## 417. Bronchial hyperresponsiveness and exhaled and sputum biomarkers

P4031 Efficiency of a laser-based sensor for FeNO measurements and multiple flows analysis Julien Mandon<sup>1</sup>, Peter J.F.M. Merkus<sup>2</sup>, Simona M. Cristescu<sup>1</sup>, Frans J.M. Harren<sup>1</sup>. <sup>1</sup>Life Science Trace Gas Research Group, Molecule and Laser

Physics, Institute for Molecules and Materials, Nijmegen, Netherlands; <sup>2</sup>Division of Respiratory Medicine, Dept of Paediatrics, Radboud University Medical Centre, Nijmegen, Netherlands

Fractional exhaled nitric oxide (FeNO) is a useful indicator in the diagnostic and management of asthma in children. Up to now, despite the availability of standardized ATS/ERS recommendations, numerous ways to collect breath samples and several NO sensors have been reported. The aims of this study were to compare different analyzers by measuring the FeNO in asthmatic children and to calculate the NO parameters in healthy people by using NO sampling at various expiratory flow rates.

A laser-based sensor with sub-ppb (sub-part-per-billion by volume) detection limit [1] was compared with two market sensors, a chemiluminescent analyzer (model 280, Sievers) and a portable hand-held electrochemical analyzer (MINO<sup>®</sup>, Aerocrine AB), respectively. FeNO from 20 children (6-16 years of age) diagnosed with asthma and treated with inhaled steroids was simultaneously measured with these devices. The data analysis was used to validate the accuracy, precision, sensitivity and reproducibility of the optical sensor. The finding shows that FeNO values are comparable between the different analyzers. However, the variability of the electrochemical analyzer should be considered for clinical decisions as changing current treatment.

NO values from three expiratory flows (10, 100 and 300 mL.s<sup>-1</sup>) from healthy people were used to calculate the flow-independent parameters NO, i.e. the alveolar region, airway wall, diffusing capacity and flux with the linear and non-linear models, respectively. This study tries to provide guidelines to the clinical physician about the measuring techniques for FeNO, as well as the sampling flow rates to be considered.

#### **Redference:**

1. Cristescu et. al. Appl. Phys. B 92: 343-349 (2008)

#### P4032

# The impact of diurnal variations, atopy, pollen exposure and pharmacotherapy on exhaled nitric oxide levels

Alica Bencova<sup>1</sup>, Martina Antosova<sup>2</sup>, Eva Rozborilova<sup>1</sup>. <sup>1</sup>Clinic of Pneumology and Phtisiology, Jessenius Faculty of Medecine, Comenius University, Martin, Slovakia (Slovak Republic); <sup>2</sup>Institute of Pharmacology, Jessenius Faculty of Medecine, Comenius University, Martin, Slovakia (Slovak Republic)

Objectives and aims: The aim of the study was to investigate the impact of diurnal variations in healthy subjects and impact of atopy, pollen exposure and pharmacological treatment on exhaled nitric oxide levels (eNO) in patients with allergic rhinitis (AR).

**Methods:** eNO levels were measured using analyzer NIOX. Measurements of eNO were performed in 81 nonasthmatics with seasonal AR outside and during the pollen season, before and 3 weeks after treatment and in 52 healthy controls in 4-hour intervals.

**Results:** Diurnal variations of eNO in healthy individuals were not confirmed. Patients with AR had significantly higher levels of eNO than healthy controls not depending on pollen season or pharmacotherapy. Increased eNO levels (p<0,0001) were also found in patients with AR during the pollen season (21,25 ppb; IQR=20,3) compared to the levels outside the season (14,2 ppb; IQR=12,45) before treatment. In AR patients treated by nasal corticosteroids and antihistamines in the season were levels of eNO (18,6 ppb; IQR=14,63) significantly lower (p=0,044) than in the season before treatment (23,15 ppb; IQR=16,63). No difference was found in eNO levels in AR patients outside the season (14,2 ppb; IQR=13,38) and in the season after treatment (16,1 ppb; IQR=15,6).

**Conclusion:** This study has shown that patients with AR have significantly higher levels of eNO compared to healthy subjects and the levels of eNO increasing after pollen exposure. Application of topic corticosteroids and antihistamines caused significant decrease of eNO (almost to the starting levels) in the pollen season and depressed suspicious inflammation in the lower airways. Support: VEGA 1/0055/08, Grant MZ 2007/46 UK 11

#### P4033

# Variability of sputum inflammatory mediators in alpha-1-antitrypsin deficiency and usual COPD

Helen Stone<sup>1</sup>, Gillian McNab<sup>2</sup>, Robert Stockley<sup>1</sup>, Elizabeth Sapey<sup>2</sup>. <sup>1</sup>ADAPT Project/Department of Lung Function and Sleep, Queen Elizabeth Hospital Birmingham, Birmingham, United Kingdom; <sup>2</sup>Cardiovascular and Respiratory Medicine, School of Medical and Dental Science, University of Birmingham, Birmingham, United Kingdom

Introduction: There is inherent variability in the concentrations of inflammatory mediators in stable state sputum of usual COPD patients. Patients with alpha-l-antitrypsin deficiency (ATD) have a similar spectrum of lung disease and more inflammation but variability has not been assessed. Evidence for the efficacy of treatment in A1AT is lacking; proof of concept (POC) studies indicate that augmentation increases AAT levels and reduces local mediator release. Our aims were to assess the daily variability of mediators in sputum in ATD, compared to usual COPD and to study the effects of sequential sampling to determine sample size for POC studies.

Methods: Interleukin 8 (IL8), myeloperoxidase (MPO) and leukotriene B4 (LTB4) were measured in the stable state spontaneous sputum of 12 patients with ATD

and 12 usual COPD patients on 9 days over 1 month. The intra-patient variability was calculated (CV), compared between the groups, and the effects of combining results from multiple days for each patient were assessed.

