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Review Article

Serum Biomarkers in Interstitial Lung Disease Associated with Connective Tissue Diseases

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Abstract

Interstitial lung diseases (ILDs) are a heterogeneous group of multiple pulmonary disorders. ILDs are characterized by an irreversible architectural distortion and impaired gas exchange; however, there is great variability in the clinical course. ILD diagnosis requires a combination of clinical data, radiological imaging and histological findings. It is necessary to find reliable predictors for the disease progression. Peripheral blood biomarkers offer the advantages of being readily obtained, non-invasive, and serially monitored. At the same time, successful management of ILD patients strictly depends on an accurate and confident diagnosis. In this context, the detection of reliable biomarkers able to identify ILD subtypes. This review focuses on selected validated and/or potentially interesting biomarkers investigated in the peripheral blood and lung tissue of patients with ILD associated with Connective tissue disease (CTD)
Keywords: ILD, CTD.

INTRODUCTION

Interstitial lung diseases (ILD) are protean conditions with substantial overlap in terms of diagnosis, prognostic evaluation, and management. Interstitial lung disease (ILD) can be defined by the presence of diffuse parenchymal opacities on chest imaging and restrictive physiology not attributable to cardiac disease, infection, exposures or other identifiable cause.

The causes of interstitial lung disease can be classified into one of the following four categories: 1) diseases associated with a condition that affects other parts of the body (for example, autoimmune or collagen vascular disease), 2) diseases associated with a specific exposure to an agent known to damage the lungs (for example, medications such as bleomycin, occupational exposures such as asbestos, tobacco smoke, or agents in the environment that cause an immune reaction called hypersensitivity pneumonitis), 3) diseases associated with known genetic abnormalities (for example, Hermansky–Pudlak syndrome), and 4) idiopathic diseases (diseases of an unknown cause).

Although high resolution CT (HRCT), lung function tests (PFTs), bronchial lavage fluid (BALF), lung biopsy can be used to detect –ILD. It is important to provide accurate diagnosis and patient selection for prognostication and timely treatment, preferably at baseline. However, the management of idiopathic pulmonary fibrosis is different from that of more immunologically driven ILD patterns, such as ILD associated with connective tissue diseases. Validated non-invasive biomarkers fulfilling these unmet clinical needs are warranted.

Biomarkers associated with ILD associated with Systemic Sclerosis

Systemic sclerosis (SSc) is a disorder of the connective tissue characterized by fibrosis of the skin, vascular abnormalities, and presence of auto-antibodies [1]. Pulmonary involvement is the leading cause of death in SSc patients due to pulmonary fibrosis or pulmonary hypertension [2].

Though it is not clear about the pathogenesis of systemic sclerosis associated with interstitial lung diseases, serum biomarkers, genes and lifestyle are involved in SSc-ILD progression [3]. Unknown factors initiate immune dysfunction between epithelial and vascular endothelial cells. The combination of epithelial and endothelial cell injury as well as inflammatory and immune activation progress pulmonary fibrosis in SSc [4].

KL-6 is a glycoprotein expressed on and released from type 2 alveolar cells. KL-6, surfactant

protein (SP)-A, SP-D, and monocyte chemoattractant protein-1 (MCP-1) are reported to be sensitive markers for interstitial lung diseases (ILD). Hiroshi Ohnishi did comparative analysis of diagnostic value of biomarkers. Subjects consisted of 33 patients with ILD (21 cases of idiopathic pulmonary fibrosis and 12 associated with collagen vascular diseases) and 82 control subjects (12 cases of bacterial pneumonia and 70 healthy volunteers). There results suggested that KL-6 is the best serum marker for ILD. The serum levels of SP-A and SP-D, but not of KL-6, were significantly higher in patients with bacterial pneumonia than in healthy volunteers [5].

Hideaki *et al.*, retrospectively analyzed the medical records of 29 patients with SSc-ILD. Measurement of serum KL-6 levels, pulmonary function tests, and HRCT performed in parallel were reviewed. They found serum KL-6 correlated positively with diffusing capacity of the lung for carbon monoxide (DLCO)(% predicted) and disease extent on HRCT, and the changes in serum levels of KL-6 were significantly related to the changes in forced vital capacity (FVC) in SSc -associated ILD. Their study suggests KL-6 can be a useful monitoring tool of SSc-ILD activity [6].

Yuichiro Shirai investigated clinical utility of serial KL-6 measurement in patients with SSc-ILD using a prospective cohort**.** They found that elevated KL-6 level at baseline is an independent predictor of %FVC decline and mortality, Baseline serum KL-6 level was significantly elevated in SSc patients with ILD than in those without and was higher in extensive than in limited disease in patients with ILD. Yearly change of %FVC was inversely correlated with both the final KL-6 level. Some patients with ILD received treatment with oral or intravenous cyclophosphamide during disease course. In these patients, KL-6 level significantly reduced at 12 months after initiation of the treatment [7]. This suggests that its level can help to detect the treatment response.

