SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2013; 30; Suppl 1; 27-32

Molecular biomarkers in idiopathic pulmonary fibrosis and disease severity

B. Crestani[1]

[1] Bichat-Claude-Bernard Hospital, France, Inserm U700, Univ. Paris Diderot, Paris 7

Abstract. Despite major accomplishments in our understanding of idiopathic pulmonary fibrosis (IPF), its diagnosis, and management continues to pose significant challenges. The clinical management of IPF remains a major challenge due to a limited number of effective drug therapies, as well as accurate indicators of disease progression. Most patients die within at least five years after diagnosis. The identification of more accurate predictors of prognosis and survival in IPF is critical for physicians and would be useful to facilitate counselling of patients and their families, to aid communication among providers, and to guide optimal timing of transplantation. Improvements in molecular techniques have developed our understanding of IPF and have led to the identification of new pathways and a more targeted approach to the treatment of IPF with potentially novel anti-fibrotic agents. These insights have led to an increased interest in biomarkers of IPF disease progression. Although there are no validated biomarkers that are currently available, the need for surrogates of diagnosis, prognosis, and monitoring of disease course is great. However, there is currently no established method of combining these predictors to accurately determine prognosis or define disease stage. *(Sarcoidosis Vasc Diffuse Lung Dis 2013; 30 Suppl 1: 27-32)*

Key words: biomarkers, clinical management, idiopathic pulmonary fibrosis, prognosis

Introduction

Idiopathic pulmonary fibrosis (IPF) is defined as a specific form of chronic, progressively fibrosing idiopathic interstitial pneumonias (IIPs), and is associated with the histopathological and/or radiological pattern of usual interstitial pneumonia (UIP) (1). It is a life-threatening fibrotic lung disease of unknown aetiology with significant morbidity and mortality. The pathogenesis of IPF is not fully understood. Although IPF was initially thought to result from generalised pulmonary inflammation leading to fibrosis, the current paradigm has shifted towards alveolar epithelial cell dysfunction and disordered fibroproliferation (2). Improvements in molecular techniques have developed our understanding of IPF and with it identified new pathways and potential targets for therapeutic intervention. Current approaches looking for new biomarkers may provide new insights of underlying mechanisms of disease.

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 centres, which may be geographically distant from patients and their primary physicians. In addition, although the median survival of IPF patients ranges between 2-3 years, there is a wide spectrum of disease courses that can manifest as either long periods of stability, a steady gradual decline, and/or periods

Correspondence: Bruno Crestani
Service de Pneumologie A, Hôpital Bichat,
46 rue Henri Huchard,
75877 Paris Cedex 18, France

of acute deterioration (3). This makes it difficult for clinicians to predict the disease course for an individual patient, as there are no accepted surrogates of these clinical courses. The identification of accurate and objective biomarkers that provide prognostic information about disease status and/or and survival estimates would help clinical management and individual treatment decision-making in patients with IPF, particularly in cases where a surgical lung biopsy cannot be obtained or access to dedicated interstitial lung disease physicians is limited (4). Finally, the discovery of biomarkers that reflect disease activity would allow for serial monitoring as well as an objective marker to assess treatment efficacy.

Definition and need for biomarkers in IPF

Biomarkers may be defined as '*a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention*' (5). Biomarkers act as surrogates for clinically meaningful outcomes and may or may not reflect the pathogenesis underlying a disease. Peripheral blood biomarkers in particular are easy to obtain, can be measured longitudinally, and have the greatest likelihood of achieving clinical utility) (6). Ideally, they should also provide an advantage over currently used clinical measures in ease, timeframe or expense.

Currently there are no validated biomarkers that are routinely used in the clinical care of patients with IPF. There are limited retrospective data on the predictive value of biomarkers in patients with IPF (7, 8). To date, identification of biomarkers in IPF has been focused on single specific molecules. Studies have been retrospective and monocentric and included small cohorts of patients without adequate controls (e.g. other IIPs), independent validation, or adequate statistical analysis. However, the advent of molecular analytic techniques, e.g. microarray technology, has allowed the simultaneous monitoring of the transcriptional behaviour of thousands of genes and proteins. This technology has been repeatedly shown to be useful in the analysis of the response of a variety of cellular systems to stimuli, in the classification of human cancer, as well as the characterisation of the transcriptional profile of UIP (9-11).

