65. Biomarkers in asthma and COPD

392 Serum periostin but not airway POSTN expression is reduced in smokers with asthma

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Background
Elevated serum periostin is associated with airway eosinophilia and non-smokers with asthma and may predict response to therapies targeting Th2 inflammation. Smoking in asthma is associated with non-eosinophilic airway inflammation and corticosteroid insensitivity. We determined the effects of smoking status on serum periostin and airway expression in asthma.

Methods
Serum periostin (ELISA; Aviscera Bioscience) and airway (nasal) epithelial POSTN expression (210809_s_at on U133+2 chips) were measured in asthmatic and healthy subjects (Table). Serum periostin was also measured before and after two week oral steroids in another asthma group (n=45). Data (median IQR) was analysed by Kruskal-Wallis test.

Results
Serum periostin was reduced by smoking in both asthmatic (p=0.017) and healthy subjects (p=0.063). Periostin was not influenced by disease severity (p=0.786). Oral steroid treatment reduced serum periostin (p=0.030), particularly in non-smokers with asthma. POSTN expression was similar in non-smokers and smokers with severe asthma, but lower in healthy smokers (p=0.002).

Table 1 Serum periostin and airway POSTN expression

<table>
<thead>
<tr>
<th>Serum periostin (ng/ml)</th>
<th>Airway (nasal) POSTN expression</th>
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</thead>
<tbody>
<tr>
<td>Healthy non-smokers</td>
<td>89 (7, 1275) (n=24)</td>
</tr>
<tr>
<td>Healthy smokers</td>
<td>89 (7, 1275) (n=18)</td>
</tr>
<tr>
<td>Asthma non-smokers</td>
<td>280 (9, 4027) (n=32)</td>
</tr>
<tr>
<td>Asthma smokers</td>
<td>9 (9, 330) (n=24)</td>
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Conclusion
Serum periostin is lowered by smoking in asthma, whereas airway POSTN expression is similar in smokers and non-smokers with asthma. The role of periostin as a potential biomarker of Th2 inflammation in asthma requires further evaluation in smokers.

394 Methylated arginine derivatives can differ stable and unstable patients with COPD

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Early recognition of patients with unstable COPD is desired reducing the mortality. Asymmetric and symmetric dimethylarginines (ADMA and SDMA, respectively) are markers of endothelial dysfunction. In addition, increased protein arginine methylation is related to hypoxia. We aimed to compare the serum level of L-arginine, ADMA and SDMA in patients with stable and unstable COPD. Methods: A total of 45 patients with COPD were prospectively investigated. Venous blood was taken for evaluation of biomarkers (L-arginine, ADMA, SDMA and hsCRP). All markers were compared with normal controls (NC, n=64). Beside, capillary blood gas analysis was recorded. Unstable patients were defined based on presence of acute exacerbation (COPD-U, n=12). Statistics: ANOVA, chi-square test and Spearman correlation. Results: Both capillary pO2 and SpO2 were significantly lower (p<0.01) in patients with COPD-U. ADMA and SDMA were significantly higher (p=0.004 and p=0.001, respectively) in COPD-U compared to stable patients, however neither the precursor molecule L-arginine, nor the hsCRP showed significant difference. Both ADMA (AUC: 0.81, p=0.001) and particularly SDMA (AUC: 0.91, p=0.001) with high sensitivity and specificity differed patients with COPD-U. A cut-off for SDMA ≥ 0.57 was found as an independent variable in a regression model to verify COPD-U (OR: 39.2, p=0.001). All markers were significantly higher in patient groups compared to NC. Conclusions: COPD is associated with elevated L-arginine, ADMA and SDMA serum level. In unstable patients, production of ADMA and SDMA are more pronounced probably due to more severe hypoxic insults. Thus methylated arginine derivatives may help in recognition of unstable patients.
395 Altered inflamasome activity in bacterial acute exacerbations of COPD (AECOPD)
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Inflamasome and its products, as part of the innate immune system, can be triggered to assist in defence against invading pathogens. We have previously shown increased levels of IL-18 in induced sputum of stable COPD patients, which were decreased in acute exacerbations of COPD (AECOPD), implying a possible dysregulation of inflamasome in AECOPD. Aim of this study was to assess the inflamasome activity in AECOPD in the case of proven bacterial infections versus AECOPD where only bacterial colonization was proved.