Kumanovics G *et al*., did retrospective longitudinal study to investigate the predictive value of KL-6 serum levels for the outcome of interstitial lung fibrosis in a large systemic sclerosis (SSc) patient cohort. Serum titer of KL-6 was negatively correlated with lung function parameters, independent of the time of investigation. There was a significantly higher probability of death among patients with high level of baseline KL-6. Serum levels of KL-6 significantly decreased in patients receiving cyclophosphamide treatment in spite of the fact that the spirometry results (FVC and DLCO) did not show a significant change [8].

Masataka *et al*., investigated the relationship between SSc-ILD and prognosis. They chose fifty patients with early-stage SSc-ILD who had never received disease-modifying drugs and were either observed for ≥ 10 years or died from ILD-related causes. They found elevated KL-6 at initial assessment was highly correlated with ESLD development and the initial KL-6 level correlated with the forced vital capacity decline rate [9].

The relationship between KL-6 and the severity and the activity of SSc-ILD in bronchoalveolar lavage fluid was studied Fifteen patients with early SSc and 12 healthy controls were subjected to BALF. Serum KL-6 were increased in SSc patients and correlated with BALF concentration of eosinophils. Patients with more widespread ground glass opacities (GGO) on HRCT were characterized in BALF by a higher eosinophil count and in serum by higher KL-6. Therefore, KL-6 levels from BALF and serum being correlated to findings on HRCT suggest that KL-6 can be used as a useful biomarker of presence and extent of lung fibrosis in SSc patients [10]. Roger Hessel strand et al estimated CXCL5, CXCL8 and S100A8/A9 were performed in BALF and serum. COMP and KL-6 were measured in serum. HRCT of lungs was quantified for ground glass opacities (GGO), reticulation and traction bronchiectases. Serum concentration of KL-6 was also increased in patients, in contrast to CXCL5, CXCL8, S100A8/A9 and COMP that were not. The relation between serum KL-6 and BALF eosinophil concentration is of interest since BALF eosinophils have been associated with a worse prognosis. Eosinophil count in BALF was associated with decreased diffusion capacity for carbon monoxide [11].

Stefania Celeste *et al*., showed that Ca 15.3 is a rapid and inexpensive candidate biomarker for SSc–ILD being proportional to the extent of lung injury and specific and sensitive in assessing meaningful extents of the disease with prognostic significance [12].

Dr. Assassi conculuded that higher CCL2 levels in the circulation, after adjusting for other potential biomarkers, do predict faster ILD progression and poorer survival, supporting the notion CCL2 plays a role as a biomarker and may be a therapeutic target [13].

Chun Geun Lee showed that the levels of chitotriosidase (Chit1) bioactivity and protein are significantly increased in the circulation and lungs of SSc patients compared with demographically matched controls. In vitro studies also demonstrated that Chit1 interacts with TGF-b1 to augment fibroblast TGF-b receptors 1 and 2 expression and TGF-b–induced Smad and MAPK/ERK activation. These studies indicate that Chit1 is potential biomarker for ILD in SSc and a therapeutic target in SSc-associated lung fibrosis and demonstrate that Chit1 augments TGF-b1 effects by increasing receptor expression and canonical and noncanonical TGF-b1 signaling [14].

Biomarkers associated with ILD associated with Polymyositis and Dermatomyositis

Polymyositis (PM) and dermatomyositis (DM) are characterized by muscle weakness and inflammation of the striated muscles. Systemic inflammation affecting organs such as skin in DM, and the gastrointestinal tract, heart or lungs in myositis (both PM and DM) is common. The lungs are among the most frequently involved organs, and interstitial lung disease (ILD) is reported to be common. Standard tests to screen for ILD in patients diagnosed with myositis are pulmonary function tests, chest radiography and high-resolution computerized. Tomography (HRCT) of the lungs myositis-specific autoantibodies, in particular the antisynthetase antibodies of which the anti-Jo-1. Elevated serum levels of KL-6 have been reported in patients with myositis-associated ILD [15-17]. Furthermore, serum levels correlated with the severity of ILD as measured by pulmonary function tests.

Kubo *et al*., reported that serum levels of KL-6 were inversely correlated with the percentages of VC and DLco [15]. Bandoh *et al*., demonstrated that the highest levels of KL-6 were measured in patients with diffuse alveolar damage and the levels changed according to the progression or improvement in ILD [16]. M. Fathi *et al*., investigated whether Caucasian patients with polymyositis (PM) or dermatomyositis (DM) and interstitial lung disease (ILD) have elevated serum levels of KL-6 compared with patients without ILD. Changes in KL-6 levels showed a significant inverse correlation with changes in percentage FEV1, TLC, DLco and RV [18].

Hozumi H *et al*., found that serum YKL-40 levels were significantly higher in patients with PM/DM-ILD compared with controls. In addition, serum YKL-40 was linked with arterial oxygen pressure in these patients. Furthermore, higher serum YKL-40 and lower percent-predicted forced vital capacity were both linked with poor prognosis. Immunohistochemistry analysis showed YKL-40 expression was heightened in aggregated intra-alveolar macrophages and hyperproliferative alveolar epithelial cells in patients with PM/DM-ILD [19].

