## 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**

Mario Malerba[1], Beatrice Ragnoli[2], Alessandro Radaeli[2], Chiara Ricci[3], Francesco Lanzarotto[3], Alberto Lanzini[3]. *[1]Medicina Interna, Università di Brescia, Brescia, Italy; [2]Dipartimento di Emergenza, Spedali Civili di Brescia, Brescia, Italy; [3]Unità di Gastroenterologia, Università di Brescia, Brescia, Italy*

**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.

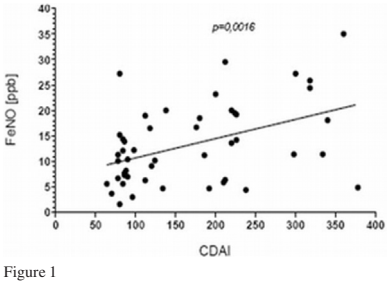



Figure 1

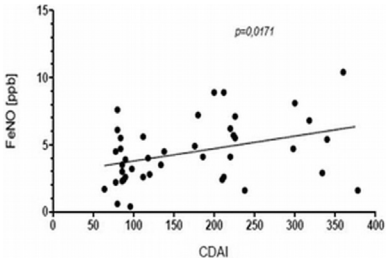



Figure 1 (continued)

**Methods:** Thirty CD patients (age 43.10±14.6 yrs) without clinical evidence of pulmonary diseases and 21 non smokers, not atopic healthy controls (age 35.1±13.2 yrs) were enrolled.
**Results:** Bronchial (14.9±10.2 ppb vs 10.1±6.3 ppb, p=0.049) and alveolar FENO (4.4±2.2 ppb vs 2.6±1.9; p=0.006) were significantly higher in CD patients than in healthy controls, respectively. Both bronchial (p=0.0016) and alveolar FENO (p=0.017) were positively correlated with Crohn's Disease Activity Index.
**Conclusions:** Our results for bronchial and alveolar FENO confirm subclinical pulmonary involvement in Crohn's disease. FENO may be of clinical value during follow-up of these patients as a surrogate marker of systemic inflammation.

**P4780**
**Are the flow independent NO parameters flow dependent?**
Marieann Högman[1,2], Pekka Meriläinen[2]. [1]*Centre for Research and Development, Uppsala University/County Council of Gävleborg, Gävle, Sweden;* [2]*Department of Medical Sciences, Uppsala University, Uppsala, Sweden*

**Background:** The interest in alveolar NO, one of the flow independent parameters, is increasing since it is elevated in severe and nocturnal asthma and in COPD. The nonlinear approach (Högman-Meriläinen Algorithm) with low, medium and high flow rates has been used to calculate $C_ANO$, $D_{aw}NO$ and $C_{aw}NO$. The aim was to set how low and how high these flow rates should be.
**Methods:** Subjects (n=32), without classifications of disease, volunteered to exhale with eight flow rates between 10 and 350 mL/s. NO was measured by CLD 88sp NO analyzer (ECO Medics AG, Switzerland). The subjects were grouped according to low, normal and high $F_ENO_{0.05}$. The correction for axial diffusion was applied (Condorelli *et al.* 2004).
**Results:** There was a significant difference (p=0.001) in $C_{aw}NO$ and $D_{aw}NO$ (p=0.001) with the use of 30 instead of 10 mL/s. The $C_ANO$ values were significantly different with the use of 250 instead of 350 mL/s (p=0.001). The three groups had $C_ANO$ of 0.9±0.2, 0.9±0.1 and 0.9±0.3 ppb respectively. With correction of axial diffusion the $C_ANO$ became 0.3±0.2, -0.5±0.2 and -2.7±0.6 ppb with majority of values being negative.
**Conclusion:** The flow independent NO parameters are flow dependent. With the use of the nonlinear model with the HMA approach to calculate $C_ANO$, $D_{aw}NO$ and $C_{aw}NO$ the flow rates to be used use are 10, 100 and 350 for adults. Further studies have to be done in children that cannot perform these low and high flow rates. Most importantly, the axial diffusion correction is of limited value with the HMA method.
Abbreviation: HMA=Högman-Meriläinen algorithm, $C_ANO$= alveolar NO, $C_{aw}NO$=airway wall NO, $D_{aw}NO$=airway transfer factor of NO

