417. Bronchial hyperresponsiveness and exhaled and sputum biomarkers

P4031
Efficiency of a laser-based sensor for FeNO measurements and multiple flows analysis
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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 recommendations, numerous works using FeNO 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 226, Sievers) and a portable hand-held electrochemical analyzer (Mingo® Aeroscience 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 analyzer’s 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) from healthy people were used to calculate the flow-independent parameters NO, i.e. the alveolar region, airway wall, diffusion capacity and flow 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.

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.

**References:**

**P4032**

**The impact of diurnal variations, atopy, pollen exposure and pharmacological treatment on exhaled nitric oxide levels**

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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.

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 24-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<0.001) were also found in patients with AR during the pollen season (21.25 ppm; IQR=20.3) compared to the levels outside the season (14.2 ppm; IQR=12.45) before treatment. In AR patients treated by nasal corticosteroids and antihistamines in the season were also found in patients with AR during the pollen season (21.25 ppm; IQR=20.3)

Conclusion: There was significant daily variability in all mediators, which was greater in usual COPD, compared to IL5 and LTβ4 concentrations being higher (p<0.01) in ATD (medians 11.29 vs 3.72 nM; and 12.16 vs 6.10 nM respectively) or 3-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.

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 atopic-pneumoses patients using mass spectrometry, as well as to compare proteome identification.

**Conclusions:**

In conclusion, each of abnormal peptides, as well as their combinations, may have diagnostic value.

**References:**
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**P4033**

**Circadian variation of exhaled breath temperature in healthy subjects**

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**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, range age 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 handheld device (X-halo, Delmedica, Singapore). They took it home and were instructed to do measurements at four time points (±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):
Exercise test with dry air inhalation compared to mannitol test as marker of exercise induced asthma

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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®, Sweden) was compared to inhaled mannitol test in a randomized trial (NCT 00898833) 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 ≥ 15% 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 peak fall FEV1 after EIA test was 16.34% at baseline and 13.11% after 6 weeks treatment. There was a significant improvement in B/F (A/EIA test at 90% CI) 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, 5.59)). 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 AstraZeneca.

Ectopic cell inflammatory pattern in induced sputum in patients with mild-moderate asthma for five years

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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 (5 pred, no exacerbations/year (EY), use of relief therapy (RT), dose of inhaled corticestroid/day (ECs), 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), paucigranulocytic (<2% eosinophils and <61% neutrophils), paucigranulocytic (<2% eosinophils and <61% neutrophils). Paucigranulocytic, 22%agressive forms (neutrophilic, eosinophilic o mixted) and >2% eosinophils and >61% neutrophils). Results: The study began with 24 patients and in 18 we repeated the induced sputum 5 years after. The probability of were 100%: 55% showed the same cellular inflammatory pattern in the sputum (11% eosinophilic, 11% neutrophilic y 33% paucigranulocytic). In 45% were found different patterns: 11% benign forms (paucigranulocytic), 22%agressive forms (neutrophilic, eosinophilic o mixed) and a 11% changed the inflammatory pattern but with aggressive 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 aggressive forms. Non significative changes towards neutrophilic patterns has been observed that warrant further study in larger groups.

Prostaglandin E2 and cysteinyl leukotriene concentrations in sputum supernatant in smoking asthma

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Background: Smoking may modify airway inflammatory pattern. There is some evidence that the elevated levels of PGE2 in the induced bronchial condensate of patients with asthma are mainly related to smoking habit [Kostikas et al ERJ 2003].

Objective: To evaluate the concentrations of PGE2 and cysteiny-functionalities 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 NeNO and BHR to methacholine expressed as PD20...

Results: Median (IQR) sputum cysteiny-functionalities concentration was significantly higher in asthmatic smokers compared to non asthmatic smokers and both smoking and non smoking controls [303 (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 PGE2 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 cysLT concentration and sputum eosinophils and PGE2 concentration and sputum neutrophils.
Conclusions: The increased concentrations of PGE$_2$ and cys-LTs 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, cys-LTs 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 pulmonary dysfunction

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Introduction: Volatile substances (VOC's) in exhaled breath are target for identification of new biomarkers for disease and metabolic processes. Respiratory insufficiency could be a good example of an illness with exhaled markers of an internal disease. The aim of the study was to demonstrate standardized sampling, reproducibility and the discrimination of groups of volunteers by exhaled markers. Further studies are needed to identify certain markers and metabolites.

Methods: A DMS of sNEXI 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, were found.

Discussion: Characteristic breath pattern could be detected. The method is non-invasive and safe 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)

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Introduction: Non invasive biomarkers from exhaled breath became high interest. The ion mobility spectrometry has better sensitivity and differentiation of volatile compounds than their gas-chromatography. The high sensitivity of the method brings a couple of ions by IMS with 270 sec. Spectra were discriminated by detection of clusters and calculation of significance using support vector machine.

