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INTERSTITIAL LUNG DISEASE (E ALHAMED, SECTION EDITOR)

Biomarkers in interstitial lung disease: moving towards composite indexes and multimarkers?

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Abstract Interstitial lung disease (ILD) covers a large spectrum of lung disorders that affects the parenchyma and is often associated with inflammation and/or fibrosis. Clinically, there is a great need for biomarker development for these disorders, to help diagnosis, treatment selection and assessment of efficacy as well as to predict progression. Thus far, no broadly validated biomarker exists for ILD, due to the existence of a very large number of disorders of often-unknown etiology, overlapping symptoms and disorders associated with a spectrum of multi-morbidities involving similar chronic inflammatory and fibrotic biochemical processes. We discuss here the development of biomarkers in IPF, sarcoidosis, connective tissue disease-associated ILD (CTD-ILD), and chronic hypersensitivity pneumonitis. We further discuss the need and opportunity to develop a multimarker approach that would be clinically meaningful for patients with ILD. Such composite index could include clinical symptoms, pathological assessment, and lung physiology measurements added to molecular information derived from bronchoalveolar lavage and serum. We also discuss the increased opportunity for patients to be involved in research as recent technological advances now allow the serial measurement of lung function using handheld spirometers, combined with handheld devices allowing measurement of patient reported outcomes, mobility, exercise, and sleep.

Keywords Biomarkers · Interstitial lung disease · Composite index · Multimarker

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Introduction

The term interstitial lung disease (ILD) refers to a large group of heterogeneous disorders characterized by inflammatory and/or fibrotic infiltration of the alveolar septa [1]. As the degree of involvement varies between diagnoses and among individual patients with similar diagnosis, the natural history of disease is highly variable, complicating the diagnostic and therapeutic approach to patient care [2, 3]. Many of the ILDs are seen on a spectrum of fibrotic lung disease with a propensity to progress towards end-stage parenchymal fibrosis. Although significant strides have been made in understanding the mechanisms responsible for the development of pulmonary fibrosis, no therapeutic options exist that can reverse established fibrosis. Therapeutic agents such as pirfenidone and nintedanib, which target key pro-fibrotic signaling pathways, have recently been shown in large randomized trials to be safe and efficacious in patients with idiopathic pulmonary fibrosis (IPF) [4, 5]. Rather than reversing established fibrosis, however, these agents slow disease progression. With an evolving understanding of the basic cellular processes responsible for pulmonary fibrosis, numerous putative molecular biomarkers, associated with disease progression have been identified. A NIH working group defined a biomarker as "a characteristic that is objectively measured and evaluated as an

indicator of normal biological processes, pathological processes, or pharmacologic response to a therapeutic intervention". In recent years, extensive research has focused on identifying clinically relevant molecular biomarkers that can be detected in easily accessible patient specimens such as blood, sputum, or urine. Although a number of putative markers have been identified in small cohorts of patients with ILD, no molecular biomarker has reached FDA approval in the realm of any of the ILDs. Rather than represent epiphenomena, it is critical that molecular biomarkers should reflect the pathobiologic mechanisms driving progressive fibrosis. An ideal molecular biomarker is characterized by the following features: easily attainable with low to no risk to the patient; objectively detected and quantified; biochemically stable for routine processing; available for serial measurement; clinically impactful from a screening, diagnostic, therapeutic, or prognostic perspective; provide a safety assessment; predict patient adherence; cost-effective; and predict treatment response. This last point is critical as molecular biomarkers are at the forefront of the "personalized medicine" movement, permitting the development of therapies to target the responsible disease processes and the patients most likely to benefit [6].

Considering that the differential diagnosis for ILD has grown to include 200–300 diverse entities, the task of identifying and characterizing useful biomarkers is challenging. In this review, we summarize the usefulness of selected biomarkers in the following ILDs: IPF, sarcoidosis, connective tissue disease-associated ILD (CTD-ILD), and chronic hypersensitivity pneumonitis. We further discuss the need and opportunity to develop composite and biomarkers including molecular biomarkers, with physician-evaluated clinical information and patient-generated data.