**P4781**
**There is no need for complicated equations and mathematical models. Exhaled nitric oxide can be partitioned with two simple exhalations**
Paolo Paredi, Omar Usmani, Katharine Hurt, Neil Pride, Peter Barnes. *Airway Disease, National Heart and Lung Institute, Imperial College, London, United Kingdom*

**Background:** By measuring eNO at multiple flows and applying mathematical models of eNO exchange dynamics, the signal can be partitioned into its proximal airway [Jno (nl/sec)] and distal airway/alveolar contributions [CA(NO) (ppb)]. This method is time consuming, requires at least 3 exhalations and is affected by a number of limitations such as axial diffusion and turbulent flow. We eveloped a more practical method based on the production of NO (Vno) at two exhalation flow rates.
**Methods:** In a group of 27 normal subjects (38±2 yr; 20 male), 13 steroid naive asthmatic patients (34±3 yr; 6 male, FEV1 70±4% predicted), 14 patients with chronic obstructive pulmonary disease (COPD) (65±2 yr; 10 male, FEV1 58±3%), and 12 patients with cystic fibrosis (21±4 yr; 8 male, FEV1 60±3%) we compared CA(NO) and Jno with the variation of total NO production at 50 and 200 ml/s [Vno50-200 (nl/s)]. Vno was measured by calculating the average area under the curve (NO concentration/time) of two successive exhalations at each flow rate.
**Results:** Vno50-200 was strongly correlated with Jnoin normal subjects (r=0.94, p<0.001), asthma (r=0.98, p<0.001), COPD r=0.93, p<0.001), and CF patients (r=0.74, p<0.05). This agreement was confirmed by the Bland and Altman test. Vno50-200 did not correlate significantly with CA(NO) in any of the study groups.
**Conclusions:** The flow dependent component of exhaled NO, which is affected by its bronchial production, can be estimated by measuring Vno50-200. This method is simple, does not require sophisticated equipment or equations and is in agreement with Jno calculated mathematically.

**P4782**
**Can nasal-NO be used to differentiate between primary and secondary ciliary dyskinesia?**
Christina Krantz[1], Andrei Malinovischi[2], Annika Hollsing[1], Kjell Alving[1]. [1]*Women and Childrens Health, Uppsala University Hospital, Uppsala, Sweden;* [2]*Medical Sciences, Clinical Physiology, Uppsala University Hospital, Uppsala, Sweden*

Bakground: Nasal nitric oxide (n-NO) 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 NIOX Mino in 21 patients with clinically diagnosed 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 with PCD while in the group with high n-NO there were both patients with SCD and PCD, including 2 with Kartagener's syndrome. Regarding EM findings, the only 3 subjects with no dyneinarms 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 heterogenous 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 dissociate 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**
**Comparison of alveolar nitric oxide concentrations using two different methods for the assessment of small airways inflammation in asthma**
Daiki Kobayashi, Yoshihiro Tochino, Hiroshi Kanazawa, Yukikazu Ichimaru, Kyoh Shigenori, Kazuhisa Asai, Kazuto Hirata. *Graduate School of Respiratory Medicine, Osaka City University, Osaka City, Osaka Prefecture, Japan*