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 different hospitals.

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.

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P4043

Exhaled hydrogen sulfide in patients with chronic airway inflammatory disease

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Background: Chronic obstructive pulmonary disease (COPD) and asthma are two chronic airway inflammatory diseases. Recent studies showed that endogenous hydrogen sulfide (H$_2$S) might be the third signaling gasotransmitter playing an important role in inflammation.

Objective: To investigate whether exhaled H$_2$S level is related to airway inflammation in COPD and asthma.

Methods: Clinical data, inflammatory cell in induced sputum, plasma level of TNF-α, IL-8 and LTB4, exhaled and plasma H$_2$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$_2$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$_2$S level was decreased by 21.64% in patients with AECOPD than stable COPD patients (P<0.05). Exhaled H$_2$S was positively correlated with the percentage of predicted inspiratory capacity (r=0.321, P=0.026), negatively correlated with plasma H$_2$S levels (r=-0.348, P=0.012) and SGRQ activity score (r=0.226, P=0.05). Exhaled H$_2$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). Smokers in asthma had lower exhaled H$_2$S levels than non-smokers (P<0.05). Exhaled H$_2$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), NCEF (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

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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

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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 were 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. Group 1 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). 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

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Background: Osteopontin (OPN) has been associated with inflammation and fibrosis. OPN is increased in asthma and is related to the underlying severity and to the expression of smoking to baseline. 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-β1, 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 (50, 156) pg/ml respectively; p<0.0001]. Regression analysis provided significant associations between log OPN and sputum neutrophils, IL-8, IL-13 and TGF-β1. The most significant association of TGF-β1 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-β1, 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

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

Methods: Biochemical parameters of sputum (medium weight molecules (MWM), trypsin, cysteine proteases cathepsin B, cathepsin L, α1-protease inhibitor (α1-PI)) were researched for MCC’s evaluation. Results: Dynamics of indices are in table 1.

Table 1

<table>
<thead>
<tr>
<th>Indices</th>
<th>Patients with COPD</th>
<th>Mild (n=14)</th>
<th>Moderate (n=34)</th>
<th>Severe (n=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MWM, mg/ protein</td>
<td>1114±21.5</td>
<td>1228±6.4</td>
<td>1443±6.3</td>
<td>555±35.5</td>
</tr>
<tr>
<td>Trypsin, nmol/sec L/g protein</td>
<td>7.1±0.4</td>
<td>5.8±0.4</td>
<td>4.5±0.2</td>
<td>4.5±0.2</td>
</tr>
<tr>
<td>Cysteine proteases cathepsin B, cathepsin L, α1-protease inhibitor (α1-PI)</td>
<td>49±1±9</td>
<td>44±3.3</td>
<td>37±6</td>
<td>11±1</td>
</tr>
<tr>
<td>Cathepsin L, DQD protein</td>
<td>78±4.7</td>
<td>86±2.0</td>
<td>100±2.0</td>
<td>80±2.0</td>
</tr>
<tr>
<td>α1-PI, nmol/sec L/g protein</td>
<td>2.07±0.07</td>
<td>2.20±0.05</td>
<td>2.30±0.03</td>
<td>2.30±0.03</td>
</tr>
</tbody>
</table>

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), α1-PI (n=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 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 disease

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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 play 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 inflammatory cytokines in exhaled breath condensate (EBC) in children with IBD.

Materials: 47 children with IBD (24 boys and 23 girls, mean age 13.8±3.3) and 37 healthy volunteers (20 boys, 17 girls, mean age 13.9±4.3) were enrolled into the study.

Methods: EBC was collected during 10 minutes of tidal breathing. IL1β, IL6, IL8 and TNFα were measured with ELISA.

Results: The concentration of inflammatory cytokines in study group was as follow: TNFα - mean 0.8 pg/ml, SD 0.4, IL1β - 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 - TNFα - 0.16 pg/ml, SD 0.3 IL1β - SD 0.1, IL 6 - 0.0 pg/ml, SD 0.0, IL 8 - 0.0 pg/ml. The levels of all cytokines were significantly higher in the study group (p<0.05).

Conclusions: 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

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This study tried to evaluate whether outdoor daily temperature (T) and humidity (H) influence methacholine test results in outpatients living in temperate climate areas. 4,723 subjects (2391 males; age 35.1±6.1; F shows that an increase in temperature (excluding extreme values) is associated 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 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.
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 ≥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.

**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 CAIVNO. 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 ≤34ppb clearly does not rule out bronchial hyperresponsiveness and should prompt the clinician for asking methacholine challenge.