30 patients hospitalized for an infectious AECOPD according to Antoniussen’s criteria were included in the study. We examined the inflammatory properties of induced sputum and assessed bacterial infection using PCR. IL-18, caspase-1, TLR-2, and IL-1b were measured in induced sputum and serum by immunosorbent analysis (ELISA). Immunocytochemistry of IL-18 expression in sputum cells was performed using a mouse monoclonal IL-18 antibody.

IL-18 levels in sputum were found significantly lower in AECOPD caused by a pathogen compared to colonized AECOPD (207 pg/ml (range 47-2301) vs 88 (2.9-989), p<0.05). Similarly, although non statistically significantly, decreased levels of caspase-1 and TLR2 were found in infectious versus colonized AECOPD (1.6 pg/ml (1.22-19) vs 3.6 pg/ml (1.27-117), p>0.05).

IL-18 was statistically significantly increased in induced sputum of infectious AECOPD (539 pg/ml (1.5-973) vs 88 (2.9-898), p>0.05). Positive staining of IL-18 was observed in macrophages in immunocytochemistry.

Our data show that in bacterial AECOPD there may be a dysregulated activation of inflamasome mediating IL-18 production.

396 Local and systemic inflammation in chronic obstructive pulmonary disease (COPD)
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Background
The aim of the study was to explore to what extent local inflammatory processes in the mouth (saliva, clinical assessment of periodontitis) and the respiratory tract (sputum, bronchoalveolar lavage (BAL), lung function) are associated with systemic inflammatory responses (blood) in smokers with and without COPD.

Method
Healthy controls (n=23), smokers with (n=28) and without (n=29) COPD performed spirometry and dental examinations. Saliva, induced sputum, BAL fluid and serum were collected. Inflammatory mediators were measured using ELISA. Soluble and cell bound tumor necrosis factor receptors (TNFRs) in sputum, BAL fluid and serum were detected by flow cytometry. The mRNA-expression of tumor necrosis factor-α (TNF-α) and its receptors on BAL macrophage were analyzed by real-time PCR.

Result
A negative correlation between lung function and saliva IL-8/MMP-9 was found in smokers with COPD (p<0.01). There were positive correlations between these mediators (IL-8/MMP-9) in saliva and periodontitis as assessed by bleeding index in non-smokers (p<0.01). Sputum IL-6 and IL-8 were significantly positively correlated with soluble TNFRs (sTNFRs) in non-smokers (p=0.01) and with sTNFR2 in smokers with COPD (p=0.01). There was a close positive correlation between soluble TNFR1 and TNFR2 receptors in sputum, BAL and serum in all groups (p=0.01).

Conclusion
Inflammatory markers in saliva, which is easy to collect, seem to reflect disease severity in COPD patients. Shedding of TNFR is similarly regulated locally and systemically, both in healthy subjects and in smokers, irrespective of airflow limitation.

397 Pro-surfactant protein B, a promising BAL biomarker of COPD progression in heavy smokers, is increased by budesonide/formoterol short-term therapy
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Rationale & Aim: Reduced levels of surfactants in lung and bronchoalveolar lavage fluid (BAL) generally signal disease progression. The aim of this study was to determine the effects of Symbicort® therapy on the BAL levels of pro-surfactant protein B (pro-SPB) and other biomarkers in heavy smokers with or without COPD.

Methods: We recruited 37 heavy smokers (3 current and 34 former; >30 pack-years), age 65±6 years (mean±SD), free of exacerbations for 2 weeks, with FEV1 of 73.1±18.3% predicted and FEV1/FVC ratio 66.3±9.4% (clinical trials. gov: NCT006569712). COPD was defined as FEV1/FVC <70%. BAL was obtained at baseline and after 4 weeks of Symbicort Turbuhaler® 400/12 mcg (budesonide/formoterol) BID therapy. Lung-predominant proteins: pro-SPB, surfactant protein D (SP-D) and Club Cell Secretory Protein (CCSP)-16 were measured in BAL supernatants.