**Results:** There was significant daily variability in all mediators, which was greater in usual COPD, despite IL8 and LTB4 concentrations being higher (p<0.01) in ATD (medians 11.29 vs 3.72 nM; and 12.16 vs 6.10 nM respectively). 3 or 5-day rolling means reduced the median CV in both groups compared to a single days' data (p<0.01) and reduced the number needed to show a 50% reduction in mediator as part of a POC study.

**Conclusion:** There is greater variability in usual COPD than ATD; though mediator levels were higher in ATD. Sequential sampling reduced intra-patient variability in both groups. Averaging 3 consecutive samples per patient was optimal.

### P4034

# The application of mass spectrometry to the analysis and characterization of protein and peptide composition in exhaled breath condensate of pneumo-oncological diseased persons

Guzel Kireeva<sup>1</sup>, Anna Ryabokon<sup>1</sup>, Aleksey Kononikhin<sup>2</sup>, Vladimir Bagrov<sup>4</sup>, Oleg Pikin<sup>4</sup>, Evgenij Nikolaev<sup>2</sup>, Eldar Anaev<sup>3</sup>, Sergey Varfolomeev<sup>1</sup>. <sup>1</sup>Department of Kinetics and Mechanisms of Enzymatic and Catalytic Reactions, Emanuel Institute of Biochemical Physics, Moscow, Russian Federation; <sup>2</sup>Department of Ion and Moleculare Physics, Institute for Energy Problems of Chemical Physics, Moscow, Russian Federation; <sup>3</sup>Department of Oncological Biomarkers, Research Institute of Pulmonology, Moscow, Russian Federation; <sup>4</sup>Department of Pulmonary Oncology, Herzen Oncological Research Institute, Moscow, Russian Federation

In recent years exhaled breath condensate (EBC)has been investigated more and more extensively as a matrix that reflects the composition of the airway-lining fluid and may contain biomarkers of diseases of respiratory system.

The aim of this study is to compare identify proteins and peptides in EBC samples collected from two group of people with healthy pulmonary system and with verified oncological diseases patients of pneumonic system from Moscow Scientific Research Oncology Institute with using mass spectrometry, as well as to compare proteome identification.

Bathes were collected using R-Tube, freeze dried, treated by trypsin and analyzed by nanoflow LC-MS/MS with a 7-Tesla Finnigan LTQ-FT mass spectrometer (Thermo Electron, Germany), by means of Bioworks Browser 3.1 SR1 (Thermo Electron, Germany) were generated list of direct peptide mass and mass of their fragments, with following identification of proteins in the databases thought Mascot (Matrix Science version 2.0.04, the UK). At collected samples were identified peptides from more that 40 proteins of different nature, e.g. keratins and non-keratins. Peptides of nuclear ubiquitous casein and cyclin-dependent kinases substrate (NUCKS) which located in the nuclei in proliferating cells, glutamine synthetase (GS)-an enzyme that plays an essential role in the metabolism of nitrogen, alpha 1-Antitrypsin (A1AT) is a protease inhibitor belonging to the serpin superfamily were discovered in disease EBC samples. They are uncharacteristic of healthy EBC samples. In conclusion, each of abnormal peptides, as well as their combinations, may have diagnostic value.

#### P4035

Circadian variation of exhaled breath temperature in healthy subjects Tanya Kralimarkova, Miroslava Rasheva, Tanya Grigorova, Zlatko Dimitrov, Dimitar Tihomirov, Roxana Mincheva, Vasil Dimitrov, Todor Popov. Medical University Sofia, Clinic of Allergy and Asthma, Sofia, Bulgaria

**Background:** Evaluation of the exhaled breath temperature (EBT) has been suggested as surrogate biomarker of airway inflammation, but there is no data on its circadian variation in health and disease. Measuring it by portable handheld device has been proven to be precise and highly reproducible. The aim of the study was to identify peaks and troughs in EBT around the clock in healthy individuals.

**Methods:** Forty two subjects (24 women; median age 26 years, age range 3 to 80 years) without history and objective signs of respiratory disease volunteered for the study. Subjects and/or their parents were trained to measure EBT with a portable hanheld device (X-halo, Delmedica, Singapore). They took it home and were instructed to do measurements at four time points ( $\pm$ 30 min.): 7, 13, 19 and 1 hours (h). All values were stored in the memory of the devices and were subsequently retrieved and analyzed by the research team. Axillary temperature (AxT) was measured and analyzed in parallel.

**Results:** EBT values showed a circadian pattern different from the one of AxT: the acrophase (peak temperature) was registered at 19 h for EBT and at 13 h for AxT. The bathyphase (trough temperature) was the same for both circadian rhythms at 1 h. Repeated measures analysis found both circadian fluctuations to be statistically significant (table):

	7 h	13 h	19 h	1 h	Р
EBT, °C	33,78±0,17	33,57±0,28	33,84±0,22	33,42±0,24	< 0,001
AxT, ℃	$36,15{\pm}0,07$	36,37±0,06	$36,33{\pm}0,06$	$36,10{\pm}0,08$	< 0,01

**Conclusions:** Healthy subjects EBT have a different circadian course during the day and night compared with AxT. EBT circadian influences need also to be identified in inflammatory airway diseases.

