493. Role of the biomarkers in airway diseases

P4779
Bronchial and alveolar exhaled nitric oxide as a marker of systemic involvement in patients with Crohn’s disease
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Background: Crohn’s Disease (CD) is an inflammatory bowel disease often associated with a variety of systemic manifestations, including airways involvement. Fractional Exhaled Nitric Oxide (FENO) can be measured non invasively at low (bronchial air) and high (alveolar air) flows to reflect proximal airway inflammation, and systemic inflammation.

The aim of our study was to compare both bronchial and alveolar FENO as an index of pulmonary involvement and of systemic inflammation in CD patients with different stages of clinical activity, with a group of healthy subjects.
Our results for bronchial and alveolar FENO confirm subclinical inflammation in healthy controls, respectively. Both bronchial (p=0.0016) and alveolar FENO (p=0.007) were positively correlated with Crönh’s Disease Activity Index.

Methods: Our results for bronchial and alveolar FENO confirm subclinical pulmonary involvement in Crönh’s disease. FENO may be of clinical value during follow-up of these patients as a surrogate marker of systemic inflammation.

**P4784**

**Comparison of alveolar nitric oxide concentrations using two different methods for the assessment of small airways inflammation in asthma**

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Background: Assessment of exhaled breath temperature (EBT) has been suggested as a screening test for primary ciliary dyskinesia (PCD) as patients with PCD have lower n-NO levels than healthy controls. Recent studies on n-NO in PCD and secondary ciliary dyskinesia (SCD) show an overlap between these two groups.

Aim: To investigate in a pilot study if n-NO can differentiate between patients with diagnosed PCD and SCD treated at our centre.

Methods: n-NO was measured in duplicate by aspiration at 5 ml/s with NRB 50 in 21 patients with clinical symptoms suggestive of PCD (n=12) or SCD (n=9). The electron microscopy (EM) results on brush samples from the nose were available in all subjects.

Results: Reproducibility of n-NO was good in each patient. A group of patients (n=6) had clearly low n-NO (5-69ppb) compared with the rest (216-857ppb). In the group with low n-NO there were only patients while PCD with high n-NO there were both patients with SCD and PCD, including 2 with Kartagener’s syndrome. Regarding EM findings, the only subjects with no dyskinetica were in the low n-NO while 8 subjects without structural abnormalities had high n-NO values. The relatively large group with more atypical or inconclusive results was also heterogeneous with regard to n-NO levels.

Conclusion: A low n-NO value had a good positive predictive value for PCD in our material, but some PCD patients had normal n-NO levels. As structural findings on EM and n-NO appear to disassociate in some subjects, we continue to investigate all PCD and SCD patients at our centre including even functional tests of ciliary clearance, to see if n-NO correlates more to the cilia function than structural abnormalities.

**P4783**

**Exhaled breath temperature increases at COPD exacerbation and correlates with sputum neutrophilia**

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Introduction: Assessment of exhaled breath temperature (EBT) has been suggested as a novel tool to detect airway inflammation. EBT and the rate of temperature increase are altered in stable states of asthma and COPD compared to healthy controls. In asthma EBT correlates with eosinophil percentage in sputum. However, it is not known if EBT changes at acute exacerbation of COPD and how it relates to airway inflammation, spirometric values and patient's health status.

Methods: Ten patients with previously diagnosed COPD (age 63±11 years) were recruited 24 h within the onset of exacerbation (Anthonisen type II) and after systemic steroid and/or antibiotic treatment at recovery (7.6±1.4 days). At visits
We enrolled 79 patients affected by lung cancer and exhaled breath condensate (EBC) of lung cancer patients to investigate, for the first time to our knowledge, the presence of HPV in the patients compared to controls. Significantly higher concentrations were found as LTB-4 and IL-8 resulted higher in breath condensate and the blood of NSCLC patients.

**Background:**
We enrolled 50 NSCLC patients and 25 controls. LTB-4 and IL-8 concentrations play a role in cancer development and progression. The aim of this study was to give our contribution in this field by investigating inflammatory information in tumor biology remain complex and not completely known.

**Purpose:**
Today an increasing interest is being generated by the study of lung cancer markers for monitoring of lung cancer. Targeted eicosanoid lipidomics of induced sputum as compared to exhaled breath condensate in asthmatics is well known that the airway inflammation is enhanced at exacerbations. The identification of VOCs that characterise the enhanced airway inflammation. The identification of VOCs that characterise the acute exacerbations could be used as diagnostic tool for an exacerbation apart from the clinical criteria.