**Background and objectives:** Fractional exhaled nitric oxide ($F_ENO$) is considered a potentially useful biomarker to detect airway inflammation. Two-compartment model (2CM) of pulmonary NO dynamics has been used for the evaluation of bronchial NO flux ($J'aw_{NO}$) and peripheral airway/alveolar NO concentration ($CA_{NO}$) in asthmatic patients. Recently, the trumpet shape of airway tree and axial diffusion model (TMAD) has been reported as the correction of 2CM. This study was designed to determine the validity of measurement of $CA_{NO}$ using the TMAD model for the assessment of small airways obstruction in asthma
**Methods:** A total of 52 asthmatic patients and 12 normal controls were included in this study. Methacholine inhalation challenge testing, pulmonary function tests, sputum induction, and exhaled NO measurements at several flow rates were performed. And then $J'aw_{NO}$ and $CA_{NO}$ were calculated using both the 2CM ($CA_{NO\ 2CM}$, $J'aw_{NO\ 2CM}$) and the TMAD model ($CA_{NO\ TMAD}$, $J'aw_{NO\ TMAD}$) respectively.
**Results:** Both $J'aw_{NO}$ and $CA_{NO}$ were significantly higher in asthmatic patients than normal controls. $CA_{NO\ 2CM}$ was significantly correlated with $FEV_1$/FVC (r=-0.35, p=0.01), $FEF_{25-75}$ (r=-0.45, p<0.001), and sputum eosinophils (r=0.32, p=0.02). In contrast, $CA_{NO\ TMAD}$ was significantly correlated with $FEF_{25-75}$ alone (r=-0.42, p=0.002), and not with $FEV_1$/FVC or sputum eosinophils.
**Conclusions:** $CA_{NO\ TMAD}$ is more selective as an indicator of small airways obstruction than $CA_{NO\ 2CM}$. Assessment of small airways obstruction using the TMAD model may reveal the role of the small airways in the pathogenesis of asthma.

**P4784**
**Exhaled breath temperature increases at COPD exacerbation and correlates with sputum neutrophilia**
Zsófia Lázár, András Bikov, Gabriella Gálffy, Márta Orosz, György Losonczy, Ildikó Hováth. *Dept. of Pulmonology, Semmelweis University, Budapest, Hungary*

**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 I) and after systemic steroid and/or antibiotic treatment at recovery (7.6±1.4 days after). At visits

EBT was recorded with a breath thermometer (X-Halo, Delmedica Investments Ltd, Singapore), spirometry was done, patients completed the COPD Assessment Test (CAT) and the first spontaneous sputum in the morning was collected and processed (Yamamoto C et al. Chest 1997). Paired t-test and Spearman correlation were used.
**Results:** EBT at exacerbation was higher compared to that at recovery (34.42±0.73°C vs. 34.03±0.56°C, p=0.03). Sputum neutrophil percentage at exacerbation showed a positive correlation with EBT (r=0.78, p=0.02), and fell after treatment (57±12% vs. 39±18%, p=0.04). EBT at exacerbation was not related to spirometric variables or CAT score.
**Conclusion:** EBT rises at acute exacerbation of COPD and it is associated with increased airway inflammation. Measuring exhaled breath temperature might be useful for monitoring airway inflammation in COPD.
The study was supported by Hungarian Respiratory Society grant (to Zsófia Lázár) and OTKA 68808.

**P4785**
**Neutrophilic airways inflammation in lung cancer: The role of exhaled LTB-4 and IL-8**
Giovanna E. Carpagnano, Donato Lacedonia, Pierluigi Bonfitto, Stefano Notarangelo, Mariella Grimaldi, Gianluca Ciliberti, Maria P. Foschino Barbaro. *Occupational and Medical Sciences, Section of Respiratory Diseases, University of Medicine, Foggia, Italy*

Recent advances in lung cancer biology presuppose its inflammatory origin. In this regard, LTB-4 and IL-8 are recognized to play a crucial role in neutrophil recruitment into airways during lung cancer.
Notwithstanding the intriguing hypothesis, the exact role of neutrophilic inflammation in tumor biology remain complex and not completely known.
The aim of this study was to give our contribution in this field by investigating LTB-4 and IL-8 in the breath condensate of NSCLC patients and verifying their role in cancer development and progression.
We enrolled 50 NSCLC patients and 25 controls. LTB-4 and IL-8 concentrations were measured in the breath condensate and the blood of all the subjects under study using EIA kits. Thirty NSCLC patients and ten controls underwent induced sputum collection and analysis.
LTB-4 and IL-8 resulted higher in breath condensate and the blood of NSCLC patients compared to controls. Significantly higher concentrations were found as the cancer stages progressed. A positive correlation was observed between exhaled IL-8 and LTB-4 and the percentage of neutrophils in the induced sputum.
**Conclusion:** The high concentrations of exhaled LTB-4 and IL-8 showed the presence of a neutrophilic inflammation in the airways of NSCLC patients and gave a further support on the inflammatory signalling in lung cancer. These exhaled proteins could represent a suitable non -invasive marker in the diagnosis and monitoring of lung cancer.