Idiopathic pulmonary fibrosis

IPF is the most common idiopathic interstitial pneumonia and the best-studied fibrotic lung disorder. IPF is characterized by the progressive accumulation of matrix molecules in the interstitial spaces, leading to increased stiffening of the lungs and reduced ability to exchange gas. Despite intense investigation over the last 30 years, the etiology of IPF remains unknown. Although we have advanced our ability to describe disease by radiographic, pathologic, and physiologic means, we remain unable to predict the course of disease in individual patients who have a median survival from the time of diagnosis of 3–5 years. The topic of biomarker development and utilization in the IPF population has been covered in several excellent reviews including a recent update by Hambly et al. [6–15, 16••, 17, 18]. Of the multiple candidate markers, the most promising are the identification of a specific mucin 5B (MUC5B) promoter variant and the matrix metalloproteinase 7 (MMP7), both of which having great promise. In 2011, Seibold et al. identified a single nucleotide polymorphism (SNP) in the promoter region of MUC5B (rs35705950) that was strongly associated with both familial and sporadic IPF [19]. The association appears to be specific for IPF, as no similar relationship has been identified in patients with either sarcoidosis or CTD-ILD [20]. The MUC5B promoter variant also has prognostic value, as it is associated with decreased mortality when compared to the wild-type form [21]. MMP7 is the only marker to date to show promise in a combined personal clinical and molecular prediction index (PCMI) index, where the authors demonstrated that higher MMP7 levels combined with gender, forced vital capacity (FVC), and diffusing capacity yielded a severity score that was associated with poor outcome [22]. To date, only MUC5B and MMP7 have been studied in large validation cohorts and, perhaps more importantly, demonstrated clinical utility beyond that achieved with conventional clinical predictors. Krebs von den Lungen (KL-6) is a circulating glycoprotein released from alveolar and bronchial epithelial cells that have shown promise as a biomarker for IPF patients [38]. Although it has already been used as a diagnostic marker in Japan, clinical trials has yet to validate the clinical efficacy of KL-6 [23] and further prospective studies are warranted. Recently, both hospital admissions [24••] and co-morbid obstructive sleep apnea were associated with poor outcome in IPF patients [10]. This highlights the importance of combining clinical, physiologic, and molecular variables to optimize test characteristics, rather than looking at each value in isolation.

Sarcoidosis

Sarcoidosis is a systemic inflammatory disease of unknown etiology that commonly manifests as a fibrotic lung disease with variable progression [25]. The clinical diagnosis relies on the histologic identification of non-necrotizing granulomas in biopsied samples, combined with specific clinical, radiologic, and laboratory findings [26]. The importance of a multimodality assessment cannot be understated as non-necrotizing granulomas are encountered in numerous disease states including berylliosis, lymphoma, and chronic hypersensitivity pneumonitis. In the thorax, sarcoidosis most commonly affects the pulmonary parenchyma and the mediastinal/hilar lymph nodes. Granulomas may also be detected in the heart, liver, spleen, skin, and eyes. The observed granulomatous inflammation may spontaneously resolve, remit with treatment or progress to scar tissue. In the lungs, progressive, long-standing, severe disease can result in pulmonary fibrosis and ultimately respiratory failure and death. The identification of clinically relevant diagnostic, prognostic, and treatment-responsive biomarkers in patients with sarcoidosis is imperative given the challenges encountered when caring for this population. Sarcoidosis is primarily thought to be a disease of T-helper-1 (Th1)-mediated inflammation [27]. The non-

specific nature of this biochemical process, involved in a large number of chronic inflammatory disorders, makes the identification of a single sarcoidosis specific, molecular biomarker troublesome. The current state of biomarkers in sarcoidosis was recently reviewed [28]. The authors report that although the search for biomarkers has improved our understanding of the pathophysiological processes involved in sarcoidosis, most tests available are limited by a lack of reproducibility, specificity, and sensitivity. Recent proteomic profiling built on 3072 protein fragments from BALF and serum indicated four antigens associated with sarcoidosis with a high level of inter-individual heterogeneity [29]. Further exploration of larger cohorts of patients with sarcoidosis using various "omics" methods and the generation of multi-modality prediction tools may assist in generating high yield clinical instruments.