#### P4036

# Exercise test with dry air inhalation compared to mannitol test as marker of exercise induced asthma

Kai-Håkon Carlsen<sup>1,2,3</sup>, Leif Bjermer<sup>4</sup>, Barbro Dahlén<sup>5</sup>, Tommy Ekström<sup>6</sup>, Gunilla Hedlin<sup>7</sup>, Trine Stensrud<sup>1</sup>, Leif Jörgensen<sup>6</sup>, Kjell Larsson<sup>8</sup>. <sup>1</sup>Section of Sport Medicine, Norwegian School of Sport Sciences, Oslo, Norway; <sup>2</sup>Dept. of Paediatrics, Oslo University Hospital, Oslo, Norway; <sup>3</sup>Institute of Clinical Medicine, University of Oslo, Oslo, Norway; <sup>4</sup>Dept. of Resiratory Med. & Allergology, Lund University Hospital, Lund, Sweden; <sup>5</sup>Dept. of Medicine, Division of Respiratory Med. and Allergy, Karolinska University Hospital Huddinge, Karolinska Institutet, Stockholm, Sweden; <sup>6</sup>Astra Zeneca Nordic, Södertälje, Sweden; <sup>7</sup>Dept. of Women's and Children's Health, Centre for Allergy Research, Karolinska Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

**Objective:** To compare exercise test with dry air inhalation and mannitol test to discriminate between different asthma treatments.

**Methods:** Exercise test with dry air inhalation (EIA test) (Aiolos bronchial challenge<sup>®</sup>, Sweden) was compared to inhaled mannitol test in a randomized trial (NCT 00989833) on budesonide/formoterol (B/F) as needed (n=23), budesonide (B; n=21) once daily, terbutaline (T) as needed (n=22) on exercise induced asthma (EIA) in mild asthmatic adults and adolescents. EIA test: 6 minutes treadmill run at 90% of max aerobic capacity; FEV1 measured before, and 0, 5, 10, 15, 30, 45 minutes after exercise. EIA test was positive with max fall in FEV1  $\geq$  10% of baseline. Dry powder mannitol was inhaled in cumulative doses from 5 - 635 mg, to find the dose causing 15% FEV1 fall (PD15). EIA test was performed at trial start, after 3 and 6 weeks, mannitol test at trial start and after 6 weeks.

**Results:** Mean max FEV1 fall after EIA test was 16.34% at baseline and 13.11% after 6 weeks treatment. There was a significant improvement in B/F ( $\Delta$ FEV1: LS mean (95% CI) -5.4 (-8.93, -1.83) and B (-6.6 (-10.3, -2.96)) groups after 6 weeks, but not in the T group (+1.48 (-2.1, 5.09)). The mannitol test was positive (PD15<635 mg) in only 31/66 subjects at start, and in 22 at 6 weeks. All 66 subjects had a positive EIA test.

**Conclusion:** All patients had positive EIA test at baseline. The response to the EIA test improved significantly in two of the treatment groups (B/F and B) and discriminated between the treatments. The mannitol test was positive in less than 50% of the subjects, and could not be used as outcome in the present study. The study was sponsored by AstraZencca.

#### P4037

# Evolution of cellular inflammatory pattern in induced sputum in patients with mild-moderate asthma for five years

Elena Forcen, Elisabet Vera, Angela Fernandez, Jose Angel Carretero, Juan Antonio Domingo, Enrique Chacon, Salvador Bello. *Pulmonology, Hospital Universitario Miguel Servet, Zaragoza, Spain* 

**Background:** Prospective study of cellular inflammatory pattern in samples of induced sputum can identify inflammatory changes in the natural history of asthma. The aim of our study is to know whether there are modifications of cellular inflammatory pattern in mild-moderate asthma along 5 years.

Materials and methods: The patients with mild- moderate asthma were studies along 5 years.

The outcomes were: lung function FEV1 (% pred), no. exacerbations/year (EY), use of relief therapy (RT), dose of inhaled corticosteroid/day (ICs), bacterial and viral cultures and total and differential cell count in induced sputum.

Cellular inflammatory pattern was classified as eosinophilic (>2% of eosinophils), neutrophilic (>61% of neutrophils), paucigranulocytic (<2% eosinophils and < 61% neutrophils) or mixed (>2% eosinophils and > 61% neutrophils).

**Results:** The study began with 24 patients and in 18 we repeated the induced sputum 5 years after. The profitability were of 100%. 55% showed the same cellular inflammatory pattern in the sputum (11% eosinophilic, 11% neutrophilic y 33% paucigranulocytic). In 45% were found out differents patterns; 11% being forms (paucigranulocytic), 22% agressive forms (neutrophilic, eosinophilic o mixted) and a 11% changed the inflammatory pattern but with agressive forms too.

**Conclusions:** In stable patients with mild- moderate asthma, the most recurrent inflammatory pattern is thepaucigranulocytic. The positivity of bacterial cultures in sputum may be the cause of the changes in the induced sputum to agressive forms. Nonsignificant changes towards neutrophilic patterns have been observed that warrant further study in larger groups.

### P4038

# Safety and efficacy of inhaled mannitol as a bronchial provocation test in children

Ioanna Vasilopoulou, Michael Anthracopoulos. Pediatric Respiratory Unit, University Hospital of Patras, Patras, Greece

**Background:** Bronchial provocation with inhaled mannitol is a new and simple test for osmotic bronchial challenge. The aim of this study was to determine the efficacy and safety of inhaled mannitol in asthmatic children.

**Methods:** Sixty five children (54% males) aged 6-12 years, with established diagnosis of asthma, were studied. An asthma control questionnaire (C-ACT) was completed before bronchial provocation. A commercial preparation of dry powder

mannitol was administered in progressively increasing doses according to the standard protocol, and the FEV<sub>1</sub> was measured 60 seconds after each dose. The procedure ceased when a 15% fall in FEV<sub>1</sub> was achieved (positive challenge) or the cumulative dose of 635 mg was reached (negative challenge). In case of a positive provocation, the dose of mannitol (mg) to provoke a 15% fall in FEV<sub>1</sub> (PD<sub>15</sub>) was calculated. Adverse events during and 2 hours after the test were also monitored.

