Paediatric Respiratory Reviews xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Paediatric Respiratory Reviews

Mini-symposium: Biomarkers and Phenotype

# Biomarkers in Interstitial lung diseases

Nadia Nathan [1,2], Harriet Corvol [1,3], Serge Amselem [2,4], Annick Clement [1,2,*]

[1] Assistance Publique-Hopitaux de Paris (AP-HP), Hôpital Trousseau, Service de pneumologie pédiatrique; Sorbonne Universités, Université Pierre et Marie Curie (UPMC) Paris06
[2] Institut National de la Santé et de la Recherche Médicale (INSERM), UMR_S 933; Sorbonne Universités, Université Pierre et Marie Curie (UPMC) Paris06
[3] Institut National de la Santé et de la Recherche Médicale (INSERM), UMR_S 938; Sorbonne Universités, Université Pierre et Marie Curie (UPMC) Paris06
[4] Assistance Publique-Hopitaux de Paris (AP-HP), Hôpital Trousseau, Unité de génétique moléculaire; Sorbonne Universités, Université Pierre et Marie Curie (UPMC) Paris06

## EDUCATIONAL AIMS

The reader will be able to:

- Understand the pathophysiology of interstitial lung diseases (ILD) as may relate to potential biomarkers
- Learn the basis for selection of potential biomarkers, including MMP7, SP-A and SP-D, KL-6, CCL-18, YKL-40 and MUC5B
- Describe what is needed to make one or more of these markers clinically useful for diagnosing or following the progress of ILD.

ARTICLE INFO

*Keywords:*
Interstitial lung diseases
Biomarkers
Idiopathic pulmonary fibrosis

SUMMARY

Interstitial lung diseases (ILD)s represent a heterogeneous group of rare respiratory disorders, mostly chronic and associated with high morbidity and mortality. They are complex diseases that remain, in children, largely underdiagnosed and difficult to manage. Therefore, identification of biomarkers, which could be used for ILD diagnosis, measurements of disease severity and progression, and responsiveness to treatments, is a major challenge for clinical practice and for translational research. The present review focuses on blood biomarkers and provides an overview on the current information on molecular parameters of interest for ILD patient management.



## INTRODUCTION

Interstitial lung diseases (ILD)s represent a heterogeneous group of respiratory disorders that are mostly chronic and associated with high morbidity and mortality [1]. These diffuse infiltrative lung diseases are characterized by a progressive remodelling of the alveolo-capillary barrier. The clinical presentation includes dyspnoea, a restrictive pattern on pulmonary function testing, and diffuse lung infiltration on chest imaging.

ILDs are rare diseases that can be observed at all ages, from infancy to the elderly. They are, however, more frequently diagnosed in adult patients [2]. In children, information on their global epidemiology remains extremely limited [3]. Extrapolation from small studies has suggested an approximate incidence of 1 case per 100.000 population. However, this estimation is not reliable as pediatric ILDs remain largely underdiagnosed [4]. Nevertheless, common underlying mechanisms are currently being described, supporting the view that tools developed to improve the diagnostic approach and clinical management of ILD should benefit to all patients regardless of age. Based on this understanding, potential disease biomarkers derived mainly from studies performed in adult patients are discussed in this review. Their translation into clinical practice should ultimately benefit all forms of ILD including paediatric diseases.

* Corresponding author. Pneumologie Pédiatrique, Hôpital Armand Trousseau, 26 avenue du Dr Arnold Netter, 75012 Paris-France Tel.: +33144736668.
*E-mail address:* annick.clement@trs.aphp.fr (A. Clement).

http://dx.doi.org/10.1016/j.prrv.2015.05.002
1526-0542/

Please cite this article in press as: Nathan N, et al. Biomarkers in Interstitial lung diseases. Paediatr. Respir. Rev. (2015), http://dx.doi.org/10.1016/j.prrv.2015.05.002

## ILD PATHOPHYSIOLOGY: CURRENT UNDERSTANDING FOR BIOMARKER DEVELOPMENT

ILDs display a wide range of phenotypic expression that is influenced by age of onset and instigating factors. However, in all situations, disease progression shares the common features of lung remodelling. Based on numerous experimental studies and human tissue analysis, the working concept of ILD pathogenesis highlights the central role of alveolar epithelial cells and fibroblast aberrant activation [5–7].

Repeated injuries of "vulnerable" alveolar epithelial cells and the failure of the alveoli to correctly respond to injury lead to aberrant lung repair and progressive fibrosis. The current understanding of ILD physiopathology suggests a multiple hit model, with genetic and non-genetic insults contributing to increase the vulnerability of alveolar epithelial cells to repeated injuries [8]. This scenario is associated with changes in cellular phenotype and function. Important consequences include modification of the crosstalk between alveolar epithelial cells and mesenchymal cells as well as induction of epithelial mesenchymal transition. The transformation of epithelial cells acquiring phenotypic and functional characteristics of fibroblasts contributes to an exaggerated production of collagen and extra cellular matrix components. An acceleration of the aging process that could impact on progenitor cells leading to stem cell exhaustion is also suggested [9].

