INFLAMMATORY MARKERS IN THE EXHALED BREATH CONDENSATE OF PATIENTS WITH PULMONARY SACROIDOSIS

Pulmonary sarcoidosis may progress to fibrosis in some patients, so that close monitoring of its activity is essential for recommending clinical strategy. Examination of airway inflammatory markers in bronchoalveolar lavage (BAL) is one of the methods applied to assess the disease severity. Recently, the expired breath condensate (EBC) has become another source of cytokines and mediators. In sarcoidosis, except for NO and oxidative stress markers, no other mediators have yet been estimated in the exhaled air. In the present study we attempted to answer the question of whether airway inflammatory markers in pulmonary sarcoidosis patients might be assessable in EBC and to what extend these markers might reflect the disease activity in the lungs IL-6, TNF-α, PAI-1, and IGF-1 were measured by Elisa method in EBC and BALF samples from 9 patients with newly-diagnosed pulmonary sarcoidosis. TNF-α, IGF-1, and PAI-1 levels in EBC and BAL samples were comparable and closely positively correlated [TNF-α (r=0.79, P<0.001), IGF-1 (r=0.94, P<0.001), and PAI-1 (r=0.81, P<0.001)]. In contrast, IL-6 concentration in EBC was significantly lower compared with that in BALF, while the correlation between both materials was negative (r=−0.47, P<0.05). An important distinction in IL-6 performance, which might explain this inconsistency, is its tendency to form more complex molecular forms of a higher weight than that of other cytokines. Our study shows that EBC reflects cytokine production in the lung as effectively as BALF, providing that the characteristics of proteins evaluated allow their easy transfer into the exhaled air. Further studies are required before accepting EBC samples as an equivalent to BALF.

Key words: bronchoalveolar lavage, exhaled breath condensate, IGF-1, IL-6, PAI-1, pulmonary sarcoidosis, TNF-α
INTRODUCTION

Sarcoidosis is a multisystem disease of unknown origin, most often localized in the pulmonary system, but also in lymph nodes, eyes, skin, and other organs (1). It is characterized by granulomatous inflammation, with activated T lymphocytes accumulation and increased production of TH1 type cytokines in the involved organ, followed by granuloma formation. In about 75%, pulmonary sarcoidosis assumes a benign course with spontaneous regression or stable clinical presentation. In some patients, treatment is necessary due to the disease progression, which may finally lead to lung fibrosis. Therefore, close monitoring of the disease activity is considered a key element of the recommended strategy for the pulmonary sarcoidosis patient’s follow up and treatment. Lung function testing and HRCT (high-resolution computer tomography) scanning are accepted tools for the monitoring of disease dynamics (clinical level), while a direct examination of airway inflammatory markers is performed via their assessment in bronchoalveolar lavage fluid (BALF) material (cellular level) (2). Although BALF analysis provides detailed information concerning pathological events in the lungs, and thus gives an insight into current disease activity, it is an invasive technique with limitations and risks for side effects.

Recently, a number of cytokines and growth factors (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, VEGF, PDGF-AA, EGF), eicosanoids and nitric oxide (NO), some of them corresponding with clinical disease markers, have been demonstrated in EBC from cystic fibrosis, bronchial asthma, and COPD patients (3-7). In contrast to BALF, the technique of the exhaled breath condensate (EBC) collection is simple, quick, safe, non-invasive, and suitable for patients of any age and clinical status (8). However, except for oxidative stress (H₂O₂) and NO, no other mediators have been evaluated in EBC from sarcoidosis patients, although, as pointed above, airway monitoring is considered a key element of the recommended medical strategy (9, 10).

In the present study we attempted to answer the question of whether airway inflammatory markers in pulmonary sarcoidosis patients might be assessable in EBC and, if so, to what extend they might reflect the disease activity in the lungs, compared with the assessment based at the content of these markers in BALF.

MATERIAL AND METHODS

The study was approved by a local Ethics Committee and informed consent was obtained from all study subjects. Nine patients (4 women and 5 men) aged 30-57 years with newly-detected pulmonary sarcoidosis were used for the study. The diagnosis was confirmed by typical clinical representation, histology, and HRCT findings. No patient was on steroid treatment at the time of the study or during the preceding 3 months.
EBC collection was performed according to a standard protocol (8). Subjects breathed tidally for 10-15 min, using a-nose-clip, into a special chamber of a condenser (EcoScreen, Jaeger, Germany). The collected condensate was stored in aliquotes at -70°C until further examination.

BALF collection was performed as part of routine diagnostics. The recovered BALF was filtered through sterile gauze and centrifuged (4°C, 400 x g for 15 min.). The supernatant was collected and frozen at -70°C. According to the study protocol, EBC was collected the day before or in the morning prior to bronchoscopy.

The measurement of cytokines in EBC and BAL was performed by a quantitative enzyme immunoassay technique (ELISA) using commercial kits (R&D) according to manufacturer recommendations. Optical density was measured at 450 nm using a spectrophotometric reader Elx800 (Bio-Tek Instruments, Winooski, VT, USA). The cytokine concentration was expressed in pg/ml (IL-6, TNF-α, PAI-1) or in ng/ml (IGF-1).