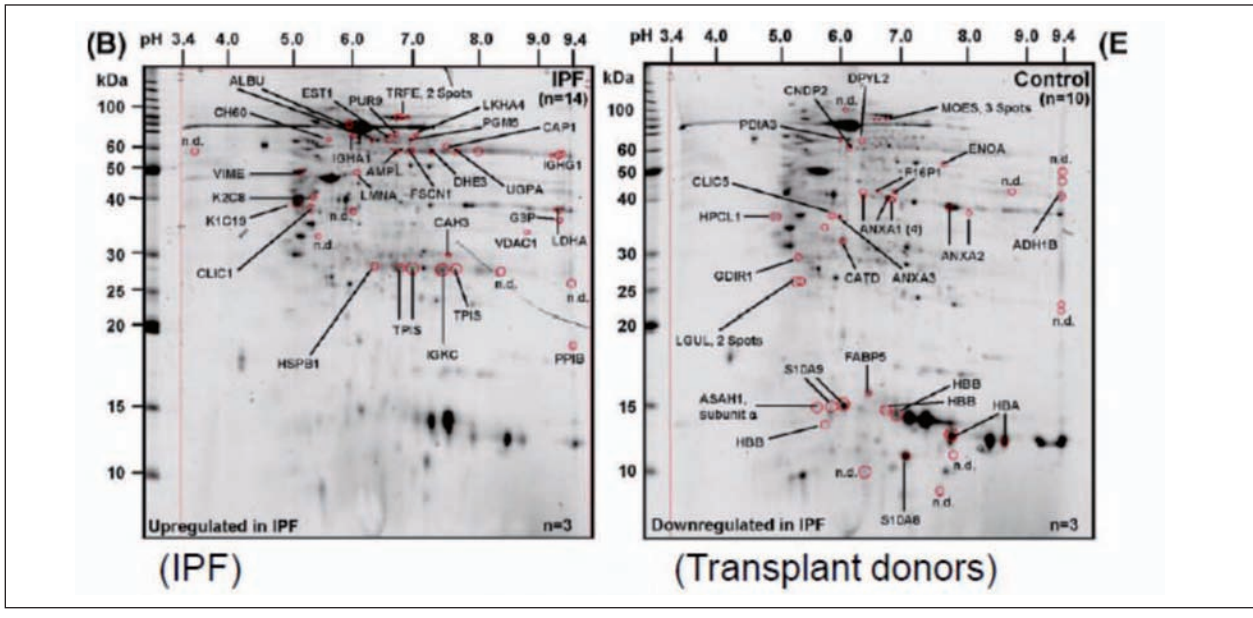


Fig. 1. Representative 2-DE maps of 400 μg of proteins extracted from lungs of IPF patients and control lungs (12). Reprinted (adapted) with permission from Korfei M, et al. Comparative proteomic analysis of lung tissue from patients with idiopathic pulmonary fibrosis (IPF) and lung transplant donor lungs. J Proteome Res 2011; 10: 2185-205. Copyright 2011 American Chemical Society