Connective tissue disease

Connective tissue diseases (CTD) represent a diverse group of heritable and idiopathic disorders where the pathologic target is the extracellular matrix that supports tissue structure [30]. This has immediate complications in the lung where the interstitium, which supports the framework of the alveolar network, is primarily composed of collagenous and non-collagenous proteins and proteoglycans. The CTDs that are most commonly associated with fibrotic ILD include rheumatoid arthritis, systemic sclerosis, idiopathic inflammatory myopathies (IIM), mixed-connective tissue disease, Sjøgren's disease, and rarely systemic lupus erythematosus. Although pulmonary manifestations typically develop in patients with a pre-existing established CTD diagnosis, a significant proportion will initially present with isolated respiratory complaints. At present, our ability to isolate those patients with a CTD predisposed to develop ILD, and predict prognosis and treatment response in patients with established CTD-ILD is primitive, reflecting a great need for the development of biomarkers and molecular signatures to appropriately characterize individual patients. The autoimmune element of many of these disorders has prompted many investigations to examine biomarkers in serum or plasma, either by measuring immune activation, autoantibodies, degradation products from tissue destruction, or the actual enzymes that drive the pathologic change. For example, the identification of the anti-Jo-1 antibody in patients with IIM isolates a group of patients at increased risk of developing ILD. This antibody is directed against the histidyl-tRNA synthetase that catalyzes the binding of histidine to its cognate tRNA during protein synthesis. Anti-Jo-1 antibodies are commonly encountered in the antisynthetase syndrome, which is characterized by ILD, myositis, non-erosive arthritis, Raynaud's phenomenon, fever, and "mechanic's hands". In this scenario, serum levels of anti-Jo-1 antibodies correlate to disease activity [31]. The increasing access to novel screening tools permits large-scale proteomic analysis, such as aptamer technology that allows the rapid and unbiased detection of a large number of autoantibodies (SOMAscan) and viral infections (VirScan) that may promote the identification of composite clinical and molecular signatures. Phenotyping patients in this fashion will truly advance and propel the development of biomarkers in this field. This is highlighted by a recent international consensus statement which provided a multi-modality set of measurements in the domains of lung physiology, imaging, survival, dyspnea, cough, and health-related quality of life as appropriate outcome measures in future clinical trials [32].

Hypersensitivity pneumonitis

Hypersensitivity pneumonitis (HP) is a complex syndrome caused by exposure to sensitizing organic molecules small enough to reach the alveolar airspace [33]. In susceptible individuals, these antigens provoke an exaggerated immune response within the small airways and pulmonary interstitium. Unlike other inflammatory diseases such as sarcoidosis and CTD, the manifestations in HP are restricted to the lung. Causative antigens include fungal, bacterial, protozoal, animal, and insect proteins, not to mention a handful of low-molecular-weight chemical compounds such as isocyanates or also drugs such as methotrexate and others.

In its acute form, HP is characterized by an influenza-like illness occurring a few hours following exposure to antigen in high-concentrations. The disease is usually non-progressive with spontaneous resolution with antigen avoidance. Recurrence is common on re-exposure. Patients with unrecognized and untreated acute HP with ongoing low-level antigen exposure may subsequently develop chronic HP. Interestingly, many patients with chronic HP have no history of acute flares and present with a slowly progressive chronic respiratory disease. Chronic HP is provoked by T-lymphocytes through a Th-1 immune response that results in a granulomatous interstitial bronchiolocentric pneumonitis [34]. Typically, the resultant non-necrotizing granulomas are small, poorly formed, and loosely arranged, which differentiates them from the well-formed lymphangitic granulomas observed in sarcoidosis. Progressive disease results in a pattern of lung fibrosis that may mimic IPF, from both a radiological and histopathological perspective [35].

