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# Biomarkers from bronchoalveolar lavage fluid in systemic sclerosis patients with interstitial lung disease relate to severity of lung fibrosis

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**Summary**
*Objectives:* Decision on treatment of systemic sclerosis (SSc) related interstitial lung disease (ILD) largely relies on the findings on high resolution computed tomography (HRCT) and there is a need for improvement in assessment of the fibrotic activity. The objectives of this study were to study biomarkers in bronchoalveolar lavage fluid (BALF) from SSc patients with ILD and to relate the findings to the severity and activity of lung fibrosis.
*Methods:* Fifteen patients with early SSc and 12 healthy controls were subjected to BAL. Cell counts and analyses of 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.
*Results:* BALF concentrations of CXCL8 ($p < 0.001$), CXCL5 ($p = 0.002$) and S100A8/A9 ($p = 0.016$) were higher in patients than controls. Serum KL-6 ($p < 0.001$) was increased in SSc patients and correlated with BALF concentration of eosinophils ($r_S = 0.57$, $p = 0.027$). Patients with more widespread GGO on HRCT were characterised in BALF by a higher eosinophil

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count ($p = 0.002$) and in serum by higher KL-6 ($p = 0.008$). Patients with more fibrosis were characterised in BALF by higher eosinophil count ($p = 0.014$), higher CXCL8 ($p = 0.005$) and S100A8A/A9 ($p = 0.014$) concentration and in serum by a higher serum COMP ($p = 0.023$).
*Conclusions:* In SSc related ILD, biomarkers from BALF and serum correlate to findings on HRCT suggesting usefulness as markers of presence and extent of lung fibrosis.


## Introduction

Systemic sclerosis (SSc, scleroderma) is characterised by fibrosis in skin and internal organs, progressive vascular obliteration and the production of autoantibodies.[1] Pulmonary complications have been recognised as the leading cause of death in SSc.[2,3] The identification of interstitial lung disease (ILD), often developed early on in the disease, has been facilitated by the widespread usage of high resolution computed tomography (HRCT) of the lungs. Identification and characterisation of activity in ILD is however very uncertain. Furthermore, it is still debated which patients might benefit from any possible therapy since reliable markers of severity are lacking.

HRCT can identify patients with SSc and ILD with a poor prognosis.[4] However, the prognostic value of bronchoalveolar lavage (BAL) cellular profiles in SSc associated ILD is still unclear.[5–7] Analyses of soluble biomarkers in BAL fluid (BALF) might provide prognostic information. BALF analysis does also offer the possibility to study local disease mechanisms.[8]

Radiological characterisation of ILD in SSc is usually consisted of the two major forms usual interstitial pneumonia (UIP) and nonspecific interstitial pneumonia (NSIP), the latter being the most common. In NSIP the prognosis worsens in the presence of eosinophilia in BALF.[9] Eosinophil count in BALF was associated with decreased diffusion capacity for carbon monoxide ($DL_{CO}$)[7] and some SSc patients display eosinophilia in peripheral blood.[10] Patients with idiopathic pulmonary fibrosis (IPF) have a negative correlation between both the absolute numbers and percentage of eosinophils in BALF and survival,[11] and biopsy studies of IPF and pulmonary fibrosis related to collagen vascular diseases show a correlation between eosinophil concentrations in peripheral blood and in lung tissue.[12] Increased levels of eosinophils may be seen in lung tissue from patients with ILD, especially in IPF and hypersensitivity pneumonitis and also to a lesser degree in SSc.[13]

CXCL5, also known as epithelial derived neutrophil activating peptide 78 or ENA-78, is a chemokine produced by epithelial cells following stimulation by interleukin-1 or tumour necrosis factor-alpha. It is also expressed in eosinophils, which supports the notion that eosinophils are not just mere targets for chemokines, but also have immunoregulatory properties.[14] In patients with exacerbation of asthma, endobronchial biopsies have shown increased number of CXCL5 expressing cells and a relation between CXCL5 and the number of eosinophils.[15] CXCL5 has been explored in ILD, where it has been found to be elevated in BALF from patients with IPF compared to healthy controls.[16]

CXCL8, also known as interleukin-8 or IL-8, is a chemokine often produced by macrophages and epithelial cells. It is a mediator of inflammatory response and an angiogenic factor.[17] Hypoxia may result in accumulation of inflammatory cells and structural changes featured by fibrosis in the tissue surrounding the area of low oxygen. In cultured fibroblasts CXCL8 and CCL11 were both increased following hypoxic stimulation.[18] Hypoxia can also increase the levels of procollagen in the supernatant from cultured fibroblasts,[18] and CXCL8 is higher in BALF from patients with ILD than in healthy controls.[16] In SSc associated ILD CXCL8 levels from BALF have been found to be elevated[19,20] although one study found CXCL8 to be overexpressed only in vitro.[21]

The S100A8/A9 heterodimer is also referred to as calprotectin, MRP8/14 or calgranulin A and B. It is considered to be a damage associated molecular pattern molecule and as such, an activator of the innate immune system via Toll-like receptor 4. In SSc, faecal level of S100A8/A9 has been explored as a possible biomarker of gastrointestinal disease.[22] In IPF, increased levels of the monomer S100A9 have been identified in BALF.[23,24] The level of S100A9 in BALF has also been proposed to be a possible biomarker of fibrosis in IPF.[25]

Cartilage oligomeric matrix protein (COMP or thrombospondin-5) is a large disulfide-linked pentameric glycoprotein discovered and characterised in cartilage.[26] It has gained increasing attention in SSc as a biomarker for activity in skin fibrosis and to a lesser extent in ILD.[27,28]

Krebs von den Lungen-6, or simply KL-6, is a glycoprotein antigen expressed mainly on type II pneumocytes in alveoli and respiratory bronchiolar epithelial cells. The serum level of KL-6 correlates to the presence of ILD and is also related to activity of ILD in SSc.[29]

There is a need for improvement in assessment of the fibrotic activity in ILD. Based on our previous work on immunological and fibrotic effects of S100A8/A9 and COMP we wanted to explore the relationship to other biomarkers known to be associated with neutrophil and eosinophil activation. Thus, the aim of this study was to analyse CXCL5, CXCL8 and S100A8/A9 in the circulation and in BALF from SSc patients with ILD and to relate the findings to the severity of ILD using imaging, and activity of ILD, using cellular profile in BALF and measurement of COMP and KL-6 in serum.

## Patients & methods

### Patients

We included 15 previously untreated SSc patients with short disease duration (Table 1) and signs of ILD on HRCT of the lungs. They underwent examination with BAL before initiation of treatment and 5 patients also had a second BAL