**P4786**
**HPV in exhaled breath condensate of lung cancer patients**
Giovanna E. Carpagnano, Elio Costantino, Donato Lacedonia, Rossella Decembrino, Viviana Turchiarelli, Gerardo Rago, Maria P. Foschino Barbaro. *Occupational and Medical Sciences, Section of Respiratory Diseases, University of Medicine, Foggia, Italy*

**Background:** A recent intriguing carcinogenetic hypothesis for lung cancer foresees its viral aetiology. The human papilloma virus (HPV) is the main virus actually recognized in the pathogenesis of lung cancer. The aim of this study was to investigate, for the first time to our knowledge, the presence of HPV in the exhaled breath condensate (EBC) of lung cancer patients.
**Material and methods:** We enrolled 79 patients affected by lung cancer and 68 controls. HPV were investigated in their EBC and paired bronchial brushing through genotyping.
**Results:** We were able to detect HPV in the EBC and bronchial brushing. We described the presence of HPV infection in 16.4% of the subjects affected by non-small cell lung cancer, but in none of the controls. HPV 16 and 31 turned out to be the most widespread genotypes. The HPV positivity in airways as well as in the smoking habit was seen to independently increase the individual's susceptibility to developing lung cancer.
**Conclusion:** In conclusion, we demonstrated the possibility to identify an HPV infection in the EBC of lung cancer patients, and supported the notion that the EBC is a suitable tool to study airways colonization. Although further studies are needed to confirm our results, we retained the study of HPV in EBC very interesting in terms of future programs involving lung cancer screening.

**P4787**
**Could exhaled ferritin and SOD be used as markers for lung cancer and prognosis prediction purposes?**
Giovanna E. Carpagnano, Anna Koutelou, Annarita Depalo, Laura Bergantino, Cinzia Ruggeri, Donato Lacedonia, Maria P. Foschino Barbaro. *Occupational and Medical Sciences, Section of Respiratory Diseases, University of Medicine, Foggia, Italy*

Today an increasing interest is being generated by the study of lung cancer markers in the exhaled breath condensate as this sample seems to lend itself to lung cancer early-screening and follow-up. Ferritin and SOD have recently been recognized to play a role in lung cancerogenesis and patients' survival.
**Purpose:** The aim of this study was to evaluate the clinical value and the prognostic power of exhaled ferritin and exhaled SOD in lung cancer patients.
**Material and methods:** 40 NSCLC patients and 15 controls were enrolled in the study. All subjects under study underwent exhaled breath condensate collection and analysis of ferritin and SOD. A total of 36 patients were either given a follow-up of at least 102 weeks, or were followed up until death.
**Results:** Exhaled ferritin and SOD resulted higher in NSCLC than in controls and influenced by the stage of cancer. A pronounced survival difference was found in the presence of exhaled ferritin 300 ng/ml and exhaled SOD >13.5 U/μl.
**Conclusions:** In conclusion, although results need to be confirmed on a larger population, we supposed that the measure of ferritin and SOD in the EBC could be clinically suggested in the monitoring of lung cancer and as an outcome predictor.

**P4788**
**Volatile organic compounds (VOCs) in COPD patients with exacerbation**
Vasiliki Besa[1], Kaid Darwiche[1], Helmut Teschler[1], Urte Sommerwerck[1], Isabella Kurth[1], Thomas Rabis[1], Jörg Ingo Baumbach[2], Lutz Freitag[1].
[1]*Pulmonology, Ruhrlandklinik, University Hospital Essen, Essen, Germany;*
[2]*KIST Europe, University of Saarland, Saarbruecken, Germany*

**Introduction:** COPD exacerbations are events in the natural course of the disease characterised by change in patients' baseline symptoms which are beyond normal day to day variability and may warrant medical treatment. The early diagnosis and treatment of acute exacerbations is of major importance since they have a given impact on the morbidity, mortality and health care costs associated with COPD.
**Objectives:** It is well known that the airway inflammation is enhanced at exacerbations. The purpose of this study is to investigate if the volatile organic compounds (VOCs) detected in the exhaled breath of patients with an acute exacerbation of COPD are different from those detected in patients with a stable disease.
**Methods:** Breath samples were collected via a side-steam 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 from patients with stable disease.
**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.
**Conclusions:** 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.

