL-6 may leak into the circulation where it can be measured in the serum. In IPF patients, serum KL-6 levels are elevated compared to healthy volunteers [57]. Because KL-6 promotes human pulmonary fibroblast migration and proliferation [58], it can be hypothesized that changes in KL-6 levels are not only an epiphenomenon, but may play a role on the progression of the disease. KL-6 is not useful for the diagnosis of IPF as it is also elevated in patients with other ILDs [57, 59, 60]. Moreover, KL-6 is not only a marker for ILD, as elevated levels are also observed in cancer and tuberculosis [61–64]. In a small study including 14 IPF patients, serial increases in serum KL-6 levels were associated with poor survival [65]. Data obtained from a prospective study of 152 patients with idiopathic interstitial pneumonias and 67 patients with ILD associated with connective tissue disease showed that patients with a serum KL-6 level >1,000 U/ml had a worse survival compared to those with lower levels [66]. These results support some usefulness for KL-6 as a potential prognostic biomarker.

SP-A and SP-D

SP-A and SP-D are lipoprotein complexes synthesized in the lung mainly by AECII and secreted into a liquid layer lining the epithelium. In addition, they play an important role in the host defense against pathogens and are important constituents of the innate immunity of the lung [67]. SP-A and SP-D serum concentrations are increased in different pulmonary diseases including IPF. The mechanisms underlying serum elevation of these proteins likely include a combination of epithelial injury and breakdown together with an increased accumulation of AECII due to hyperplasia [68]. SP themselves might play a role in IPF pathogenesis. Aberrant SP processing by the endoplasmic reticulum has been involved in IPF pathogenesis, and genetic defects in genes encoding SP-A1 and SP-A2 were associated with familial IPF [69–71]. With respect to their diagnostic and/or prognostic potential as biomarkers, both serum SP-A and SP-D are significantly elevated in IPF patients compared to healthy controls and patients with other ILDs [57, 72], although SP-D is also elevated in patients with nonspecific interstitial pneumonia [60]. Increased serum SP-A and SP-D levels obtained at the time of IPF diagnosis were independently associated with increased mortality [72–74]. More specifically, in a population of 52 IPF patients (mean follow-up time 11.4 months for the subjects who died and >3 years for the survivors), the concentration of SP-A and SP-D was within the normal range in the group of survivors (n = 10). Amongst the patients who died, only 25% had protein concentrations within the normal range [73]. In another study, the use of SP-A and SP-D was validated in a population of 142 IPF patients as a predictor of survival. They used a Cox proportional hazard model and found that an elevated concentration of SP-D (but not SP-A) correlates with increased death rate [72]. Finally, in a recent study in 82 IPF patients, increased serum SP-A (but not SP-D) levels were independently associated with death or lung transplantation within 1 year (HR 3.27 for each standard deviation increase, 95% CI 1.49–7.17, p = 0.003) [74]. Thus, multiple studies have demonstrated that both SP-A and SP-D can distinguish IPF patients from those with other ILDs, and their levels are correlated with disease progression or mortality. However, large standard deviations of surfactant concentrations in the different studies and some yet unexplained differences between the populations and SP-A versus SP-D alterations preclude their present routine use as diagnostic and/or prognostic biomarkers.

Vascular Endothelial Growth Factor

Many ILDs are associated with aberrant angiogenesis, but its role in the pathogenesis of fibrosis has not yet been fully elucidated [75]. In IPF, capillary density was increased in nonfibrotic usual interstitial pneumonia lesions, and AECII adjacent to these vessels were shown to produce vascular endothelial growth factor (VEGF)-B [76]. VEGF is a crucial factor for the homeostasis of the alveolus via the control of surfactant homeostasis and endothelial cell tropicity. The role of VEGF in lung fibrogenesis is poorly understood. Recent data demonstrate that exogenous VEGF-B can protect against pulmonary hypertension de-
velopement while it increased fibrosis in an experimental model of fibrosis [77]. In a cohort of 41 IPF patients, an increase in serum VEGF-A was shown in patients with a high compared to patients with a low alveolar-arterial difference of oxygen (241.0 vs. 141.4 pg/ml, respectively, p = 0.030) and compared to healthy volunteers (162.2 pg/ml, p = 0.007) [78]. Interestingly, serum VEGF levels did not correlate with baseline pulmonary function tests, but negatively correlated with changes in vital capacity during follow-up (r = –0.38, p = 0.044). IPF patients with serum VEGF levels above the median tended to have a shorter survival compared with patients with levels below the median. These data suggest that serum VEGF may reflect the severity of lung disease as well as predict declines in pulmonary function. Noteworthy, measuring the relative levels of the different VEGF isoforms might be very interesting given the gap of knowledge on the contribution of VEGF in IPF. Other markers of endothelial cell activation (such as VCAM-1) [79] or vascular remodeling (such as circulating endothelial cell precursors) [80] have been studied and might prove useful in the future.

