# Sputum Proteomics in Asthma

The identification of markers of inflammatory activity at the early stages of pulmonary diseases which share common characteristics that prevent their clear differentiation is of great significance to avoid misdiagnosis, and to understand the intrinsic molecular mechanism of the disorder. The combination of electrophoretic/chromatographic methods with mass spectrometry is currently a promising approach for the identification of candidate biomarkers of a disease. Since the fluid phase of sputum is a rich source of proteins which could provide an early diagnosis of specific lung disorders, it is frequently used in these studies.

## 1. Asthma

As underlined above, asthma leads to increased irritability of the mucosa and the episodes of shortage of breath or coughing make this disorder overlap with COPD for a few traits [1]. The frequent observation of neutrophilic airway inflammation in both controlled asthma (CA) and severe uncontrolled asthma (UA) prevents the use of sputum biomarkers to differentiate the two conditions. Thus, to identify biomarkers of severe UA with neutrophilic airway inflammation, Lee et al. [2] applied two-dimensional electrophoresis coupled with MALDI-TOF/MS to the analysis of sputum samples from patients with CA and severe UA. From among the proteins that exhibited differences in relative intensity between patients of the two cohorts, the most impressive was S100 calcium binding protein A9 (S100A9), a calcium- and zinc-binding protein which plays a prominent role in the regulation of inflammatory processes and immune response. The fact that it was detected at a higher level in neutrophilic sputum from patients with severe UA vs CA led the authors to hypothesize that sputum S100A9 could be considered a potential biomarker of neutrophilic inflammation in severe UA.

Non-smokers, healthy non-smokers, ex-smokers (ESA) and current smokers (CSA), were the components of the cohorts of patients examined by Takahashi et al [3] to define severe asthma molecular phenotypes. The broad range of analyses performed by the authors included exploratory proteomic analysis of sputum supernatants and transcriptomic analysis of bronchial brushings, biopsies, and sputum cells. Based on the differentially expressed proteins identified, the usefulness of sputum proteomics in distinguishing CSA from ESA subjects was reaffirmed despite little difference in their clinical characteristics. The peculiarity of this difference was an increase of the expression of colony-stimulating factor 2 protein in CSA patients and a remarkable loss of epithelial barrier processes in ESA patients. Based on prespecified clinic-physiologic variables, Lefaudeux et al. [4] could compare cohorts of patients with moderate-to-severe asthma and stratify them into four clusters that showed significant differences in sputum proteomics and transcriptomics. The data relative to severe asthma clusters indicated that sputum eosinophilia was higher than in cluster T1, while no differences was observed in sputum neutrophil counts and exhaled nitric oxide and serum IgE levels.

The improvement of patient stratification by the identification of molecular sub phenotypes of asthma defined by proteomic signature was undertaken by Schofield et al. [5]. They applied an unbiased label-free quantitative MS technique combined with topological data analysis to analyze the proteomes of sputum supernatants from asthmatic patients. Based on similarity in proteomic features, patients were stratified in ten clusters representing three sub-phenotypes of asthma: highly eosinophilic, highly neutrophilic, and highly atopic with relatively low granulocytic inflammation. The insight on granulocytic inflammation provided by these data could be useful for detection of targets for novel therapies.

## 2. Asthma and Gastro-Esophageal Reflux

Gastro-esophageal reflux disease (GORD) is one of the numerous co-morbidities of asthma. Despite being clear that there is a significant association between these two disorders, the paucity of data on the direction of causality makes the role of GORD poorly understood [6]. In this context, based on the assumption that severe asthmatics with active GORD inhale oropharyngeal refluxate into their lower airways where it causes severe biological problems, Tariq et al. [7] performed a proteomic analysis on induced sputum of mild/moderate asthmatics and healthy controls and of a subset of

severe asthmatics. Quantitative LC coupled with untargeted MS was used to perform the experiments, and proteins associated with GORD in the cohort of severe asthmatics were identified by means of univariate and multiple logistic regression analyses. The data evidenced that GORD was three- and ten-fold more prevalent in severe asthmatics compared to mild/moderate asthmatics and healthy controls, respectively. A further comparison of the sputum proteome in active GORD severe asthmatics with that of patients without active GORD showed five differentially abundant proteins with roles in anti-microbial defenses, systemic inflammation, and epithelial integrity. Multiple linear regression analysis revealed that three of these (Ig lambda variable; plasma protease C1 inhibitor, and lipocalin-1) were associated with active GORD. The evidence provided by this study indicates that severe asthmatics with GORD may represent a distinct phenotype of asthma, and that reflux can cause subtle perturbation of proteins detectable in the airways lining fluid. The list of articles commented in this paragraph is summarized in Table 1.

**Table 1.** List of articles dealing with proteomics of sputum in patients with asthma.

| Subjects Investigated | Method of Sputum Collection and Processing | Proteomic Technique Applied | Target of the Rersearch | Finding | Reference # |
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| Patients with controlled asthma and severe uncontrolled asthma | Induced. Sputum was centrifuged and loaded on 2-DE. | 2 DE coupled to MALDI-TOF | Identifying biomarkers to differentiate the two conditions | S100 calcium binding protein A9 was considered a potential biomarker of neutrophilic inflammation in severe UA | [2] |
| Non-smokers; healthy non-smokers; ex-smokers and current smokers | Induced. Sputum plugs were separated into cells and supernatant. This latter was submitted to MS analysis. | LC-MS | Define severe asthma molecular phenotypes | The differentially expressed proteins identified allowed to distinguish current smokers from ex-smokers | [3] |
| Patients with moderate-to-severe asthma | Induced. Sputum plugs were selected and liquefied with DTT.Transcriptomic analysis was performed on extracted RNA from sputum cells derived from cell pellets | Affymetrix HT HG-U133 + PM GeneChip | Stratify patients into clusters | Four clusters were identified that showed significant differences in sputum proteomics and transcriptomics | [4] |
| Asthmatic patients | Induced. Sputum was treated with DTT, and cellular and aggregated material removed by centrifugation. Proteins in the supernatant were alkylated and digested with trypsin. | LC-MS | Patient stratification | Patients were stratified in 10 clusters representing 3 sub phenotypes of asthma: highly eosinophilic, highly neutrophilic, and highly atopic with relatively low granulocytic inflammation | [5] |
| Mild/moderate asthmatics, healthy controls and of a subset of severe asthmatics | Induced sputum was acquired and processed DTE as a mucolytic to obtain supernatant for mass spectrometric analysis. | LC-MS | Identify proteins associated with Gastro-oesophageal reflux disease (GORD) in asthmatic patients | GORD was three- and ten-fold more prevalent in severe asthmatics compared to mild/moderate asthmatics and healthy controls | [7] |

* In all cases of induced sputum, induction was performed after inhalation of hypertonic (0.9% to 4.5%) saline with a nebulizer according to the procedure.

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## References

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