**Results:** Twenty four children (36.9%) were positive to the mannitol challenge, with a maximum mean% FEV<sub>1</sub> fall of 18.7±4.1%, and a median PD<sub>15</sub> of 125 mg (range 18 to 470 mg). There was a significant correlation between PD<sub>15</sub> and C-ACT score (r = 0.58, p < 0.001). The most common adverse events were: cough (83.1% - 100% in those with a positive challenge), nausea (9.2%), headache (4.6%), and abdominal pain (1.5%). No adverse effect was present at 2 hours after test.

**Conclusion:** Bronchial challenge with mannitol is safe and correlates well with the level of asthma control in asthmatic children.

### P4039

# Comparison of two devices and two breathing patterns for exhaled breath condensate sampling

Charles Rembert Kozulla<sup>1</sup>, Eva Hüttmann<sup>1</sup>, Timm Greulich<sup>1</sup>, Akira Hattesohl<sup>1</sup>, Sarah Noeske<sup>1</sup>, Severin Schmid<sup>1</sup>, John Gerrit<sup>2</sup>, Rudolf Jörres<sup>3</sup>, Bernd Müller<sup>1</sup>, Claus Vogelmeier<sup>1</sup>. <sup>1</sup>Department of Internal Medicine, Division for Pulmonary Diseases, Philipps University Marburg, Marburg, Hessen, Germany; <sup>2</sup>Comprehensive Pneumology Center, Institute of Lung Biology and Disease, Helmholtz Zentrum München, München, Bayern, Germany; <sup>3</sup>Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, Ludwig Maximilian University, München, Bayern, Germany

Background: Analysis of exhaled breath condensate (EBC) provides a noninvasive access to the lung epithelial lining fluid.

**Aim:** Comparison of two commercially available portable devices (RTube, ECo-Screen turbo), and evaluation of different breathing patterns with regard to marker proteins and the source of EBC in healthy subjects.

**Methods:** EBC was collected from 10 subjects 4 times in a cross-over design, for each device once during tidal breathing and once hyperventilating. Conductivity, pH, surfactant protein-A (SP-A), clara-cell protein (CCP) and total protein in EBC were assessed. Data on the volatile organic compound (VOC) profile of the EBC were obtained using the electronic nose Cyranose 320TM.

**Results:** RTube provided a higher sample volume compared to the EcoScreen. Hyperventilation yielded higher volumes than tidal breathing. Neither devices nor breathing patterns affected pH. Although conductivity appeared to be affected by both, average measurements remained rather stable. Hyperventilation led to an increase of total protein. The ECoScreen showed a trend towards higher protein amounts. CCP and SP-A amounts were not influenced by the breathing pattern. The electronic nose was capable of distinguishing between breathing patterns and devices.

**Conclusion:** EBC pH and (to a lesser extent) conductivity are fairly stable measures that do not depend on device or breathing pattern. Hyperventilation increases total protein concentration possibly via augmented shear forces and increased aerosol production, but not through increased alveolar ventilation, as SP-A amounts were not altered by hyperventilation. The VOC content of EBC seems to be influenced by device and breathing pattern.

### P4040

### Prostaglandin E<sub>2</sub> and cysteinyl leukotriene concentrations in sputum supernatant in smoking asthma

Konstantina Kontogianni<sup>1</sup>, Petros Bakakos<sup>1</sup>, Konstantinos Kostikas<sup>2</sup>, Georgios Hillas<sup>1</sup>, Anastasia Papaporfyyriou<sup>2</sup>, Philipos Emmanouil<sup>1</sup>, Konstantinos Matzouranis<sup>1</sup>, Manos Alchanatis<sup>1</sup>, Spyros Papiris<sup>2</sup>, Stelios Loukides<sup>2</sup>. <sup>1</sup>Ist Respiratory Medicine, University of Athens Medical School, Athens, Greece; <sup>2</sup>2nd Respiratory Medicine, University of Athens Medical School, Athens, Greece

**Background:** Smoking may modify airway inflammatory pattern. There is some evidence that the elevated levels of  $PGE_2$  in the exhaled breath condensate of patients with asthma are mainly related to smoking habit [Kostikas et al ERJ 2003].

**Objective:** To evaluate the concentrations of PGE<sub>2</sub> and cysLTs in sputum supernatants of patients with asthma and to determine whether smoking affects significantly their measurements.

**Methods:** We studied 98 patients with asthma (47 smokers), under optimal treatment with ICS. We also studied 40 control subjects (20 smokers). All subjects underwent sputum induction, pulmonary function tests, measurement of FeNO and BHR to methacholine expressed as PD<sub>15</sub>.

**Results:** Median [IQR] sputum cysLTs concentration was significantly higher in asthmatic smokers compared to non asthmatic smokers and both smoking and non smoking controls [503 (400,731) vs. 345 (210-509) vs. 93 (75-121) vs. 121 (95,175) pg/ml, respectively; p<0.0001]. Similar results were observed for PGE<sub>2</sub> concentrations which were significantly higher in smoking asthmatics [754 (654,901) vs 532 (345,654), vs 212 (132,342) vs 164 (102,207) pg/ml, p<0.001]. In patients with smoking asthma, significant positive associations was observed between cysLTs concentration and sputum neutrophils.

**Conclusions:** The increased concentrations of  $PGE_2$  and cysLTS in sputum supernatants of smoking asthma are consistent with the hypothesis that these two mediators are up-regulated in this specific phenotype of asthma. Furthermore, cysLTs are associated with the persistent eosinophilic inflammation observed in smoking asthma, while  $PGE_2$  is associated with the neutrophilic one.