Matrix Metalloproteinases 1 and 7
Matrix metalloproteinases (MMP) are a structurally and functionally related family of zinc-dependent proteases involved in the breakdown of ECM components and are thought to play a crucial role in ECM during pulmonary fibrosis [81]. MMP1, the most highly expressed interstitial collagenase, degrades fibrillar collagens, while MMP7, the smallest member of the MMP family, is capable of degrading multiple ECM components. Several lines of evidence point to MMP7 as a major player in IPF. MMP7 expression has been localized to the cell surface of AEC and alveolar macrophages from lung tissue of IPF patients, but it is not detected in the healthy lung. Accordingly, BALF MMP7 levels are higher in IPF patients than in healthy controls, suggesting that BALF levels may correlate with lung activity [82]. In the murine model of bleomycin-induced lung injury, genetic ablation of MMP7 is protective against pulmonary fibrosis [82–84]. On the other hand, a polymorphism in the promoter region of the gene encoding MMP1 is linked with IPF [85].

With respect to their biomarker potential, serum MMP1 and MMP7 levels are significantly elevated in IPF patients compared to healthy controls. MMP7 is not specific for IPF, as MMP7 expression in BALF and lung tissue is not significantly different between patients with IPF and other ILDs [84]. By contrast, combining the measurements of both serum MMP1 and MMP7 levels allowed distinguishing between IPF and hypersensitivity pneumonitis. The results of this study also showed that MMP7 concentrations were elevated in patients with subclinical ILD and negatively correlated with forced vital capacity and carbon monoxide diffusion capacity, suggesting that increased MMP7 concentration may be indicative of asymptomatic ILD and reflect disease progression [86]. In a recent larger study including 241 patients with IPF (140 derivation and 101 validation), the concentrations of 92 proteins were analyzed. The results showed that high concentrations of MMP7, ICAM-1, IL-8, VCAM-1 and S100A12 were significantly associated with mortality and/or disease progression. Of note, plasma MMP7 levels >4.3 ng/ml were independently associated with increased mortality (adjusted HR 2.9, p = 0.0013) in the derivation cohort and tended towards an association in the validation cohort [79]. Altogether, these data suggest that MMP7 is unlikely to be a diagnostic marker, but it might be a prognostic tool, either alone or in combination, with other proteins.

Osteopontin
Osteopontin is a key proinflammatory cytokine involved in tissue repair [87]. Interest for this protein in lung diseases was ignited by the observation that osteopontin mRNA was upregulated in IPF lungs compared to healthy controls [83]. Accordingly, immunohistochemical staining demonstrated osteopontin expression by AEC and alveolar macrophages [88]. In the bleomycin model, osteopontin promotes migration, adhesion and proliferation of fibroblasts [89]. In vitro, osteopontin induces the growth rate and migration of fibroblasts and epithelial cells, and promotes ECM deposition [88].

In a small study including 17 patients with ILD (9 with sarcoidosis and 20 healthy controls), it was shown that plasma osteopontin concentrations were significantly higher in patients with ILD compared to the other patients. Despite the small study cohort, osteopontin levels of 300–380 ng/ml allowed to distinguish between patients with ILD and healthy controls with 100% sensitivity and specificity. However, there were no significant differences in plasma osteopontin levels between subjects with IPF and other ILDs. Plasma osteopontin was inversely correlated with PaO₂, but not vital capacity or DLCO [90]. Overall, although the data are limited, the role of osteopontin in IPF pathogenesis, together with its remarkable sensitivity and specificity as a diagnostic marker for ILDs, suggests that further studies on this intriguing molecule are warranted and may reveal its novel function as a biomarker.
**Circulating Fibrocytes**

Circulating fibrocytes do not directly constitute a biomarker reflective of epithelial injury. However, because epithelial injury modulates fibrocyte recruitment to the lungs, it would be neglectful not to mention the potential role of these cells as a biomarker. Fibrocytes are circulating bone marrow-derived mesenchymal progenitor cells that produce ECM components and have the ability to differentiate into fibroblasts and myofibroblasts during wound healing [91]. Fibrocytes have been detected in IPF tissue and are possibly attracted by the lungs via a mechanism involving the CXCL12-CXCR4 and CCL2-CCR2 axes [92, 93]. Their value as biomarker was assessed in a study involving a cohort of 58 patients with IPF. An increased proportion of circulating fibrocytes was found in patients compared to controls (2.72 vs. 1% of peripheral blood leukocytes, respectively) [49]. During acute exacerbation of IPF in 7 patients, fibrocyte numbers were significantly increased compared to 51 subjects with stable disease. Finally, in 3 IPF subjects who recovered from an acute exacerbation, fibrocyte counts returned to pre-exacerbation levels. Overall, it was shown that survival was worse in patients with >5% circulating fibrocytes than in subjects with less fibrocytes. It is, however, noteworthy that the majority of this subset comprised patients with an acute exacerbation. Fibrocytes can be detected and quantified in BALF, but the number of alveolar fibrocytes was associated with less severe disease but not with a better outcome in IPF patients [94].

