Neutrophil elastase: alpha-1-proteinase inhibitor complex in serum
and bronchoalveolar lavage fluid in patients with pulmonary fibrosis


ABSTRACT: It was hypothesized that neutrophil elastase released from activated
neutrophils may play an important role in the pathogenesis of pulmonary fibrosis.
In the present study, we measured the neutrophil elastase:alpha-1-proteinase
inhibitor complex (E-PI) in serum and bronchoalveolar lavage fluid (BALF) in 26
patients with pulmonary fibrosis and evaluated the correlation between E-PI levels
and several parameters.
E-PI levels in serum of patients with pulmonary fibrosis (635.8±112.0 ng·mL⁻¹) (p<0.01). E-PI levels in serum significantly correlated with hepatocyte growth factor (HGF) levels in serum, C-reactive protein (CRP), and negatively correlated with arterial oxygen tension (PₐₐO₂), and arterial carbon dioxide tension (PₐₐCO₂). E-PI/albumin levels in BALF significantly correlated with HGF/albumin levels in BALF, lactate dehydrogenase (LDH)/albumin in BALF, total number of inflammatory cells (alveolar macrophages and neutrophils) in BALF, and several markers derived from epithelial cells in BALF.

Our data demonstrated: 1) neutrophil elastase:alpha-1-proteinase inhibitor complex
in serum increased in patients with pulmonary fibrosis; and 2) neutrophil elastase:alpha-1-proteinase inhibitor complex in serum and bronchoalveolar lavage fluid correlated with clinical parameters in pulmonary fibrosis. These results suggest that neutrophil elastase may play a significant role in the process of lung injury in pulmonary fibrosis.

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Materials and Methods

Subjects
The protocols of this study were approved by the institutional review board for human studies and informed written consent was obtained from the subjects. We studied 20 patients with a diagnosis of pulmonary fibrosis in Kagawa Medical Hospital (613 beds) and Matsuyma Citizen Hospital (538 beds) from 1990–1996. Of the 20 patients, 14 had IPF and six had PF-CVD, three had rheumatoid arthritis, two had dermatomyositis, and one had Sjogren syndrome. The patients (11 males and nine females)
Blood samples

Peripheral venous blood samples with and without ethylenediaminetetra-acetic acid (EDTA) were obtained before breakfast. After centrifugation at 1,000g for 10 min at 4°C, the serum was frozen and stored at -70°C until used. Arterial blood samples were analysed for \( P_{\text{a}}\text{O}_2 \) and arterial carbon dioxide tension \( (P_{\text{a}}\text{CO}_2) \) using a blood gas analyser.

BAL

After the upper airway was anaesthetized with topical lidocaine, a flexible fiberoptic bronchoscope was wedged into the lower respiratory tract. To sample the lower respiratory tract, BAL was performed by infusing three 50 mL aliquots of sterile saline at the site of the anterior segment of the right lower lobe. The last two aliquots were saved for evaluation. The fluid was filtered through gauze, and cells were separated from alveolar lavage fluid by centrifugation (300g for 10 min). BALF was frozen and stored at -70°C until used.

Measurement of elastase: alpha-1-PI complex (E-PI) levels in serum and BALF and correlations with other parameters

At first, free elastase activity in BALF was measured using a synthetic substrate, methoxy succinyl-l-alanyl-l-alanyl-l-prolyl-l-valine p-nitroanilide. As free elastase activity was not detected in all BALF samples, we used E-PI levels to evaluate elastase burden in both serum and BALF [11]. E-PI concentration was determined using enzyme-linked immunosorbent assay (ELISA) kits (Diagnostica Merck, Darmstadt, Germany) as described in detail by Niemann and co-workers [18, 19]. Briefly, the samples were added to wells coated with sheep anti-neutrophil elastase immunoglobulin (Ig)G. This antibody does not cross-react with cathepsin G or other neutrophil proteinases. After incubation and washing, the solid phase-bound E-PI were further incubated with alkaline phosphatase-labelled rabbit anti-alpha-1-PI IgG. After further washings, p-nitrophenylphosphate was added to measure the amount of solid phase-bound E-PI. This assay was calibrated using a standard solution of known E-PI concentration. The lower detection limit of this assay was 2.5 ng·mL\(^{-1}\). Data were expressed as mean values from duplicate determinations.

Peripheral blood samples were measured for white blood cell (WBC) counts and erythrocyte sedimentation rate (ESR). The serum samples were analysed for hepatocyte growth factor (HGF), E-PI, C-reactive protein (CRP), lactate dehydrogenase (LDH), and IgG. The BALF samples were analysed for HGF, E-PI, LDH, carcinoembryonic antigen (CEA), carbohydrate antigens sialyl Lewis (CA) 19-9, squamous cell carcinoma related antigen (SCC), IgG, IgA, albumin, and cellular profiles. HGF was measured by ELISA with monoclonal and polyclonal antibodies against human HGF (Otsuka Assay Laboratories, Tokushima, Japan). Tumour markers were measured by radioimmunoassay. Data in BALF were corrected by albumin (divided each datum by predicted albumin concentration). Pulmonary function measurements, vital capacity % predicted (VC) and forced expiratory volume in one second (FEV\(_1\)) as a percentage of forced vital capacity were obtained for all patients.