**P4789**
**Targeted eicosanoid lipidomics of induced sputum as compared to exhaled breath condensate in asthmatics**
Marek Sanak, Lucyna Mastalerz, Anna Gielicz, Barbara Sokolowska, Natalia Celejewska-Wójcik, Andrzej Szczeklik. *Medicine, Jagiellonian University Medical College, Kraków, Poland*

Eicosanoids are mediators of arachidonic acid pathway. Induced sputum (IS) is a non-invasive material from the lower airways; its sampling is well standardized. Advantage of exhaled breath condensate (EBC) is shorter collection time and low protein content, but this is compromised by extreme dilution and inter-individual variation. Both matrices have been introduced for assessment of inflammatory mediators in asthma.
We compared eicosanoids concentration in IS and EBC samples collected according to the current guidelines and estimated redistribution of eicosanoids and their metabolites as a result of sample processing.
EBC was collected from asthmatics using Jaeger ECO Screen I; IS according to the most recent ERS Task Force recommendations. The same validated quantitative mass spectrometry was used for both matrices ecosanoids' measurements. Random IS samples were split to test enzymatic inhibition during solubilisation.
We quantified 29 eicosanoids, including major prostaglandins, leukotrienes, and their metabolites. Average concentration of eicosanoids was 82 [1-400] times lower in EBC than in IS. IS differed form EBC by higher HETE and undetectable LTC4. Conversion of LTB4 into 5-oxo-LTB4 and increase of tetranor-PGEM was observed during IS solubilisation, non-physiological pH prevented these redistributions only partially.
Although processing of IS shifts eicosanoid profile toward metabolites, significant amounts of mediators are present within detection levels of common immunoassays. A strict adherence to the IS collection protocols is recommended to avoid a pre-analytical bias.
In collaboration with U-BIOPRED within the Innovative Medicine Initiative.

**P4790**

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

Thomas Rabis[1], Olaf Anhenn[1], Jörg-Ingo Baumbach[2], Isabella Kurth[3], Gerhard Weinreich[1], Helmut Teschler[1], Lutz Freitag[3], Kaid Darwiche[3], Uwe Mellies[4], Peter-Michael Rath[5], Urte Sommerwerck[1]. *[1]Dept. of Pneumology - Lung Transplantation, Ruhrlandklinik - West German Lung Centre at the University Clinic Essen, Essen, Germany; [2]Microfluidics & Clinical Diagnostics, KIST Europe, Campus E71, Saarbrücken, Germany; [3]Dept. of Interventional Pneumology, Ruhrlandklinik - West German Lung Centre at the University Clinic Essen, Essen, Germany; [4]Dept. of Pediatric Pulmonology, Sleep Medicine and Cystic Fibrosis Center, University Clinic Essen, Essen, Germany; [5]Institute of Medical Microbiology, University Clinic Essen, Essen, Germany*

**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 inpatient stays 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 (Pac,n=3) or infected (Pai,n=9) by *Pa* (Pac+Pai=Pa+,n=12) compared to hc (n=39) and compared Pac with Pai. In addition we analysed VOCs from *Pa* cultures growing on agar plates.
**Results:** Using IMS for VOC analysis, differences between Pa+ and hc could be found. Different peaks were detected between Pac and hc, Pai and hc as well as Pac and Pai. VOC analysis from *Pa* cultures 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 colonised or infected with *Pa* seems to be possible, as well. However, 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.

**P4791**

**Metabolomic analysis of exhaled breath condensate in diagnostics of obstructive airway diseases**

T.N. Anokhina[1], E.K. Anaev[1], A.A. Rodionov[2], A.I. Revelsky[2], I.A. Revelsky[2], V.B. Kudriavtsev[3], A.G. Chuchalin[1]. *[1]Clinical Department, Research Institute of Pulmonology, [2]Chemistry Faculty, M.V. Lomonosov Moscow State University, [3]Faculty of Mechanics and Mathematics, M.V. Lomonosov Moscow State University, Moscow, Russian Federation*

Metabolomic 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-phenoxyethanol, decanol-1, ethyl citrate, 2,3–dihydro-1-H-inden-1-on 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:** Metabolomic 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.