Analytical techniques in identifying molecular biomarkers

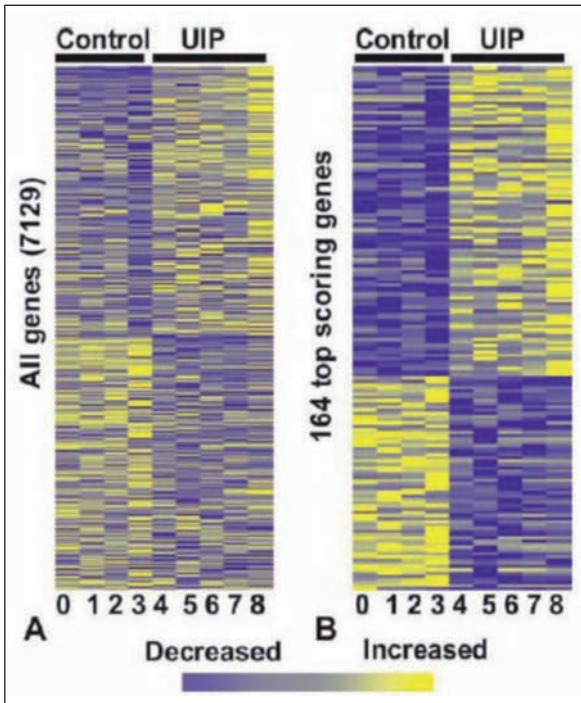
Protein electrophoresis

One comparative proteome analysis of lung tissue from patients with sporadic IPF and human donor lungs using two-dimensional gel electrophoresis and MALDI-TOF-MS identified 89 differentially expressed proteins of which 51 were up-regulated and 38 down-regulated in IPF (Figure 1) (12). Increased expression of markers for the unfolded protein response (UPR), heat-shock proteins, and DNA damage stress markers indicated a chronic cell stress-response in IPF lungs. In contrast, up-regulation of heat-shock protein 27 (Hsp27) was exclusively observed in proliferating bronchiolar basal cells and associated with aberrant re-epithelialisation at the bronchio-alveolar junctions. Among the down-regulated proteins in IPF were antioxidants, members of the annexin family, and structural epithelial proteins. These results indicate that IPF is characterised by epithelial cell injury, apoptosis, and aberrant epithelial proliferation.

Microarray technology

One proof of concept study using oligonucleotide microarrays demonstrated a clear distinction between gene expression patterns of muscle markers, extracellular matrix remodelling proteins, cytokines and growth factors, complement, and immunoglobulins in the lungs of patients with UIP and normal control lung tissue (Figure 2). In particular, this study demonstrated a coordinated induction of genes that encode metalloproteases (MMP1, MMP2, MMP7, MMP9), and identified matrilysin (MMP7) as a gene that was most distinctive between fibrotic and normal lungs (13).

Other studies have identified statistically significant differences in gene expression signatures characterising IPF from hypersensitivity pneumonitis (HP), and nonspecific interstitial pneumonia (NSIP). The IPF signature was characterised by the expression of tissue remodelling, epithelial, and myofibroblast genes, whereas the HP gene expression signature was enriched for genes that are functionally associated with inflammation, T-cell activation, and immune responses. Gene expression signatures



For all 7,129 genes (*A*) and for the 164 most informative genes (*B*). To eliminate outlier effect, genes were normalised to a range of (0,1), meaning that the maximum value for every gene was set to be 1, the minimum value to be 0, and the rest of the values were linearly fitted to this range. Yellow is maximal expression and blue is minimal.

Fig. 2. Gene expression infogram in IPF and normal lung tissue (13). From Zuo F, et al. Gene expression analysis reveals matrilysin as a key regulator of pulmonary fibrosis in mice and humans. Proc Natl Acad Sci USA 2002; 99: 6292-7. Copyright 2002 National Academy of Sciences, USA

of NSIP, a histological pattern that is often difficult to differentiate consistently from HP and IPF, was less persuasive (14, 15). Similarly, studies employing Serial Analysis of Gene Expression (SAGE) indicate that molecular signatures from IPF lung parenchyma are distinct from normal lung tissue and other chronic lung diseases at the time of diagnosis and may be beneficial in predicting disease progression and/or further elucidating the pathophysiology of IPF (16). Indeed, a further recent study used a Bayesian probit regression statistical method to analyse differences in multi-dimensional gene expression data to develop a provisional but validated diagnostic model for IPF (17).

Microarray analyses have also been used to investigate the gene expression profiles of peripheral blood RNA from IPF patients. Preliminary results

suggest that the peripheral blood transcriptome has the potential to distinguish normal individuals from patients with IPF, as well as extent of disease classified by percent predicted diffusing capacity of the lung for carbon monoxide (DL_{CO}), but not forced vital capacity (FVC) (18). Another study of the peripheral blood protein signature supports these findings and also endorses the involvement of MMP7 and MMP1 as two main components of the IPF signature (Figure 3). Moreover, it suggests that increased MMP7 concentration may be indicative of asymptomatic ILD and reflect disease progression (19).

