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Journal club

Could airway basal cells be a novel predictor of mortality in IPF?

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Commentary on:

Prasse A, *et al*. BAL cell gene expression is indicative of outcome and airway basal cell involvement in IPF. *Am J Respir Crit Care Med* 2019; 199: 622–630.

Context

Idiopathic pulmonary fibrosis (IPF) is a progressive and fatal interstitial lung disease that exhibits a variable trajectory, depending on the individual [1]. Currently, it is very difficult to predict the prognosis of IPF, making the clinical management of the disease a major challenge. Over the years, there have been numerous studies to identify peripheral blood biomarkers, gene variants or clinical features of IPF that correlate to disease outcome [2, 3]. We now understand that the development and progression of IPF occurs due to an abnormal regenerative response in response to damaged epithelium [4]; however, there has been very limited research into specific changes or potential biomarkers that reflect the mechanism of alveolar derangement and disease progression. Whilst lung biopsies taken at diagnosis would be ideal to investigate for potential alveolar-derived biomarkers, they are highly invasive and can be high risk to the patients' health due to post-operative complications. They therefore tend to be avoided in clinic. Bronchoalveolar lavage (BAL), on the other hand, is a minimally invasive procedure, while still providing a sample of the cellular environment with the alveolar region of the lung. Therefore, Prasse *et al*. [5] sought to investigate the gene expression of BAL samples from IPF patients to address this problem.

Methods

In this study, BAL samples were assessed in confirmed IPF-patients (total n=176) from three different referral centres: a derivation cohort in Friedburg, Germany (n=62), and replication cohorts in Siena, Italy (n=50) and Leuven, Belgium (n=64). Patients were naïve to treatment prior to sample collection, taken at diagnosis. Gender Age Physiology (GAP) index and lung function were obtained for all patients, and survival status was monitored for up to 3 years. BAL cell transcriptome was determined using microarray sequencing, and validated with nCounter expression analysis system. Cellular composition of BAL samples was assessed *via* immunocytology. Airway basal cells (ABC) were obtained *via* bronchial brushings for additional gene expression profiling. Lung tissue sections from 15 IPF patients, 3 sarcoidosis and 3 healthy donors were characterised for ABC markers ΔNP63 and cytokeratin 5/6 *via* immunohistochemistry. COPD patients and sarcoidosis BAL gene expression, alongside healthy controls, were used as active disease control cohorts. Genes associated with mortality were first identified in the derivation

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cohort using a Cox proportional hazard model, adjusted for age and sex, to generate a multivariable risk predication signature. Patients in the replication cohort were divided into high risk (above median gene expression) and low risk (below median gene expression) to determine prediction performance using log rank test. Survival analysis was performed with BAL prediction signature alone or in combination with the GAP score.

Main results

This study examined the changes in BAL cell transcriptomic across three separate cohorts of IPF patients (total of 176 samples used, average age 68.1±9.5 years and % predicted FVC of 71±21%). After adjustment for age and sex, a total of 1582 genes were found to be associated with mortality in the derivation cohort (n=62). Using component-wise likelihood-based boosting, a predictive signature of nine genes was identified from this dataset and examined from predicative performance in the replication cohorts. This signature found individuals with above median expression of these genes *i.e.* high risk, had greater overall mortality than low-risk individuals (Siena cohort p<0.0032, c-index 0.66; CI 0.55–0.76) and Leuven cohort (p=0.0033; c-index 0.63; CI 0.54–0.72). Resampling and validation of the signature with nCounter showed six of these nine genes were reproducibly expressed across all cohorts (n=168) and predictive of mortality (p<0.00001, hazard ratio 3.951, CI 2.132–7.323). This signature also showed stronger predictive ability of mortality than the GAP index (c-index 0.63; 95% CI 0.58–0.69), and was able to significantly reduce the rate of error when combined with the GAP index. Interestingly, closer examination of the BAL signature found 921 of the genes identified in the microarray were also upregulated in airway basal cells. A gene signature was then developed using isolated ABC from healthy controls, compared to alveolar macrophages and bronchial epithelial cells. A total of 165 genes from the ABC signature were among the 1582 genes associated with mortality (p<0.0001). Further analysis of a signature for ABC-derived genes revealed that 16 genes were significantly associated with increased mortality across all three cohorts, with increased mortality risk in those within the high-risk group (Friedberg: c-index 0.73; 95% CI 0.68–0.77; Leuven: c-index 0.67; 95% CI 0.56–0.76; Siena: c-index 0.66, 95% CI 0.57–0.74). Further investigation of the BAL cellular composition in IPF patients revealed the presence of cytokeratin 5/6 and ΔNP63, markers of ABC, which were also present in the alveolar compartment of IPF tissue. These cells were rarely observed in healthy controls, nor COPD or sacroidosis patients.