### P4041

### Adaptation of differential ion mobility spectrometry (DMS) for discrimination of specific biomarkers in exhaled breath in patients with severe renal-pulmonary dysfunction

Gunther Becher<sup>1</sup>, Anne Hillmann<sup>2</sup>, Roman Purkhart<sup>2</sup>, Martina Graupner<sup>3</sup>, Rolf Graupner<sup>2</sup>. <sup>1</sup>*R&D*, BecherConsult GmbH, Bernau, Germany; <sup>2</sup>*R&D*, Graupner Medizintechnik & Co KG, Geyer, Germany; <sup>3</sup>Praxis, Dialysezentrum Altenburg, Altenburg, Germany

Introduction: Volatile substances (VOC's) in exhaled breath are target for identification of new biomarkers for disease and metabolic processes. Renal insufficiency could be a good example of an illness with exhaled markers of an internal disease. The Differential Ion Mobility Spectrometry (DMS) is a method for detection of volatile compounds in exhaled air with a sensitivity in ppt-range. The method was used in a pilot study for discrimination of patients with chronic metabolic kidney diseases.

**Methods:** A DMS of SIONEX was used for analysis of exhaled breath. The measurements were performed before and after a dialysis procedure. The DMs-analysis includes a pre separation by a multi-capillary tube, ionization of the sample and measurement of ions by IMS with 270 sec. Spectra were discriminated by detection of clusters and calculation of significance using support vector machine.

**Results:** It was possible to collect sufficient samples in all patients. Specific clusters of biomarkers were found discriminating marker exhalation before and after therapeutic intervention with dialysis. Specific clusters, indicating drug-uptake, were found.

The inter-individual reproducibility was very high, which possibly represents the manifold drug-treatment and severity of renal insufficiency with enhanced blood urea.

**Discussion:** Characteristic breath pattern could be detected. The method is noninvasive and fast and could offer new possibilities for long term control of medicaments and chronic metabolic disorders. Further studies are needed to identify certain markers and metabolites.

### P4042

# Clusters of biomarkers in exhaled breath detected by differential ion mobility spectrometry (DMS)

Gunther Becher<sup>1</sup>, Roman Purkhart<sup>2</sup>, Anne Hillmann<sup>2</sup>, Rolf Graupner<sup>2</sup>, Swen Marke<sup>3</sup>, Werner Steinhaeusser<sup>4</sup>, Wolfram Scharff<sup>3</sup>. <sup>1</sup>*R&D*, BecherConsult GmbH, Bernau, Germany; <sup>2</sup>*R&D*, Graupner Medizintechnik & Co KG, Geyer, Germany; <sup>3</sup>Analytik, IFU Privates Inst. für Analytik, Oberlichtenau, Germany; <sup>4</sup>Marketing, CareFusion, Hoechberg, Germany

**Introduction:** Non invasive biomarkers from exhaled breath became high interest. The ion mobility spectrometry has better sensitivity and differentiation of volatile compounds then gas-chromatography. The high sensitivity of the method brings a couple of problems due to VOC's from ambient air and environment.

**Methods:** The aim of the study was to demonstrate standardized sampling, reproducibility and the discrimination of groups of volunteers by exhaled markers. The calculation of spectra and statistical discrimination was performed using a statistic program based on a Support-Vector Machine.

For the investigation were included 57 volunteers, whose were recruited from two completely different environmental-occupational ambient air conditions.

**Results:** There were collected repetitive samples on one day and within one week for each. Similar tests were performed on ambient air.

It was possible to demonstrate significant differences in spectra of volunteers. It was possible to differentiate clusters from human biomarkers from the clusters which represent VOC's from ambient air. Subgroups, e.g. sex, BMI, smoking, were possible to discriminate without disturbance from ambient conditions.

**Discussion:** The DMS is suitable for the detection of VOCs in exhaled breath even in different environmental conditions. The fingerprints (clusters) in each measurement are characteristic for the individuals, groups and highly reproducible. Specific VOC's from ambient are can be mostly excluded from patients markers. The ion mobility spectrometry may be a sufficient method for non-invasive detection of disease markers in breath.

Supported by a Grant of the Federal Ministry for Economy and Technology: EURONORM. Germany

### P4043

# Exhaled hydrogen sulfide in patients with chronic airway inflammatory disease

Yahong Chen<sup>1</sup>, Xinmao Wang<sup>1</sup>, Wanzhen Yao<sup>1</sup>, Chaoshu Tang<sup>2</sup>. <sup>1</sup>Respiratory Department, Peking University Third Hospital, Beijing, China; <sup>2</sup>Department of Physiology, Peking University Health Science Center, Beijing, China

Background: Chronic obstructive pulmonary disease (COPD) and asthma are two common chronic airway inflammatory diseases. Recent studies showed that endogenous hydrogen sulfide  $({\rm H_2S})$  might be the third signaling gasotransmitter playing a important role in inflammation.

**Objective:** To investigate whether exhaled  $H_2S$  level is related to airway inflammation in COPD and asthma.

**Methods:** Clinical data, inflammatory cell in induced sputum, plasma level of TNF- $\alpha$ , IL-8 and LTB4, exhaled and plasma H<sub>2</sub>S level were studied in 41 patients with AECOPD, 36 with stable COPD, 50 with acute exacerbation of asthma, 24 with stable asthma, and 11 healthy subjects.

**Results:** Exhaled H<sub>2</sub>S level were lower by 20.63% and 37.81%, respectively, in patients with stable COPD and AECOPD than healthy controls (P<0.05). Exhaled H<sub>2</sub>S level was decreased by 21.64% in patients with AECOPD than stable COPD patients (P<0.05). Exhaled H<sub>2</sub>S was positively correlated with the percentage of predicted inspiratory capacity (r=0.321, P=0.026), negatively correlated with plasma H<sub>2</sub>S levels (r=-0.384, P=0.021) and SGRQ activity score (r=-0.226, P=0.05). Exhaled H<sub>2</sub>S levels were decreased by 29.5% and 31.4%, respectively, in patients with stable and acute exacerbation of asthma than healthy controls (all P<0.05). Exhaled H<sub>2</sub>S in asthma had lower exhaled H<sub>2</sub>S levels than non-smokers (P=0.05). Exhaled H<sub>2</sub>S in asthma was negatively correlated with plasma LTB4 (r=-0.627, P=0.05).