Data are presented as means ±SD. Statistical analysis was performed using Student’s t-test for the comparison of mean values and Pearson’s test for the analysis of correlations.

RESULTS

TNF-α, IL-6, IGF-1 and PAI-1 concentrations were detectable in EBC of all sarcoidosis patients, except for IGF-1 that was not measurable in both EBC and BAL from one subject.

Interestingly, the levels of TNF-α, IGF-1, and PAI-1 in EBC and BAL did not differ statistically; the respective values were TNF-α 3.80 ±1.79 vs. 3.34 ±2.81 pg/ml, IGF-1 7.76 ±5.99 vs. 6.09 ±4.44 pg/ml, PAI-1 0.82 ±0.42 vs. 0.94 ±0.44 ng/ml. By contrast, the IL-6 concentration in EBC was significantly lower compared with that in BAL; 0.23 ±0.07 vs. 4.08 ±3.48 pg/ml, respectively (P< 0.001) (Fig. 1).

There were positive correlations between the content of TNF-α (r=0.79, P<0.001), IGF-1 (r=0.94, P<0.001), and PAI-1 (r=0.81, P<0.001) (Fig. 2A, B, C) and a negative one for IL-6 (r=-0.47, P<0.05) (Fig. 2D) in EBC and BALF.

Fig. 1. Mean cytokine levels in the exhaled breath condensate (EBC) and broncholaveolar lavage fluid (BALF) in pulmonary sarcoidosis patients.
DISCUSSION

The immunopathology of pulmonary sarcoidosis is complex and involves a wide range of cell types and mediators (1, 2). Activated lymphocytes and macrophages are considered the main prominent cells regulating granulomatous inflammation in the lung and determining the further disease course to spontaneous regression or pulmonary fibrosis. Both cell types produce a considerable number of proinflammatory cytokines and growth factors. The mediators evaluated in the present study are influential in shaping several important phases in the sarcoidosis pathomechanism, such as driving alveolitis and granuloma formation (IL-6, TNF-α), persistence of interstitial inflammation (IGF-1), and progression into lung fibrosis (PAI-1).

The main aim of this preliminary study was to evaluate whether inflammatory markers might be measurable in the exhaled breath condensate from pulmonary sarcoidosis patients. Our results demonstrate, for the first time, that it is possible to assess the IL-6, TNF-α, IGF-1, and PAI-1 concentrations in EBC using a standard Elisa technique. The TNF-α, IGF-1, and PAI-1 levels in EBC were comparable with those found in BALF. It should be emphasized that both materials, EBC and BALF, were collected in close time proximity. Moreover, highly significant correlations were observed between the levels of TNF-α, IGF-1, and PAI-1 in both materials. It seems reasonable to conclude that the EBC reflects the production of these cytokines in the lungs as effectively as BALF.

*Fig. 2. Correlations between the levels of TNF-α (A), IGF-1 (B), PAI-1 (C), and IL-6 (D) in exhaled breath condensate (EBC) and bronchoalveolar lavage fluid (BALF) from sarcoidosis patients.*
However, the results concerning the IL-6 concentration were different. Its level in EBC was considerably lower than that in BALF, while the correlation between IL-6 in both materials was negative. The most likely explanation for this apparent inconsistency, as compared with other cytokines, might be a characteristic for IL-6 tendency to form molecular forms more complex than a simple monomer. While the molecular weight (Mw) of IL-6 monomer is only 25–30 kDa, the average size of more intricate structures it has a propensity to form is 100-150 kDa or 400-500 kDa (11). It has been shown that molecules detected in EBC, originating from the fluid lining the pulmonary tracts, might be no more than 100 kDa (12). All the other cytokines evaluated in the present study are either small molecule, as IGF-1 with Mw 7.5 kDa, or stay preferentially in a monomeric form, as TNF-α (Mw 17 kDa) or PAI-1 (Mw 45 kDa). That important distinction in the IL-6 performance rationalizes both its measurable, although low, levels in EBC, as compared with BALF, but also a negative correlation between its content in both materials. The higher IL-6 concentration in the airways the more it tends to form large-scale molecules that are not able to relocate into the exhaled air. Further experiments are ongoing to confirm this hypothesis.

This preliminary study confirmed that EBC might provide an easily accessible material for the assessment and monitoring of inflammatory mediators in pulmonary sarcoidosis. EBC reflects their production in the lungs as effectively as BALF, providing that the molecular characteristics of proteins being evaluated allow their easy transfer into the exhaled air. Therefore, detailed studies are required before accepting the EBC sampling as an equivalent to BALF. Since the collection of EBC is faster, less expensive, safer, and beyond any comparison much more comfortable for the patient, further efforts to assess the EBC usefulness in diagnostics and monitoring of pulmonary sarcoidosis are necessary.

REFERENCES


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