Commentary

The clinical management of IPF is still significantly hindered by a lack of tools to help identify patients at risk of disease progression. Therefore, there is still an unmet need to identify useful and measurable biomarkers in IPF to assist in the diagnosis and prognosis of the disease [6]. In this study, Prasse *et al.* [5] looked for genetic changes present in the BAL that could predict survival at diagnosis in IPF. They were able to identify a genetic signature in IPF BAL that was predictive of mortality, which was enriched with genes from airway basal cells. Importantly, this signature and predictive performance was validated in replication cohorts. Furthermore, the signature had a more robust estimation of poor prognosis than the GAP index, which predicts mortality based on patient clinical characteristics [7].

BAL samples have often been overlooked in IPF, and typically used as means of exclusion of other potential alternative conditions, such as hypersensitive pneumonia, nonspecific interstitial pneumonia, and connective-tissue disease-associated interstitial lung disease [8]. Whilst peripheral blood is a noninvasive means to examine potential genetic and protein changes, potential markers significant changes present in the lung may be either missed or confounded with other changes in the body once in circulation. BAL, on the other hand, enables access to cells from the most distal regions of the lung. There have been previous studies which have identified potential biomarkers in BAL from IPF patients; however, these have been to either validate results found in peripheral blood [9] or focus on specific cells such as macrophages [10]. This is the first study to undergo a detailed transcriptomic analysis of the BAL from IPF patients to identify potential biomarkers.

One of the most interesting things in this study is the identification of genes upregulated in ABCs within the set of genes associated with mortality. Furthermore, cells expressing ABC markers were detected in both BAL and tissue of IPF patients, whilst also not being observed in healthy individuals, nor in COPD or sarcoidosis patients. The presence of abnormal epithelial cells in the IPF lung has been recognised for some time, seen in both early and late stages of IPF [11]. In advanced IPF lesions, bronchiolisation and the formation of "honeycomb" cysts are a typical feature, which consists of mucociliary epithelium within the distorted alveolar region [12]. ABCs are thought to act as a regenerative cell type in the lung and accumulate in response to bronchiolar injury [13]. Abnormal activation of ABCs, which reside in the conducting airways down to the respiratory bronchioles and function as stem cells, might contribute to re-epithelisation of damaged alveolar epithelium and resulting bronchiolisation of alveolar spaces. The work by Prasse *et al.* [5] supports the concept that the cells may be re-programmed in

IPF, resulting in the abnormal re-epithelialisation in the IPF lung and may be a novel prognostic feature of the disease.

One of the limitations of this study is that it only examined a singular time-point, rather than assessing whether the BAL signature also changes over time in correlation to mortality. It would be interesting to replicate these findings with longitudinal samples and assess the dynamics of the gene signature in additional cohorts of patients with IPF over time, with respect to both therapeutic response and clinical disease progression. In addition, further work examining the potential roles of these genes and how the ABCs are functionally altered is needed, as it is presently unclear what mechanisms lead to tissue remodelling and altered epithelial cell fates. Pathway analysis of the genes identified in the signature would be useful to help identify how ABCs are contributing to the pathogenesis and progression of IPF.

Implications for practice

This study provides not only a panel of potential, measurable biomarkers that could significantly enhance diagnosis and early prognostic indicators of IPF, but also critical insight to mechanisms in the disease. Prasse *et al.* [5] demonstrate the utility of the BAL, beyond diagnosis of IPF. Currently, BAL is not routinely performed during IPF diagnosis. This study, however, creates a case for inclusion of routine BAL at diagnosis. BAL carries a lot lower risk than biopsy sample and also has the ability to be re-sampled for repeated measurements. However, further work is needed to validate the BAL gene signature and significance of ABC, particularly in understanding the signalling pathways, which may help identify both a specific endotype of IPF, as well as novel therapeutic targets.

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Conflict of interest

L. Organ has nothing to disclose.

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