**Conclusions:** Exhaled hydrogen sulfide was decreased in patients with COPD and asthma. Its alteration in level may be connected with airway inflammation. The study is funded by NSFC (No. 30871127), NCET (No. 985-2-082-113), and Chinese Medical Association CRD Grant (No. 08020370115).

### P4044

# Methodological aspects of nuclear magnetic resonance spectroscopy of exhaled breath condensate

Paolo Montuschi<sup>1</sup>, Debora Paris<sup>2</sup>, Dominique Melck<sup>2</sup>, Vincenzina Lucidi<sup>3</sup>, Giovanni Ciabattoni<sup>4</sup>, Andrea Motta<sup>2</sup>, on behalf of U-BIOPRED. <sup>1</sup>Pharmacology, Catholic University of the Sacred Heart, Rome, Italy; <sup>2</sup>Biomolecular Chemistry, National Research Council, Pozzuoli (Naples), Italy; <sup>3</sup>Pediatrics, Ospedale Pediatrico Bambino Gesù, Rome, Italy; <sup>4</sup>Drug Sciences, University G. D'Annunzio, Chieti, Italy

We investigated the following methodological aspects of nuclear magnetic resonance (NMR) spectroscopy-based metabolomics of exhaled breath condensate (EBC): 1) within-day, between-day and technical repeatability; 2) detection limit; 3) possible EBC contamination with saliva or cleaning solution for disinfection of EBC collection setup. A 600 MHz Bruker Avance spectrometer (Bruker BioSpin, Rheinstetten, Germany) was used. EBC was collected with a condenser (Ecoscreen, Jaeger, Hoechberg, Germany). Within-day repeatability of NMR spectroscopy, expressed according to Bland-Altman, was assessed in 5 healthy and 11 cystic fibrosis subjects collecting separate samples twice within the same day. All values were within the 2SD range. Between-day repeatability was assessed in the same subjects collecting 3 samples on day 1,3,7. Technical repeatability was assessed measuring 3 different samples 10 times. Between-day and technical repeatability were expressed as intraclass correlation coefficient which was 0.93 and 0.96, respectively. Detection limit was 0.14 µM. EBC and salivary metabolomic spectra were different as signals in 3.3-4.5 ppm region in saliva were absent in EBC. Apart from reference TSP, no peak was observed in the cleaning solution (sodium hypochlorite, 3.55 mM) spectrum.



This technique has a low detection limit for NMR spectroscopy, is reproducible and not affected by contamination with saliva or cleaning solution.

### P4045

# The nitric oxide (NO) metabolites in monitoring and therapy of bronchial asthma

Svetlana Soodaeva<sup>1</sup>, Igor Klimanov<sup>1</sup>, Alexander Lisitsa<sup>1</sup>, Elena Zaprudnova<sup>2</sup>. <sup>1</sup>Clinical and Experimental Biophysics, Pulmonology Research Institute, Moscow, Russian Federation; <sup>2</sup>Department of Biochemistry, Vladimir State University, Vladimir, Vladimir Region, Russian Federation

The aim of the study was to investigate the efficacy of the treatment of bronchial asthma using the estimation of the dynamics of stable NO metabolites in exhaled breath condensate (EBC). These parameters was also investigated for the monitoring of airway inflammation in patients with stable bronchial asthma treated by GINA-2009 guideline.

Materials and methods: 158 outpatients were enrolled in the study. Group1 contains 48 children (8-12 y.o.); group 2 contains 52 adults with the exacerbation of moderate bronchial asthma (BA). The control group consisted of 58 healthy volunteers. The EBC was collected in all patients, as well as the TNN concentration in EBC was measured before and after the course of therapy by spectrophotometric method using the Griess reaction.

**Results:** The statistically significant decrease of TNN concentration in EBC both in children and adults was demonstrated during the study (in 4.0 and 4.5 times respectively compared to baseline). There was no difference in this parameter between the children and the adult group. After the course of therapy it was shown the decrease of TNN level in EBC in 89.7% of patients in both groups (in 1.6 and 1.7 times respectively). However, the TNN level in EBC was strongly higher in patients with BA than in control.

**Conclusion:** The measurement of TNN concentration in EBC can be used as a marker of airway inflammation in patients with moderate BA for the monitoring of patient's status. During the course of therapy the statistically significant decrease of this parameter was demonstrated; that is strongly correlated with clinical status. So, the TNN level in EBC also can be considered as a sensitive marker of the efficacy of the therapy administered.

#### P4046

Increased levels of osteopontin in sputum supernatant in smoking asthma Georgios Hillas<sup>1</sup>, Stelios Loukides<sup>2</sup>, Konstantinos Kostikas<sup>2</sup>, Davina Simoes<sup>3</sup>, Konstantina Kontogianni<sup>4</sup>, Vasiliki Delimpoura<sup>2</sup>, Eleni Tseliou<sup>2</sup>, Spyros Papiris<sup>2</sup>, Manos Alchanatis<sup>4</sup>, Petros Bakakos<sup>4</sup>. <sup>1</sup>Department of Respiratory and Critical Care Medicine, Research Unit, "Sotiria" Chest Diseases Hospital, Athens, Greece; <sup>2</sup>2nd Respiratory Medicine Department, University of Athens Medical School, "Attikon" Hospital, Athens, Greece; <sup>3</sup>Marianthi Simou Laboratories, University of Athens Medical School, Athens, Greece; <sup>4</sup>1st Respiratory Medicine Department, University of Athens Medical School, "Sotiria" Chest Diseases Hospital, Athens, Greece

**Background:** Osteopontin (OPN) has been associated with inflammation and fibrosis. OPN is increased in asthma and is related to the underlying severity and to features expressing remodeling and inflammation. Smoking may modify the inflammatory pattern of the airways.