**Conclusion:**
Our data suggest that specific VOCs can be detected in the exhaled breath of COPD patients with an acute exacerbation indicating possibly the enhanced airway inflammation. The identification of VOCs that characterise the acute exacerbations could be used as diagnostic tool for an exacerbation apart from the clinical criteria.

**Materials and methods:**
Breath samples were collected via a side-stream Teflon tube and measured directly by an ion mobility spectrometer coupled to a multi capillary column (MCC/IMS). VOCs were detected and statistically evaluated in order to discriminate COPD patients with an exacerbation compared to stable disease and healthy subjects.

**Results:**
Thirteen COPD patients with an acute exacerbation, 46 COPD patients with a stable disease as well as 51 healthy subjects were included in the study. Several peaks were found to differentiate in the group of patients with an acute exacerbation compared to the COPD patients with stable disease and with healthy subjects.

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Detection of pseudomonas aeruginosa (Pa) specific peaks by ion mobility spectrometry (IMS) in exhaled breath of bronchiectasis patients

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Introduction: Colonisation or infection of airways from bronchiectasis patients by Pa results in chronic inflammation leading to a progressive destruction of the lung and to a decline in lung function. Therefore more sensitive stay for intravenous antibiotic treatment are necessary and the quality of life in these patients is severely limited.

Objectives: Aim of our study was to detect and compare volatile organic compounds (VOCs) by IMS in exhaled breath of bronchiectasis patients either colonised or infected by Pa with healthy non-smoking controls (hc).

Methods: We have analysed VOCs by IMS coupled to a multi-capillary column (MCC) for pre-separation (MCC-IMS, B+S Analytik) in exhaled breath of bronchiectasis patients either colonised or infected with Pa compared to healthy subjects.

Results: By IMS VOC analysis differences between Pa+ and hc could be found. Different peaks were detected between Pa+ and hc. Moreover Pa+ were separated from patients with Pa+ and from Pa- subjects. Pa+ VOC analysis revealed two peaks which could be found in the Pa+ bronchiectasis patients.

Conclusions: IMS seems to be a promising method for the non-invasive identification of patients which are colonized or infected with Pa. A differentiation between patients treated with antibiotics or infected with Pa seems to be possible, as well. However, further confirmation of our findings in a larger study population is needed. The comparison of Pa+ with Pa- cultures will help to identify peaks caused by the presence of Pa. Furthermore, it is required to identify the molecules representing the peaks.

Metabolic analysis of exhaled breath condensate in diagnostics of obstructive airway diseases

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Metabolic analysis provides molecular and biochemical profiles of metabolites in different biological fluids.

Objectives: The aim of this study was to assess the potential of exhaled breath condensate (EBC) molecular profiling in discrimination patients with COPD and asthma and healthy subjects.

Methods: Twenty patients with asthma, twenty patients with COPD and thirty healthy control subjects were enrolled in cross-sectional study. Every subject performed spirometry and EBC collection. EBC samples were analyzed by gas chromatography – mass spectrometry method (GC-MS). EBC profiles from patients with asthma were separated from patients with COPD and from healthy control subjects using an algorithm based on linear methods of pattern recognition theory.

Results: We have detected various profiles of semi-volatile organic compounds (SVOC) in EBC in patients with asthma, COPD and healthy subjects. Mathematical approach to available data revealed 9 SVOC which have been deemed the most appropriate for solving recognition problem (2-phenoxycethanol, decanal-1, ethyl citrate, 2,3-dihydro-1-H-inden-1-0 and others). EBC profiles of healthy subjects can be distinguished from patients with asthma with reliability 75%, healthy subjects from COPD patients with reliability 85% and asthma patients from COPD patients with reliability 83%.

Conclusion: Metabolic analysis of EBC can discriminate patients with asthma and COPD and healthy subjects. We propose that differences in SVOC profiles between asthma and COPD are disease related.

Detection of protein and peptide composition of exhaled breath condensate in patients with pulmonary disease by mass spectrometry

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Exhaled breath condensate (EBC) reflects the composition of the airway-lining fluid and may contain biomarkers of diseases of respiratory system.

The aim of this study is to identify proteins in EBC of patients with chronic obstructive pulmonary disease (COPD) and pneumonia using two techniques of proteome identification.

In fourteen COPD and thirteen pneumonia samples were collected using the Jaeger ECoScreen (VIASYS Healthcare, Germany), freeze dried, treated with trypsin and analyzed by nanoflow LC-MS/MS with a 7-Tesla Finnigan LTQ-FT mass spectrometer (Thermo-Electron, Germany). Six COPD samples were mixed and applied to 2D-PAGE by Mini-PROTEAN system (Bio-RAD, USA). Silver stained spots were analyzed by MALDI-TOF-MS using a Microflex device (Bruker Daltonics, Germany). Using 2D-PAGE and MALDI-TOF-MS we were able to show that EBC samples from patients with COPD contain whole “normal” keratins that were detected also in EBC of healthy donors.