MicroRNA microarrays

MicroRNAs are small, non-coding, post-transcriptional RNA gene regulators. MicroRNAs can be found in the peripheral blood (20). They function by binding to specific sequences, typically in the untranslated region of the target mRNAs and blocking translation or causing the rapid degradation of the target transcript (21). Considering that the lung in IPF is characterised by profound changes in the phenotype of lung fibroblasts and epithelial cells, as well as by drastic changes in global patterns of gene expression (22, 23), analyses suggest a role for microRNAs in epithelial-mesenchymal transition (EMT) in IPF (24-27). Two such studies have demonstrated that let-7d, a microRNA abundantly expressed in epithelial cells in normal lungs, is down-regulated in IPF while its target molecule, high-mobility group AT-hook 2 (HMGA2), a protein that belongs to the non-histone chromosomal high-mobility group (HMG) protein family, is over-expressed. The expression of let-7d is inhibited by transforming growth factor-β1. These results suggest that down-regulation of let-7 microRNAs may be important in determining the lung phenotype in IPF, as well distinguishing between rapidly versus slowly progressing IPF, and although currently only a research tool, may have some future clinical use (28, 29).

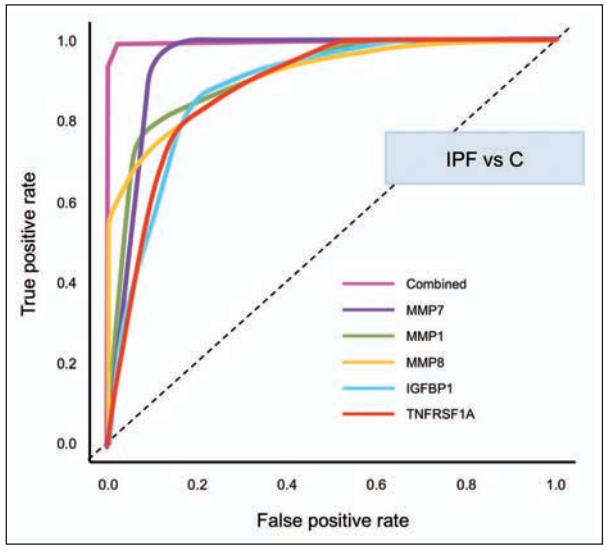


Fig. 3. Peripheral blood proteins distinguish IPF patients from controls

Composite biomarker indices

The notion that peripheral blood proteins may be informative in IPF has recently gained significant momentum (30). The use of peripheral blood proteins as potential biomarkers is supported by recent studies that demonstrate reduced survival in IPF patients with high serum concentrations of MMP-7, mucin 1 (KL-6), (31) CCL-18 (32), or surfactant protein A (33). Using a combination of five protein markers (MMP-7, VCAM-1, S100A12, ICAM-1, and IL-8), a risk score, the *Personal Clinical and Mortality Index (PCMI)*, has recently been derived that accurately distinguishes IPF from other lung diseases (chronic obstructive pulmonary disease, sarcoidosis, and HP), and predicts survival, transplant-free survival, and progression-free survival (34, 35).

Discussion

Despite major advances in our understanding of IPF, the diagnosis and management of the condition continues to pose significant challenges. The treatment of IPF remains unsatisfactory due to limited availability of effective drug therapies (only one drug, pirfenidone, is currently approved for the treatment of patients with mild-to-moderate IPF), a lack of accurate indicators of disease progression, and an absence of simple short-term measures of therapeutic response. The identification of more accurate surrogate predictors of diagnosis, prognosis and monitoring of disease course in IPF is critical for physicians and would be useful to facilitate

counselling of patients and their families, to aid communication among providers, and to guide optimal timing of transplantation. The availability of protein biomarkers and validated integrated risk scores should lead to better evaluation and stratification of patients with IPF for research, and for transplant prioritisation.