Complex underlying mechanisms are involved, with increasing evidence for an important role of endoplasmic reticulum (ER) stress [10]. ER stress occurs in situations associated with accumulation of misfolded proteins in the ER and activation of the unfolded protein response. When the load of altered proteins is excessive, the resulting ER stress can lead to alveolar epithelial cell death through apoptosis. Among factors causing ER stress are abnormal Surfactant Proteins (SP), viruses, tobacco smoke and oxidants [11]. Recent studies have demonstrated that mutant SP-C proteins could co-localise in the ER, and that a significant increase in ER-stress reaction was observed in cells expressing mutated SP-C. This understanding explains the novel interest for anti-ER stress therapeutic strategies in lung fibrosis [12].

The nature of inciting injury and subsequent alveolar epithelium dysfunction includes genetic and epigenetic factors as well as environmental and host co-morbidity components [13]. All together these contributors affect disease expression and progression. From a number of reports, there is emerging evidence that the development of all forms of ILD is, at least in part, determined by genetic factors. In children, mutations are mainly reported in the genes encoding SP-C and SP-B [14,15]. Other surfactant system defects include mutations in the genes encoding the thyroid transcription factor 1 and the member A3 of the ATP-binding cassette family of transporters [16,17]. Mutations in genes implicated in DNA repair and telomere functions, including genes encoding the telomerase RNA component and the telomerase reverse transcriptase, have been described in patients with Idiopathic Pulmonary Fibrosis (IPF) [18]. In addition to genetic causes, there is compelling evidence that factors from the environment influence disease expression and progression. Current knowledge points out the role of tobacco smoke, exposure to aero contaminants, and viruses. Of interest, several studies have showed the presence of various virus proteins in lung tissues from patients with ILD and lung fibrosis, with an expression localized to alveolar epithelial cells.

## BIOMARKERS OF LUNG PARENCHYMA DYSFUNCTION

A large variety of respiratory disease markers has been examined including chest imaging, lung function tests, and specific tissue and molecular components. Currently, much effort is devoted to the qualification and validation of biologically relevant molecular biomarkers [19,20]. A number of approaches have been used from 'omic' studies to candidate molecule analysis of lung tissues, bronchoaveolar lavage (BAL) fluids, and blood samples [21–23]. These approaches have identified several types of biomarkers with the potential to predict lung disease progression and prognosis [24,25]. The most studied components, which are listed below, include molecules implicated in matrix formation and remodelling, alveolar epithelium metabolism and immune processes. In addition, novel findings provide support for the consideration of a role for mucociliary clearance molecules.

### *Remodelling molecules*

A number of molecules have been described including Matrix Metallo Proteinase (MMP)s, which appear of particular importance for lung parenchymal homeostasis. MMPs belong to a family of zinc-dependent proteases that cleave the extracellular matrix and cell-surface proteins to regulate wound repair and a number of immune and inflammatory processes [26]. MMP production and activity are up-regulated during healing or remodeling. MMP7 (matrilysin) is the smallest of the known MMPs, Unlike many other MMPs that are highly expressed in the stroma, MMP7 is expressed in epithelial cells [27,28]. Following Injury, MMP7 synthesis is rapidly induced. An important proposed role for MMP7 is to facilitate the migration component of the re-epithelialization program, a complex process involving multiple interactions between the cell and the matrix. In MMP7-deficient mice, re-epithelialization and neutrophil recruitment into the alveolar space are almost completely abrogated, demonstrating the major role of MMP7 in the healing cascade of the lung. Increased expression of MMP7 is observed in hyperplastic epithelial cells, and elevated blood levels have been documented in various forms of diffuse parenchymal lung diseases.

### *Alveolar epithelium functional molecules*

Among the molecules of interest are the surfactant proteins (SP), and mainly the hydrophilic proteins SP-A and SP-D. These factors are predominantly produced by alveolar type 2 epithelial cells [29]. They are members of the collectin family of C-type lectins, with an amino-terminal collagen-like region and a carboxy-terminal carbohydrate recognition domain (CRD). The CRD domains are adapted to bind essential carbohydrate and lipid antigens present on the surface of microorganisms. They modulate the uptake of infectious agents by phagocytic cells as well as both the inflammatory and the adaptive immune response. SP-A also facilitates the surface tension-lowering properties of surfactant phospholipids and plays an active role in the re-epithelialisation processes of the alveolar surface. Indeed, recent studies have provided evidence that SP-A binds Transforming Growth Factor (TGF)-β1 with high affinity and stimulates the TGF-β1 pathway [30,31]. In situations of parenchymal injury and alveolitis, increased levels of SP-A and SP-D have been reported in the BAL and serum of patients [32–34].

KL (Krebs von den Lungen)-6, classified as a submolecule of MUC1, is a candidate molecule of alveolar epithelium homeostasis. It was initially suggested as a serum tumour biomarker for lung, breast and pancreatic cancers. It was then recognized and approved as a serum biomarker for ILD in Japan [35,36]. It is a high-molecular-weight glycoprotein with an extracellular domain, and is expressed on the surface of various types of epithelial cells [37]. In the lung, it is moderately expressed by alveolar type 2 epithelial cells in control conditions. Its expression dramatically increases in regenerating cells in tissue sections from ILD patients and is associated with high KL-6 levels in the serum [38,39]. The

Please cite this article in press as: Nathan N, et al. Biomarkers in Interstitial lung diseases. Paediatr. Respir. Rev. (2015), http://dx.doi.org/10.1016/j.prrv.2015.05.002