Aims and objectives: To evaluate the levels of OPN in sputum supernatants of asthma patients and to investigate the possible role of smoking as well as associations with mediators and cells involved in the inflammatory and remodeling process. **Methods:** We studied 98 asthma patients (51 smokers) and 40 healthy subjects (20 smokers) who underwent lung function tests, bronchial hyperresponsiveness to methacholine, and sputum induction for cell count identification and measurement of OPN, VEGF, TGF- $\beta$ 1, CysLTs, IL-13, ECP and IL-8 in supernatants. The concentrations of all mediators were measured using enzyme immunoassays.

**Results:** Median OPN levels (pg/ml) were significantly higher in smoking asthmatics (SA) compared to non-smoking asthmatics (NSA), and both smoking and non smoking controls [120 (651, 1793) vs 210 (120, 404) vs 50 (42, 70) vs 102 (71, 156) pg/ml, respectively; p<0.0001]. Regression analysis provided significant associations between log OPN and sputum neutrophils, IL-8, IL-13 and TGF- $\beta$ 1. The most significant association of TGF- $\beta$ 1 was the one with OPN. These associations were observed only in SA. No significant associations were observed between OPN, lung function tests and PD15 to methacholine in all groups.

**Conclusions:** OPN levels are affected by the smoking habit in asthma. The associations of OPN with sputum neutrophils,  $TGF-\beta 1$ , IL-13 and IL-8, only in SA, suggest a possible role for OPN in the inflammatory and remodeling process in SA.

### P4047

# Laboratory investigation of sputum and mucociliary clearance (MCC) condition in patients with COPD

Tetyana Pertseva, Olena Lykholat, Olena Gurzhiy. Internal Medicine Department, Dnepropetrovsk State Medical Academy, Dnepropetrovsk, Ukraine

Aim: To investigate biochemical parameters of sputum for evaluation of MCC's disorders in patients with COPD.

Materials: 90 smoking patients with COPD in stable condition.

**Methods:** Biochemical parameters of sputum (medium weight molecules (MWM), trypsin, cysteine proteases cathepsin B, cathepsin L,  $\alpha$ 1-proteinase inhibitor ( $\alpha$ 1-PI)) were researched for MCC's evaluation. **Results:** Dynamics of indices are in table 1.

Table 1

		Patients with COPD	
Indices	Mild (n=14)	Moderate (n=34)	Severe (n=42)
MWM, mg/g protein	1114,6±21,5	1226,8±47,7	1443,6±35,5
Trypsin, nmol/sec L/g protein	$7,1\pm0,1$	$5,8\pm0,4$	$4,5\pm0,2$
Cathepsin B, OD/g protein	$49,8\pm1,9$	$44,5\pm 3,2$	37,6±1,6
Cathepsin L, OD/g protein	$78,1\pm4,7$	86,7±4,0	$102,2\pm 4,0$
α1-PI, mcmol/sec L/g protein	$2,07{\pm}0,07$	$2,20{\pm}0,05$	$2,30{\pm}0,03$

**Conclusions:** Bronchial obstruction progression accompanied by decreasing of local proteolytic potential of sputum: trypsin (r= -0,61, p<0,001); cathepsin B (r= -0,42, p<0,01); cathepsin L (r=0,39, p<0,01);  $\alpha$ 1-PI (r=0,39, p<0,01). It

leads to decreasing of sputum lysis and accumulation of hyperviscosity mucus in respiratory tract. As a result, movement of ciliary epithelium is blocked. Decreasing of sputum lytic properties accompanied by accumulation of MWM (correlation between severity of COPD and MWM level – r=0.60, p<0.001). It

(correlation between sevenity of COPD and MWM level – r=0,60, p<0,001). It also enhances the viscosity of sputum and violates the optimal conditions of MCC functioning. So, bronchial obstruction progression in patients with COPD accompanied by

decreasing of common lytic activity of sputum, therefore excessive amount of viscous bronchial secretion accumulate. It slows down ciliary epithelium movement, compounding the disorders of MCC.

### P4048

# The concentration of inflammatory cytokines in exhaled breath condensate in children with inflammatory bowel diseases

Katarzyna Krenke<sup>1</sup>, Joanna Peradzynska<sup>1</sup>, Joanna Lange<sup>1</sup>, Katarzyna Grzela<sup>1</sup>, Aleksandra Banaszkiewicz<sup>2</sup>, Izabela Lazowska<sup>2</sup>, Marek Kulus<sup>1</sup>. <sup>1</sup>Department of Pediatric Pneumonology and Allergy, <sup>2</sup>Department of Pediatric Gastroenterology and Nutrition, Medical University of Warsaw, Warsaw, Poland

Introduction: Nowadays, Crohn's disease (CD) and ulcerative colitis (UC) are regarded as a systemic disorders and extraintestinal sites of inflammation have been found in various organs, including respiratory system. Chronic inflammation involving a wide variety of cytokines plays an important role in the pathogenesis of inflammatory bowel diseases (IBD).

Aim of the study: The aim of the study was to evaluate the concentration of several inflammatory cytokines in exhaled breath condensate (EBC) in children with IBD.

**Material:** 47 children with IBD (24 boys and 23 girls, mean age  $13.8\pm3.3$ ) and 37 healthy volunteers (20 boys, 17 girls, mean age  $13.9\pm3.6$ ) were enrolled into the study.