While several biomarker candidates have been proposed, it seems unlikely that a single biomarker will serve these multiple purposes; however, a panel of several biomarkers may accomplish these goals (36). Careful longitudinal phenotyping of individuals with IPF, together with the application of novel '-omics'-based technology, should provide important insights into disease pathogenesis and should address some of the major issues holding back drug development in IPF (37). The PROFILE (Prospective Observation of Fibrosis in the Lung Clinical Endpoints) study is a currently enrolling, prospective cohort study designed to tackle these issues. The PROFILE trial is generating longitudinal data to identify specific biomarkers that enable diagnosis without biopsy, predict IPF patients with more aggressive and progressive disease, and also to identify response to treatment in future clinical trials (38). The study is also designed to assess the use of daily home lung function measurement and a computerised technique for analysing lung sounds to predict the development of worsening lung fibrosis. This study is due to complete in September 2013.

Acknowledgments

The author thanks C. Trenam and M. Smith of IntraMed Europe for editorial assistance in the preparation of the manuscript.

Development of this article was supported by InterMune.

References

1. Raghu G, Collard HR, Egan JJ, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. American journal of respiratory and critical care medicine 2011; 183 (6): 788-824.
2. Kim DS, Collard HR, King TE Jr. Classification and natural history of the idiopathic interstitial pneumonias. Proceedings of the American Thoracic Society 2006; 3(4): 285-92.
3. Martinez FJ, Safrin S, Weycker D, et al. The clinical course of patients with idiopathic pulmonary fibrosis. Ann Intern Med 2005; 142: 963-7.
4. Thomeer M, Grutters JC, Wuyts WA, et al. Clinical use of biomarkers of survival in pulmonary fibrosis. Respir Res 2010; 11: 89.
5. Lesko LJ, Atkinson AJ Jr. Use of biomarkers and surrogate endpoints in drug development and regulatory decision making: criteria, validation, strategies. Annu Rev Pharmacol Toxicol. 2001; 41: 347-66.
6. Vij R, Noth I. Peripheral Blood Biomarkers in Idiopathic Pulmonary Fibrosis Transl Res 2012; 159 (4): 218-27.
7. McCormack FX, King TE Jr., Bucher BL, Nielsen L, Mason RJ. Surfactant protein A predicts survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 1995; 152: 751-9.
8. Kinder BW, Brown KK, Schwarz MI, Ix JH, Kervitsky A, King TE Jr. Baseline BAL neutrophilia predicts early mortality in idiopathic pulmonary fibrosis. Chest 2008; 133: 226-32.
9. Cojocaru GS, Rechavi G, Kaminski N. Isr Med Assoc J 2001; 3: 292-6.
10. van Berkum NL, Holstege FC. Curr Opin Biotechnol 2001; 12: 48-52.
11. Kaminski N, Allard JD, Pittet JF, et al. Proc Natl Acad Sci USA 2000; 97: 1778-83.
12. Korfei M, Schmitt S, Ruppert C, et al. Comparative proteomic analysis of lung tissue from patients with idiopathic pulmonary fibrosis (IPF) and lung transplant donor lungs. J Proteome Res 2011; 10 (5): 2185-205.
13. Zuo F, Kaminski N, Eugui E, et al. Gene expression analysis reveals matrilysin as a key regulator of pulmonary fibrosis in mice and humans. Proc Natl Acad Sci USA 2002; 99 (9): 6292-7.
14. Selman M, Carrillo G, Estrada A, et al. Accelerated variant of idiopathic pulmonary fibrosis: clinical behavior and gene expression pattern. PLoS One 2007; 2(5): e482.
15. Selman M, Pardo A, Barrera L, et al. Gene expression profiles distinguish idiopathic pulmonary fibrosis from hypersensitivity pneumonitis. Am J Respir Crit Care Med 2006; 173 (2): 188-98.
16. Boon K, Bailey NW, Yang J, et al. Molecular phenotypes distinguish patients with relatively stable from progressive idiopathic pulmonary fibrosis (IPF). PLoS One 2009; 4 (4): e5134.
17. Meltzer EB, Barry WT, D'Amico TA, et al. Bayesian probit regression model for the diagnosis of pulmonary fibrosis: proof-of-principle. BMC Med Genomics 2011; 4: 70.
18. Yang IV, Luna LG, Cotter J, et al. The peripheral blood transcriptome identifies the presence and extent of disease in idiopathic pulmonary fibrosis. PLoS One 2012; 7 (6): e37708.
19. Rosas IO, Richards TJ, Konishi K, et al. MMP1 and MMP7 as potential peripheral blood biomarkers in idiopathic pulmonary fibrosis. PLoS Med 2008; 5 (4): e93.
20. Hunter MP, Ismail N, Zhang X, et al. Detection of microRNA expression in human peripheral blood microvesicles. PLoS One 2008; 3: e3694.
21. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-97.
22. Selman M, Pardo A, Kaminski N. Idiopathic pulmonary fibrosis: aberrant recapitulation of developmental programs? PLoS Med 2008; 5: e62.
23. Zuo F, Kaminski N, Eugui E, et al. Gene expression analysis reveals matrilysin as a key regulator of pulmonary fibrosis in mice and humans. Proc Natl Acad Sci USA 2002; 99: 6292-7.
24. Burk U, Schubert J, Wellner U, et al. A reciprocal repression between ZEB1 and members of the miR-200 family promotes EMT and invasion in cancer cells. EMBO Rep 2008; 9: 582-9.
25. Korpal M, Lee ES, Hu G, Kang Y. The miR-200 family inhibits epithelial-mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2. J Biol Chem 2008; 283: 14910-14.