E-PI levels in serum and BALF were also measured in normal smokers and compared with the results of patients with pulmonary fibrosis. In patients with pulmonary fibrosis, correlations between each parameter was evaluated.

Statistical methods

Results were expressed as mean values±SEM. Comparisons of values between two groups were performed with the Mann-Whitney U-test. Correlations were evaluated using the Pearson's correlation coefficient, and Fisher's r to z method was used to calculate the p-values. A p-value of less than 0.05 was considered significant.

Results

Figure 1 shows the E-PI levels in: 1) serum; and 2) BALF of normal nonsmokers, normal smokers, and patients with pulmonary fibrosis. E-PI levels in the serum of patients with pulmonary fibrosis (635.8±112.0 ng·mL\(^{-1}\)) were significantly elevated compared to normal nonsmokers (122.0±4.0 ng·mL\(^{-1}\)) and normal smokers (132.8±8.4 ng·mL\(^{-1}\), p<0.01). E-PI/albumin levels in BALF of patients with pulmonary fibrosis (139±44 ng·mg albumin\(^{-1}\)) were not increased (although, this was nonsignificant) compared to normal nonsmokers (83±29 ng·mg albumin\(^{-1}\)) and normal smokers (156±3±34 ng·mg albumin\(^{-1}\)). Figure 2 shows that E-PI levels in serum significantly correlated with: 1) HGF levels in serum (r=0.567, p<0.01); and 2) CRP (r=0.537, p<0.05), but negatively correlated with 3) \( P_{\text{a}}\text{O}_2 \) (r=-0.513, p<0.05) and 4) \( P_{\text{a}}\text{CO}_2 \) (r=-0.574, p<0.01) in patients with pulmonary fibrosis. Figure 3 shows...
Fig. 1. – Elastase:alpha-1-proteinase inhibitor complex (EPI) levels in: a) serum; and b) bronchoalveolar lavage fluid (BALF) in normal nonsmokers, normal smokers, and patients with pulmonary fibrosis. Open symbols and bars represent the mean±SEM. NS: nonsignificant.

Fig. 2. – Correlations between elastase:alpha-1-proteinase inhibitor complex (E-PI) in serum levels in patients with pulmonary fibrosis and: a) hepatocyte growth factor (HGF); b) C-reactive protein (CRP); c) arterial oxygen tension ($P_{a,O_2}$); and d) arterial carbon dioxide tension ($P_{a,CO_2}$). 1 mmHg=0.133 kPa.
that E-PI/albumin levels in BALF significantly correlated with: 1) HGF/albumin levels ($r=0.707$, $p<0.0005$); 2) LDH/albumin ($r=0.808$, $p<0.0005$); 3) several markers derived from epithelial cells (IgA/albumin ($r=0.605$, $p<0.005$); 4) CEA/albumin ($r=0.662$, $p<0.001$); 5) CA19-9/albumin ($r=0.653$, $p<0.005$); and 6) SCC/albumin ($r=0.476$, $p<0.05$). Figure 4 shows that E-PI/albumin levels in BALF were found to significantly correlated with: 1) total number of alveolar macrophage/albumin; and b) total number of neutrophil/albumin.

**Discussion**

In the present study, it was demonstrated that serum levels of E-PI in patients with pulmonary fibrosis were...
patients with pulmonary fibrosis. Correlation between E-PI levels in serum reflected the disease activity in epithelial cells of the respiratory tract [21]. MUKAE et al. [22] showed that CA19-9 levels are high in serum and BALF correlated with total number of alveolar macrophage/albumin in BALF. Alveolar macrophages have been shown to release neutrophil chemotactic factors on stimulation with various stimuli, e.g., bacteria, dust grains, cotton, asbestos, and the immune complex [26]. It could be suggested that alveolar macrophage-derived neutrophil chemotactic factors play a role in neutrophil infiltration [26]. Then, the infiltrated neutrophil might release neutrophil elastase.

It was also aimed to evaluate the significance of E-PI as a prognostic factor. Since the follow-up period of our patient population was not long enough, data are not available at present. In addition, the usefulness of the measurement of E-PI in comparison with other inflammatory cytokines in pulmonary fibrosis (interleukin (IL)-1 β, IL-1 receptor antagonist, soluble IL-2 receptor, IL-6, IL-8, tumour necrosis factor-α, and interferon-γ) and correlations between E-PI and these markers should be evaluated in the future study.

In conclusion, the data demonstrate that: 1) elastase: alpha-1 proteinase inhibitor complex in serum is increased in patients with pulmonary fibrosis; and 2) elastase: alpha-1 proteinase inhibitor complex serum and bronchoalveolar lavage fluid correlated with clinical parameters in pulmonary fibrosis. These results suggest that neutrophil elastase may play a role in the process of lung injury and repair in pulmonary fibrosis.

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References