Methods: EBC was collected during 10 minutes of tidal breathing. IL1 $\beta$ , IL6, IL8 and TNF $\alpha$  were measured with ELISA.

**Results:** The concentration of inflammatory cytokines in study group was as follow: TNF $\alpha$ - mean 0.8 pg/ml, SD 0.4, IL1 $\beta$  - mean 0.3 pg/ml, SD 0.3, IL 6 - mean 0,1 pg/ml, SD 0.1, IL 8 - mean 0.2 pg/ml, SD 0.3. Control group - TNF $\alpha$  - 0.6 pg/ml, SD 0.3 IL1 $\beta$  - 0.0 pg/ml, SD 0.1, IL 6 - 0.0 pg/ml, SD 0.0, IL 8 - 0.0 pg/ml, SD 0.0, The levels of all cytokines were significantly higher in the study group (p<0.05).

**Conclusion:** The elevated concentration of inflammatory cytokines in EBC in children with IBD can suggest that inflammation, which plays the key role in pathogenesis of IBD, may be also present in respiratory tract.

#### P4049

Effects of outdoor temperature and humidity on methacholine challenge tests Bruno Sposato<sup>1</sup>, Marco Scalese<sup>2</sup>, Andrea Pammolli<sup>3</sup>, Maurizio Di Tomassi<sup>1</sup>, Maria G. Migliorini<sup>1</sup>, Raffaele Scala<sup>4</sup>, Mario Naldi<sup>5</sup>. <sup>1</sup>U.O. Pneumologia, Ospedale "Misericordia", Grosseto, Italy; <sup>2</sup>Istituto di Fisiologia Clinica, CNR, Pisa, Japan; <sup>3</sup>Dipartimento di Fisiopatologia, Medicina Sperimentale e Sanità Pubblica, Università di Siena, Siena, Italy; <sup>4</sup>Unità di Pneumologia e UTSIR, Ospedale Campo di Marte, Lucca, Italy; <sup>5</sup>Unità di Pneumologia e UTIP, Ospedale S.Donato, Arezzo, Italy

This study tried to evaluate whether outdoor daily temperature (T) and humidity (H) can influence methacholine test results in outpatients living in temperate climate areas. 4,723 subjects (2391 males; age 35.1±16.15; FEV1 100.36% [IQR: 92.34-108.8]) that performed a methacholine test for a suspected bronchial asthma between 2000 and 2010 were considered. Mean outdoor temperatures (°C) and relative humidity (%), registered when the test was performed, were considered. Patients with bronchial hyperresponsiveness (PD<sub>20</sub> < 3200  $\mu$ g) were 2,889 (61.2%) and median PD<sub>20</sub> were  $359 \ \mu g$  [IQR: 160-967]. On receiver operator curve (ROC) analysis, temperature and humidity did not significantly predict hyperresponsiveness (AUC: 0.5); an AUC of 0.55 for temperature was found only in subjects aged <20 and in non-smokers. When we subdivided the subjects into different subgroups, on the basis of different levels of temperature and humidity (T<10°C or between 10 and 20°C or >20°C and H<60% or between 60% and 80% or >80%), no differences in hyperresponsiveness prevalence and in PD20 were found. A significantly positive relationship between PD<sub>20</sub> and BMI was detected in subjects aged from 36 to 51 (r: 0.079; p: 0.031) and in those with severe hyperresponsiveness (PD<sub>20</sub> < 200 µg) (r: 0.110; p: 0.001). The regression logistic model showed how the maximum temperature was a significant lower risk factor for bronchial hyperresponsiveness (OR: 0.995, 95%CI: 0.982-0.998). In conclusion, this study showed that an increase in temperature (excluding extreme values) is associated to a slight, but significant, reduction of bronchial hyperresponsiveness risk.

#### P4050

# Are FENO indices useful diagnostic tools in suspected asthma? Experience of a routine lung function laboratory

Florence Schleich, Raluca Asandei, Maité Manise, Jocelyne Sele, Cédric Graas, Renaud Louis. *Respiratory Medicine, GIGA P<sup>3</sup>, CHU Liege, University of Liege, Liege, Belgium* 

Background: Asthma diagnosis is based on symptoms associated with airflow

variability. Airway inflammatory component measured by exhaled nitric oxide (FENO50) has been proposed as a diagnostic tool but remains controversial.

Aim: To assess the ability of FENO indices to identify bronchial hyperresponsiveness to methacholine (PC20M < 16mg/ml) and to establish which respiratory symptoms relate to FENO indices and PC20M.

**Methods:** We conducted a prospective study on 174 steroid naive patients addressed for PC20M. Patients with respiratory symptoms, FEV1  $\geq$ 70% pred and no proof of reversibility to inhaled salbutamol (either not done or response <12%) completed a questionnaire about their symptoms and underwent FENO measurement at different flow rates (50-100-150 and 200ml/sec) and PC20M.

reasurement at different flow rates (50-100-150 and 200ml/sec) and PC20M. **Results:** 82 had a PC20M <16mg/ml and had significantly higher FENO50, J'awNO and Intercept but did not show significant difference in CAIVNO value. By constructing ROC curve, we found that FENO50 cut-off value of 34 ppb is able to identify bronchial hyperresponsiveness with high specificity (95%) and PPV (88%) but low sensitivity (35%) and NPV (62%). For the whole group, the dose-response slope (DRS) for methacholine weakly correlated with FENO50 but not with CalvNO. Among the positive PC20M, there was no relationship between the magnitude of PC20M and the level of FENO indices. Wheezing was the symptom most convincingly associated with raised FENO50.

**Conclusion:** FENO50 >34 ppb is a good diagnostic criterion in patients with suspected asthma. However FENO50  $\leq$ 34ppb clearly does not rule out bronchial hyperresponsiveness and should prompt the clinician for asking methacholine challenge.