Biomarker development in chronic HP has been limited by the following: the variety of antigens that can precipitate disease, the non-specific nature of Th-1 inflammation, clinical and pathologic similarity to other fibrotic lung diseases, unpredictable natural history, and heterogeneous treatment response. Garcia de Alba et al. recently observed that circulating fibrocytes and plasma CXCL12 levels are significantly increased in patients with chronic HP in comparison to healthy

controls [36]. Furthermore, numerous fibrocytes were found infiltrating the lung parenchyma near fibroblasts and lymphocytes suggesting a potential pathogenic role. These findings are not unique to chronic HP, as circulating fibrocytes are also elevated and have prognostic significance in patients with IPF [37]. Furthermore, not only are circulating fibrocytes increased during an acute exacerbation of IPF, counts have been observed to return to baseline in instances of successful recovery. Similarly, KL-6 and surfactant protein-A concentrations have been observed in both diseases suggesting similar alveolar epithelial cell behavior in both diseases [38, 39]. These findings emphasize the parallel nature of fibrotic lung diseases, regardless of inciting event, and suggest that treatment strategies targeting fibrosis may have broad utility.

Conclusion and future directions

From the observations above, the primary question that persists is the following: why have we not been able to identify specific and sensitive, clinically relevant molecular biomarkers in the field of ILD? This is particularly vexing when one considers our growing understanding of the molecular pathways of fibrosis in animal and human tissues. One of the main reasons to account for our sophisticated understanding of the molecular events responsible for fibrotic lung disease is due to the fact that the fibrotic cascade is conserved across organ systems and multiple species. The significance of this finding is highlighted by the fact that 45 % of all human deaths can be attributed to fibroproliferative disorders, as all tissue and organ systems can be affected [40]. Thus, the task of identifying and developing clinically meaningful biomarkers in the broad heterogeneous ILD population is extremely complicated and likely unrealistic. Although no single marker has reached formal approval as a valid biomarker for ILD, the overall body of work in this field has taught us a great deal about the pathogenesis involved in these inflammatory and fibrotic diseases. It has also created the need to define and evaluate combined composite biomarkers that could serve as valid and reliable instruments that would inform and influence clinical management. A successful example of a composite biomarker comes from our colleagues in oncology, where a qualitative assay utilizing gene signatures weighted together with clinical variables (patient-age, tumor grade, gross pathological tumor size, nodal status, and adjuvant therapy) was approved by the FDA to assess the risk of distant (10 years) recurrence of disease (Prosigna™/PAM50). The assay was not approved to indicate diagnosis or to select and assess response to therapy. Several studies have attempted to evaluate combined indexes in ILD [41•], including a combination of serologic markers, radiographic features, physiologic parameters, and gender. It is expected that larger prospective studies combining lung specific information with BAL, sputum, or serum markers have the potential to identify novel categories of biomarkers that will have clinical utility beyond that of conventional practices.

With the advent and wide-spread utilization of hand-held devices, the potential to collect patient-generated data is enormous. Such data may compliment, and perhaps supersede, the intermittent data we collect during patient encounters. A running catalog of activity, symptoms, and disability may prove to be incredibly valuable in predicting the natural course of disease in individual patients. For instance, hand-held spirometers with bluetooth technology have allowed patients to perform multiple daily measurements of their lung function. Pedometers, smart-phones, and -watches also enable patients to document and assess the distance or the circumference of movement on a daily basis. Other means of assessing patients include electronic applications that reflect the quality and quantity of sleep, pulse oximetry during rest and exercise, and serial measurements of quality of life and symptom limitation. We believe that combination of traditional clinical data and validated molecular biomarker signatures weighted with patient-generated data will help us to better prevent, identify, treat, and council patients with ILD. Only then, after stratifying patients based on their unique biology, will the visionary goal of personalized medicine come to fruition for patients suffering from ILD.

Compliance with Ethics Guidelines

Conflict of Interest Dr. Ask declares that they have no conflicts of interests.

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- •• Of major importance

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