**P4792**

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

Anna Ryabokon[1], Viktoria Kurova[1], Eldar Anaev[2], Aleksey Kononikhin[3], Kristina Fedorchenko[1], Eugenie Nikolaev[1], Sergey Varfolomeev[1]. *[1]Department of Kinetics and Mechanisms of Enzymatic and Catalytic Reactions, Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, [2]Noninvasive Diagnostic Department, Research Institute of Pulmonology, Federal Medicobiological Agency of Russia, [3]Department of Ion and Moleculare Physics, Institute for Energy Problems of Chemical Physics, Moscow, Russian Federation*

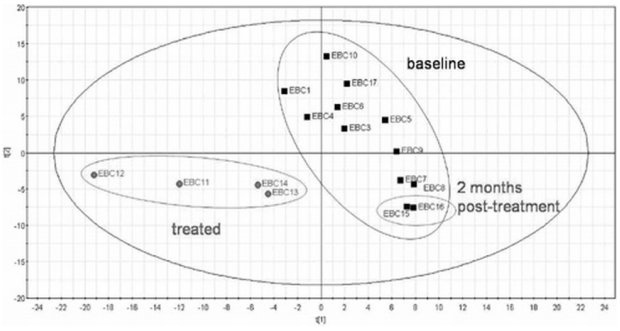
Exhaled breath condensate (EBC) reflects the composition of the airway-lining fluid and may contain biomarkers of diseases of respiratory system.
The aim of this study is to identify proteins in EBC of patients with chronic obstructive pulmonary disease (COPD) and pneumonia using two techniques of proteome identification.
Seventeen 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-PAAGE 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-PAAGE 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" keratins 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, Dynein, Collagen, Hornerin were discovered in COPD and pneumonia EBC samples. They are uncharacteristic of healthy EBC samples. None of these proteins was identified as a whole in 2D-PAA 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.

**P4793**

**NMR EBC metabonomic to assess the nutricecutic effect in COPD. A pilot study of oral administration of a curcumin based herbal preparation**

Guglielmo de Laurentiis[1,4], Giovanni Scapagnini[2], Deborah Paris[3], Dominique Melck[3], Mauro Maniscalco[1], Andrea Motta[3], Sofia Matteo[1], Andrea Bianco[2]. *[1]Dept. of Respiratory Medicine, University "Federico II" of Naples, Naples, Italy; [2]Dept. of Health Sciences, University of Molise, Campobasso, Italy; [3]Institute of Biomolecular Chemistry, National Research Council, Pozzuoli, Italy; [4]Dept. of Medicine, Rehabilitation Center, Santa Maria del Pozzo, Somma Vesuviana, Italy*

**Background:** NMR metabonomic of EBC significantly divide healthy from COPD patients, identifying specific profiles linked to cellular oxidative pattern.Curcumin is a recognized anti-oxidant agent in biochemical cellular balance.As it is still unknown if the pattern recognized by NMR metabonomic spectroscopy in COPD is sensitive to exogenous nutrients, in this preliminary open label study we evaluated the effect of daily oral administration of an herbal preparation containing high dosage of curcumin (HPC) on EBC in COPD patients.
**Materials and methods:** 10 COPD (8 M, age 63.3±7.9) were evaluated at baseline (T0) and after 12 weeks (T1) of oral administration of HPC once a day collecting each visit EBC, clinical score (BSCN) and spirometry.
**Results:** EBC collected from each patient (T0 and T1) were different in NMR spectra profiles and in comparison with spectra of a HPC. Notably, a completely different profile is observed in T1 patients, with many new resonances appearing. Moreover, 2 patients examined two months after T1 demonstrated EBC profiles similar to those at baseline.




Among clinical parameters BSCN score was 4.9±0.9 at T0 and 7.5±1.0 at T1, while FEV1 values resulted slightly but not significantly increased at T1.
**Conclusions:** NMR metabonomic is a sensitive method to explore nutriceutic effects of exogenous antioxidant on EBC in patients affected by COPD.