Using LC-MS/MS, a method of comparable sensitivity, specific peptides of “abnormal” keratin 3, 4, 8 were identified in COPD samples. Keratin set identified in samples from patients with acute pneumonia was more varied. Peptides of Plakoglobin, Desmoplakin, Alpha-1-acid glycoprotein, Filaggrin, Dynclin, Collagen, Homerin were detected in COPD and pneumonia EBC samples. They are uncharacteristic of healthy EBC samples. None of these proteins was identified as a whole in 2D-PA gels. These peptides seem to appear in airway-lining fluid due to proteolysis in respiratory tract tissue. In conclusion, each of “abnormal” peptides, as well as their combinations, may have diagnostic value.

Project Discussion

Room E104-106 - 08:30-10:30
MEF50 as well as between breath pattern and tacrolimus plasma levels (p < 0.05).

Results: A significant relationship was detected between the breathprint and clinical parameters in lung transplant recipients. The relation with the drug level draws attention to the interfering effect of treatment and to the possibility of monitoring drug level by exhaled breath testing.

Conclusions: Recovery of sputum mediators sensitive to DTT can be improved using a new sputum processing technique. Within airway inflammatory sub-phenotypes there is a differential pattern of mediator expression that is independent of disease. Whether these inflammatory phenotypes in asthma and COPD confer distinct pathogenesis, therapeutic responses and clinical phenotypes needs to be further evaluated.

Follow up of lung transplant recipients using electronic nose
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P4795

Follow up of lung transplant recipients using electronic nose

Methods: Sixteen patients following lung transplantation participated in our study (mean age 39±14). The study had a model (N=25 samples) and a validation part (N=35 samples). Hence, in average 3.7±0.2 samples per patient were analysed.

During their scheduled follow up at the outpatient clinic exhaled breath collection (N=35 samples). Hence, in average 3.7±0.2 samples per patient were analysed.

Methods: Pooled BALF from patients with sarcoidosis (n=5) was extracted by Solid Phase Extraction and analytes were quantified by LC-MS/MS (Acquity-Xerox, Waters). The method assess 87 LMs in combination with 16 deuterated internal standards.

Results: In BALF from sarcoidosis patients, 29 LMs from six fatty acid were detected; but only 8 LMs from each of the AA and LA pathways were quantifiable. The fatty acid origin and biosynthesis pathway of the 29 LMs is shown in Fig. 1.

Conclusions: LC-MS/MS methods for LM quantification are applicable for exploring the etiology and pathology of respiratory diseases as exemplified with BALF from sarcoidosis patients. These methods will be useful in examining inflammatory processes.

P4796

Profiling of sputum inflammatory mediators in asthma and chronic obstructive pulmonary disease
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Introduction: Asthma and chronic obstructive pulmonary disease (COPD) display features of overlap in airway physiology and airway inflammation. The relationship between mediator expression and airway inflammation was explored within these airway diseases.

Methods: Patients with asthma (54 patients: 21 men) and COPD (49 patients: 36 men) were studied. Clinical characteristics and sputum was collected at entry into the study. A two-step sputum processing method was performed for supernatant and cytospin preparation. The Meso Scale Discovery and Luminex platforms were used to measure cytokines, chemokines and matrix metalloproteinase levels.

Results: Analytes sensitive to dithiothreitol (DTT) that had increased recovery in the two step sputum process were IL-1b, 4, 5, 10, 13, IPN-g, TNFRII GM-CSF, CCL2, 3, 4, 5, 13 and 17. There was a differential expression in IL-8, TNFRI and TNFRII between asthma and COPD (mean fold difference (95% confidence interval) IL-8, 2.6 (1.3 to 5.4), p=0.01; TNFRI, 2.1 (1.3 to 5.4), p=0.03; and TNFRII, 2.6 (1.2 to 5.6), p=0.02). In neutrophilic and eosinophilic airway inflammation, TNFα, TNFRI, TNFRII, IL-6, IL-8 and IL-5 could differentiate between these phenotypes. However, these phenotypes were unrelated to the diagnosis of asthma or COPD.

Conclusion: Recovery of sputum mediators sensitive to DTT can be improved using a new sputum processing technique. Within airway inflammatory sub-phenotypes there is a differential pattern of mediator expression that is independent of disease. Whether these inflammatory phenotypes in asthma and COPD confer distinct pathogenesis, therapeutic responses and clinical phenotypes needs to be further evaluated.