**Inflammation and IPF**

Although inflammation is not a prominent histopathological finding in IPF, there is evidence for some diffuse infiltration of immune cells in the fibrotic lung [95, 96]. There is emerging evidence that inflammation/immune markers may provide useful information for patient stratification and to support innovative therapeutic strategies. Recent data indicate that activation of blood mononuclear cells might provide interesting markers for IPF outcome, as the level of expression of genes associated with the co-stimulatory signal during T cell activation (including CD28, ICOS, Lck and ITK) was shown to predict prognosis in two IPF cohorts [97]. Circulating autoantibodies targeting periplakin, a desmosomal protein expressed by AEC, and HSP70, a chaperone protein, were proposed as promising prognostic markers for IPF [98, 99], suggesting that auto-immunity might play a role in the course of the disease. This is supported by the demonstration that abnormalities in B cells and B lymphocyte-stimulating factor are common in IPF patients and highly associated with disease manifestation and outcome [100]. Further, downregulation of CD28 on circulating CD4 T cells, a result of repeated antigen-driven proliferation, is associated with a poor outcome in IPF patients [101]. Plasma levels of periostin, a protein produced by monocytes and fibrocytes, particularly in the context of Th2 inflammation, but also by fibroblasts, are increased in IPF patients [102]. Serum levels of periostin in IPF were significantly higher than those of healthy subjects and patients with cryptogenic organizing pneumonia. Periostin levels in IPF patients were inversely correlated with their pulmonary functions [103].

**Conclusions**

Hallmarks of epithelial cell injury have the potential to serve as diagnostic and prognostic biomarkers for IPF and assist in its clinical management. However, although considerable efforts have been made in this field, with respect to both target identification and determination of their significance, there are still several limitations that preclude their broad use for investigational and/or clinical purposes. One pitfall is the limited number of patients included in the studies, which are often retrospective, in which the biomarkers have been tested. Most results, although promising, require confirmation in larger study cohorts. More prospective studies are obviously needed. Validation of the data would then raise the question of the added value of these biomarkers compared to the tests already in use in routine clinical practice. Another drawback is the paucity of longitudinal studies assessing the changes in candidate protein levels over time. They would be of crucial importance to determine whether a potential biomarker can truly be a surrogate for clinical endpoints such as changes in lung function, disease severity and progression, mortality and, in the case of clinical trials, response to therapy. The benefit of an individual biomarker as a diagnostic tool remains questionable: to date, the majority of biomarkers proposed do not allow the discrimination between different ILDs and they have poor specificity for IPF. Besides these considerations, which are an issue for biomarker studies in general [54, 104], there are some limitations in the use of these biomarkers derived from injured epithelial cells (i.e. from epithelial cell breakdown or hyperplasia). Indeed, modulation of their plasma levels is not only noted in other ILDs but also in pathological conditions such as malignancies. Of course, these diseases may very well coincide with a poor outcome in IPF patients [105, 106]. For instance, as discussed above, the levels of KL-6 increase in different cancers, such as adenocarcinoma of the lung. Thus, it may
be tempting to hypothesize that the correlation between increases in KL-6 levels and a poor prognosis might partly reflect KL-6 increases in cancer and may not be attributable to IPF solely. These considerations raise further cautions which should be taken in account, and the future will prove or refute the importance of these biomarkers in the comorbidities associated with IPF.

Perspectives

IPF is a complex disease involving aberrant cross talk of a broad panel of pulmonary resident and recruited cells. A better understanding of its pathogenic mechanisms not only provides targets for therapy, but also allows extrapolation of these insights from the bench to clinical practice, with the possibility to identify surrogate markers to monitor the disease course. To our knowledge, there are currently no broadly accepted and established clinical applications for biomarkers of IPF. Only in Japan, KL-6, SP-A and SP-D are used in clinical practice, as they are considered to have good sensitivity and specificity to diagnose ILD, although their lack of specificity for IPF is acknowledged [107]. The combination of prospective validation of established markers, high-throughput technologies to perform unbiased screening and identification of new markers and prospective studies should allow to alleviate the limitations raised before. The results of studies like the PROFILE (Prospective Observation of Fibrosis in the Lung Clinical Endpoints) study (www.clinicaltrials.gov NCT01110694 and NCT01134822), a UK-based, multicenter, prospective cohort study of newly diagnosed IPF patients [108], which was launched in 2010, are eagerly awaited. Studies like this should help to unravel insights in IPF pathogenesis and provide tools allowing a more stratified and personalized approach to the classification, prognostication and treatment of IPF.

References


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