**P4794**

**Development of mass spectrometry approaches to quantifying lipid mediators in airway disease**

David Balgoma[1], Susanna L. Lundström[1], Anders Eklund[2], Johan Grunewald[2], Craig E. Wheelock[1]. *[1]Department of Medical Biochemistry and Biophysics, Division of Chemistry 2, [2]Division of Respiratory Medicine, Department of Medicine, Karolinska Institutet, Stockholm, Sweden*

**Background:** Lipid mediators (LMs, such as prostaglandins and leukotrienes) have been extensively studied for their role in both the onset and resolution of inflammation in airway diseases. We report here the development of a state-of-

the-art quantitative lipidomics-based method for the analysis of LMs using Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS).
**Aims:** Develop a sensitive LC-MS/MS platform to quantify LMs from multiple biological matrices including urine, bronchoalveolar lavage fluid (BALF) and sputum. The method should encompass LMs from arachidonic (AA), linoleic (LA), dihomo-γ-linolenic (DGHLA), α-linolenic (α-LA), eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acid pathways.
**Methods:** Pooled BALF from patients with sarcoidosis (n=5) was extracted by Solid Phase Extraction and analytes were quantified by LC-MS/MS (Acquity-Xevo, 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.

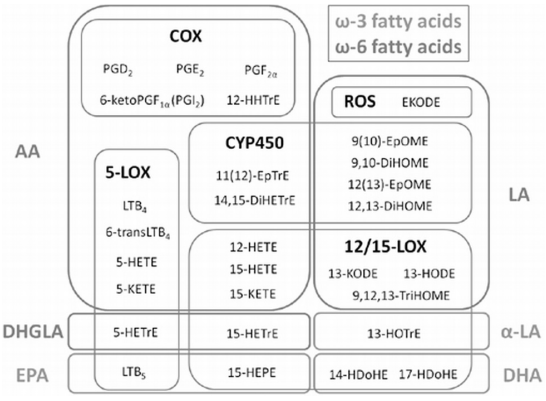


Figure 1

**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.

**P4795**
**Follow up of lung transplant recipients using electronic nose**
Dorottya Kovacs, Andras Bikov, Judit Pako, Zsuzsanna Kovats, Zoltan Sutto, Veronika Muller, Gabriella Murakozy, Gyorgy Losonczy, Ildiko Horvath.
*Pulmonology, Semmelweis University, Budapest, Hungary*

**Background:** The close follow up of lung transplant patients is essential in the recognition of both early and late complications, however today these can be confirmed only by invasive techniques. Analysis of exhaled breath might be promising, as various studies showed relationship between breath components and the development of BOS in lung transplant recipients. Electronic nose is able to analyse the molecular pattern of breath (breathprint), and it discriminated different lung diseases successfully, however lung transplant patients were not studied yet.
**Aim:** To investigate the relationship between breathprint and various clinical parameters in lung transplant recipients.
**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±2.5 samples per patient were analysed. During their scheduled follow up at the outpatient clinic exhaled breath collection for E-nose measurement (Cyranose 320) as well as blood test and lung function was performed. Breathprints were analysed using principal component analysis and the relationship between breathprint and clinical parameters was assessed by linear regression.
**Results:** A significant relationship was detected between the breathprint and $MEF_{50}$ as well as between breath pattern and tacrolimus plasma levels ($p<0.05$) which was confirmed by the validation set.
**Conclusions:** The analysis of exhaled breath can be useful in the follow-up of lower airway obstruction 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.

**P4796**
**Profiling of sputum inflammatory mediators in asthma and chronic obstructive pulmonary disease**
Mona Bafadhel[1], Margaret McCormick[2], Shiron Saha[3], Vijay Mistry[1], Susan McKenna[1], Paul Dodson[2], Andrew Lloyd[2], Paul Rugman[2], Paul Newbold[2]. *[1]Institute for Lung Health, University of Leicester, Leicester, United Kingdom; [2]AstraZeneca, R & D, Loughborough, United Kingdom; [3]Respiratory Medicine, Sheffield University Hospitals NHS Trust, Sheffield, United Kingdom*

**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, IFN-g, TNFRI, 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 subphenotypes 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.