Biomarkers associated with ILD associated with Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a systemic inflammatory disease that affects approximately 1% of the population, and pulmonary involvement is common [20]. Interstitial lung disease (ILD) is the primary pulmonary manifestation of RA, as it is for other connective tissue diseases (CTDs), such as scleroderma (systemic sclerosis), inflammatory myositis (polymyositis and dermatomyositis), Sjögren syndrome, and undifferentiated CTD [21, 22].

Chen J *et al*., showed that Levels of MMP-7 and IP-10/CXCL10 are elevated in the serum of RA patients with ILD, whether mild or advanced, supporting their value as pathogenically relevant biomarkers that can contribute to noninvasive detection of this extraarticular disease complication [23].

Harlow *et al*., identified citrullinated heat shock proteins (Hsp) 90α and Hsp90β as potential biomarkers for ILD in patients with RA [24].

Platelet derived growth factor isoforms AB and BB, interferon-alpha, and profibrotic cytokine transforming growth factor-B1. Elevated levels of these cytokines have been observed in BAL. High levels of Krebs von den Lungen-6 protein (KL-6) have been identified in serum, reflecting alveolar damage. KL-6 protein levels have demonstrated a correlation with the severity of ILD, as evaluated by HRCT [25].

Young Seok Lee *et al*., retrospectively compared with the clinical courses of 62 patients with RA-UIP. Blood levels of biomarkers (Krebs von den Lungen-6 [KL-6], surfactant protein-A [SP-A], matrix metalloproteinase-7 [MMP-7], interleukin-6 [IL-6], and interleukin-32 [IL-32]). Results of this retrospective study suggested that KL-6 and IL-6 could be used as predictors of short-term disease progression. In addition, high levels of KL-6 could be used as a predictor of mortality [26].

Doyle TJ enrolled in Brigham and Women's Hospital Rheumatoid Arthritis Sequential Study (BRASS) and American College of Rheumatology (ACR) cohorts were evaluated for ILD. Clinical risk factors and autoantibodies are strongly associated with the presence of clinically evident and subclinical RA-ILD on computed tomography scan in two independent RA cohorts. A biomarker signature composed of matrix metalloproteinase 7, pulmonary and activation-regulated chemokine, and surfactant protein D significantly strengthens this association. These findings may facilitate identification of RA-ILD at an earlier stage, potentially leading to decreased morbidity and mortality [27].

Gökhan Sargin showed that there is a relationship between ILD and tumor marker levels in connective tissue diseases. Elevated tumor markers may not be associated with hidden tumor or malignancy in RA patients. These antigens may be used as predictive biomarkers particularly in RA patients with ILD. The study included 83 RA patients (20 males, 63 females; mean age 59.3±12.1 years; range 25 to 83 years), 43 with ILD (13 males, 30 females; mean age 60.1±11.5 years; range 25 to 83 years) and 40 without ILD (7 males, 33 females; mean age 58.5±12.7 years; range 28 to 78 years). Five RA patients (11.6%) with ILD had carcinoembryonic antigen levels above the upper limit. The numbers of RA-ILD patients with above the upper

limit of CA 19-9, CA 15-3, and CA 125 levels were 10 (23.2%), 13 (30.2%), and five (11.6%), respectively. Rates of RA patients without ILD with tumor-associated antigens exceeding the upper limit were 15% for carcinoembryonic antigen, 2.5% for CA 19-9, 7.5% for CA 15-3, and 7.5% for CA 125. No evidence of any malignancy was detected by medical history, physical examination, and laboratory and imaging methods in patients who had high levels of serum tumor-associated antigen [28].

Antibodies against Peptidyl arginine deaminase (PAD) enzyme isoforms 3 and 4 (anti PAD ¾) could be associated with RA ILD.The prevalence and extent of ILD was higher in RA patients with anti PAD ¾ than those without anti PAD ¾ [29].

Biomarkers in Treatment Response in ILD associated with Connective tissue disease

Data on efficacy of immunosuppressive treatment with azathioprine (AZA) in CTD-ILD are still poor. KL-6 is an established biomarker for interstitial lung disease but its use to evaluate treatment response is still unknown.Eda B et al investigated the role of serum KL-6 as a biomarker to assess response to AZA in CTD-ILD patients. 54 patients with CTD-ILD (19 rheumatoid arthritis, 15 systemic sclerosis, 8 polymyositis/antisynthetase syndrome, 4 undifferentiated connective tissue disease, 3 sjögren syndrome, 3 mixed connective tissue disease, 1 psoriatic arthritis, 1 polymyalgia rheumatica) treated with AZA were studied. Progression was defined as decrease of FVC ≥5% or DLco ≥10% pred and worsening of clinical symptoms or new radiological findings. KL-6 was measured in serum at start of therapy. Responders showed a decrease of KL-6 by 19% over time [30].

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