Results: Symbicort therapy significantly increased pro-SPB levels in BAL (geometric mean±SD: 322±619 versus 268±394 ng/ml; p=0.0166). The pro-SPB levels (but none of the other BAL biomarkers) were significantly related to lung function expressed by FEV1% of predicted (Spearman rho=0.36; p=0.026) and FEV1/FVC ratio (rho=0.51; p=0.0013), and to the levels of SP-D (rho=0.43; p=0.0073) and CCSP-16 (rho=0.54; p=0.0065), and to body mass index (rho=0.46; p=0.0043).

Conclusions: In the current and former heavy smokers, pro-SPB levels in BAL were positively related to lung function and significantly increased by 4 weeks therapy with Symbicort. Pro-SPB is a very promising BAL biomarker to evaluate lung function in heavy smokers and thus disease progression in COPD and other chronic airway diseases.

398 Assessment of ATP degradation in bronchoalveolar lavage fluid
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Introduction: Extracellular adenosine triphosphate (ATP) via purinergic signalling plays a role in the development of airway inflammation. Elevated ATP concentration in airway samples was reported in inflammatory diseases. Airway ATP level is under tight control by enzymatic degradation, which could also affect ATP concentration measured in ex vivo biological samples.

Aim: To assess ATP degradation in bronchoalveolar lavage (BAL) fluid.

Methods: ATP was measured in BAL collected from 20 subjects (6 patients with interstitial lung diseases, 4 with asthma, 2 with COPD, 1-1 with extrinsic allergic alveolitis / Wegener granulomatosis / microaspiration / pleuritis / pulmonary hypertension / pneumonia and 2 healthy controls) within an hour after collection using luminometry. ATP degradation was assessed as the recovery of 1 μM ATP after 30 minutes. Seven samples were also collected on chelating solutions (0.32% sodium citrate or 2 mM EDTA+2 mM EGTA). Data were analyzed with non-parametric tests (median/interquartile range).

Results: BAL fluid ATP concentration was 18 nM/4-107 nM; and recovery of added ATP was 36%/29-82%. ATP concentration was higher in samples treated with citrate (530 nM /375-715 nM) or EDTA+EGTA (420 nM /234-828 nM) compared to untreated samples (186 nM /39-302 nM; p<0.05). ATP degradation was inhibited by both citrate (recovery: 99%/50-100%) and EDTA+EGTA (recovery: 89%/53-100%) in comparison with no treatment (p<0.05).

Conclusion: Extracellular ATP is degraded at a variable speed in BAL fluid, which is effectively inhibited by chelating agents. The analysis of pre-treated BAL fluid might more precisely reflect airway ATP concentration.
Profiling the proteome of the lower airway respiratory tract lining fluid in chronic obstructive pulmonary disease
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Chronic Obstructive Pulmonary Disease (COPD) is a chronic inflammatory condition of the airways associated with protease, anti-protease imbalance and oxidative stress. Relatively little is known about how underlying airway immunopathology impacts upon the protective function of the respiratory tract lining fluid (RTLF).

Aim: To investigate compositional differences in the RTLF proteome in COPD patients compared against aged and smoking history matched controls.

Broncholaveolar lavage samples were obtained from COPD smokers (n=5, 63.8±6.0 years), COPD ex-smokers (n=10, 66.0±6.8 years), healthy smokers (n=5, 61.4±6.2 years) and healthy non smokers (n=5, 66.8±5.9 years). Samples were analysed by one-dimensional gel electrophoresis and nanoliquid chromatography-tandem mass spectrometry, data processed using MASCOT and SCAFFOLD.

We identified 342 unique proteins, the greatest number, 153, observed in healthy non smokers, with only 49 proteins identified in COPD ex-smokers. Proteins were classified according to their gene ontology annotation, with COPD smokers found to possess the greatest number with roles in inflammation, immune response and protease, anti-protease balance. Employing the sum of major ion intensities permitted a semi-quantitative assessment of protein concentrations with Calgranulin A, Surfactant protein A and Alpha-1 antitrypsin decreased in COPD smokers versus non smokers. Secretoglobulin concentrations were elevated in COPD patients versus smoking and non-smoking controls.

Data suggests a simplified RTLF proteome in COPD, with a shift in protein expression in COPD smokers consistent with ongoing inflammation and protease, anti-protease imbalance.