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# Biomarker Discovery In Chronic Obstructive Pulmonary Disease (COPD) Using Epithelial Lining Fluid: A Proteomic Approach

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**RATIONALE**

Chronic Obstructive Pulmonary Disease (COPD) is the third most frequent disease worldwide with increasing mortality. Cigarette smoking is the principle risk factor and 15-20% of smokers develop COPD.

Epithelial Lining Fluid (ELF) covers the internal part of the airways and can be collected during bronchoscopy. ELF appears to be well-suited for proteomic analysis, since it contains a higher concentration of proteins (150-300 µg /mL) than other lung fluids and can be obtained from different locations of the lungs. No comprehensive proteomic analysis of human ELF has been performed to date, which makes ELF a highly interesting fluid for biomarker discovery in COPD.

**AIM**

To discover proteins that change in abundance in ELF from COPD patients versus healthy controls using a quantitative proteomics approach.

**METHODS**

The ELF proteome from COPD patients and healthy controls was studied by 1D polyacrylamide gel electrophoresis in the presence of SDS followed by in-gel tryptic digestion to establish the methodology and assess the feasibility of such an approach. Approximately 40 gel slices were obtained from each lane of the gel (corresponding to one patient). Digested samples were analyzed by nanoChip-LC-MS/MS using an ion trap.

We performed a quantitative pilot study of ELF from 4 COPD patients and 4 healthy controls (table 1) to test for statistically significant differences in protein levels. ELF samples were digested by trypsin, labeled with stable isotope-containing reagents (iTRAQ®, 8-plex) and processed by strong cation-exchange chromatography followed by nanoLC-MS/MS. In order to validate the results, a second quantitative analysis of an independent sample set (4 COPD vs 4 healthy) using the same methodological approach was done.

**RESULTS**

The 1D electrophoretic approach resulted in more than 300 identified proteins. Most of the identified proteins were present in both COPD and healthy samples, although some proteins were only identified either in healthy control or in COPD samples.

The quantitative studies showed that a number of proteins was significantly different between ELF of COPD patients and controls, including 4 up-regulated proteins in common in both studies.

**CONCLUSIONS**

This is the first study in ELF of COPD patients and healthy controls in which such a large number of proteins has been identified.

The obtained results show the feasibility of this proteomic approach and the possibility to discover proteins that are differentially expressed in ELF of COPD patients and controls. We are currently validating these proteins further by western blot and immunohistochemistry.

Patient characteristics, lung function, smoking history

| | Pilot study | Pilot study | Validation study | Validation study |
|---|---|---|---|---|
| | COPD (n=4) | Healthy (n=4) | COPD (n=4) | Healthy (n=4) |
| Female, number | 0 | 2 | 2 | 2 |
| Age, years | 63.75 | 62.75 | 60 | 55.5 |
| Co-morbidity, CCI | 2.25 | 0.25 | 1.5 | 0.25 |
| Current smoker, number | 1 | 0 | 1 | 2 |
| Ex smoker, number | 3 | 0 | 3 | 1 |
| Pack years, number | 37.5 | 0 | 37.5 | 9.52 |
| Inhaled corticosteroids, number | 2 | 0 | 1 | 0 |
| $FEV_1$/FVC, % | 58.5 | 76 | 33.25 | 84.25 |

| | | | | |
|---|---|---|---|---|
| $FEV_{1,}$ %predicted | 71.5 | 116.3 | 40.25 | 111.25 |

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