26. Park SM, Gaur AB, Lengyel E, Peter ME. The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. Genes Dev 2008; 22: 894-907.
27. Gregory PA, Bert AG, Paterson EL, et al. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat Cell Biol 2008; 10: 593-601.
28. Pandit KV, Corcoran D, Yousef H, et al. Inhibition and role of let-7d in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2010; 182 (2): 220-9.
29. Oak SR, Murray L, Herath A, et al. A micro RNA processing defect in rapidly progressing idiopathic pulmonary fibrosis. PLoS One 2011; 6 (6): e21253.
30. Prasse A, Muller-Quernheim J. Non-invasive biomarkers in pulmonary fibrosis. Respirology 2009; 14: 788-95.
31. Yokoyama A, Kondo K, Nakajima M, et al. Prognostic value of circulating KL-6 in idiopathic pulmonary fibrosis. Respirology 2006; 11: 164-8.
32. Prasse A, Probst C, Bargagli E, et al. Serum CC-chemokine ligand 18 concentration predicts outcome in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2009; 179: 717-23.
33. Kinder BW, Brown KK, McCormack FX, et al. Serum surfactant protein-A is a strong predictor of early mortality in idiopathic pulmonary fibrosis. Chest 2009; 135: 1557-63.
34. Richards TJ, Lindell KO, Klesen M, Kaminski N, Zhang Y, Gibson K. Peripheral blood biomarkers predict disease progression and mortality in IPF. Am J Respir Crit Care Med 2010; 181: A1120.
35. Richards TJ, Kaminski N, Baribaud F, et al. Peripheral blood proteins predict mortality in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2012; 185 (1): 67-76.
36. Zhang Y, Kaminski N. Biomarkers in idiopathic pulmonary fibrosis. Curr Opin Pulm Med 2012; 18: 441-6.
37. Mahendran S, Sethi T. Treatments in idiopathic pulmonary fibrosis: time for a more targeted approach? QJM 2012; 105: 929-34.
38. Maher TM. Prospective Observation of Fibrosis in the Lung Clinical Endpoints Study (PROFILE). ClinicalTrials.gov Identifier: NCT01110694; http://clinicaltrials.gov/ct2/show/NCT01110694.

Disclosures:

- Principal Investigator and coordinator for France for the BIBF 1120 trials
- Honorarium for speaking from InterMune, Boehringer Ingelheim and Sanofi-Aventis