59. Airway diseases: from invasive to noninvasive biomarkers

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Bronchial mucosal dendritic cells and VEGF expression in COPD patients

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Introduction: Dendritic cells (DCs) have a pivotal role in the onset and regulation of innate and adaptive immune responses. A decreased number of mature DCs may occur in the airways of COPD patients, thereby reducing their immune response. Moreover, DCs can affect vascularization process in different physiopathological conditions. There are no data concerning the relationship between DCs and vascular endothelial growth factor (VEGF) in COPD patients.
Objectives: We evaluated the relationship between the expression of VEGF and the density of DCs in the bronchial mucosa of COPD patients.
Methods: Twenty patients with moderate to severe COPD (age 76±10 yr, 3 F; FEV1 51±9%, FEV1/VC 48±12%) were studied. Eight healthy subjects represented a control group (CS). Bronchial biopsies were evaluated by immunohistochemistry and presence of DCs was investigated by using antibody directed against CD207, to mark immature DCs, and against CD83, to mark mature DCs.
Results: Comparisons are summarized in the table.

	COPD	CS	p value
VEGF+ cells (n/mm^2)	121±24	82±9	0,001
CD207+ cells (n/mm^2)	49±11	41±11	0,103
CD83+ cells (n/mm^2)	1,13±0,48	1,57±0,39	0,034

Data are presented as mean±SD. In all COPD patients, CD207+ cells were inversely related to FEV1 ($r=-0.52$, $p<0.05$) and CD83+ cells were inversely related to VEGF expression ($r=-0.45$, $p<0.05$).
Conclusions: Our results show that COPD airways were associated with a decrease in mature DCs and that these cells were inversely related to VEGF expression. Additionally, immature DCs were significantly related to airflow obstruction. We speculate that the DCs-VEGF interplay may play a key role both in angiogenesis and in the immune response in COPD patients.

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Correlation between airway smooth muscle (ASM) and eosinophilic inflammation in severe non-controlled asthmatics

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Background: Airway muscle hypertrophy is a surrogate of airway remodeling and eosinophilia in induced sputum (IS) is associated to lack of asthma control.
Objective: Compare the amount of ASM in endobronchial biopsies with eosinophils % (Eos) in IS in severe asthmatics after optimal treatment.
Methods: 62 severe asthmatics received a 2-week prednisone trial and high inhaled corticosteroid dose (ICs) plus LABA for 12 weeks and were classified according to lung function after the 2-week oral corticosteroid trial. Persistent airflow obstruction (PAO) was defined by FEV_1post BD <80% plus FEV_1/FVC post BD ≤0.70. IS and bronchial biopsies were performed at the end of the 12 weeks. The fractional area of ASM, assessed by quantification of alpha-smooth muscle actin, and Eos% and neutrophils% (Neu) in IS were assessed.
Results: 48 patients were classified as PAO,14 as non persistent airflow obstruction (NPAO). The fractional area of ASM was higher in PAO (p=0,041), but Neu% and Eos% in IS was not different between groups. There was a strong inverse correlation between IS Eos and total area muscle actin ($r = -0.83$, $p=0.001$) in the NPAO and a positive correlation in the PAO group ($r = 0.36$, $p = 0.038$). No correlations were found with Neu.
Conclusion: Persistent obstructed severe asthmatics have more smooth muscle in airways. In patients that normalize lung function after optimal treatment, Eo inflammation is inversely associated with ASM mass in the biopsies, whereas the opposite occurs in persistent obstructed patients. These data suggest that structural alterations, in parallalel with inflammation, are related to functional abnormalities in the persistent obstructed patients.

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The EvA study: Characteristics of the study population

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The emphysema versus airway disease (EvA) study is an EU-funded project with 15 partners in 8 European countries, which aims at identifying markers associated with subphenotypes of chronic obstructive pulmonary disease (COPD) as defined by CT image analysis. The project has recruited 280 controls (average age 59y, 36% females) and 534 patients (average age 65y, 32% females), who comply with stringent exclusion criteria in that current smoking, oral glucocorticoids, anti-coagulation, FEV1 < 30% or long term oxygen therapy is not allowed for. Although recruitment selected against asthma post-bronchodilator reversibility >200ml was seen in 184 cases (34%) and 58 controls (21%). Chest CT was done in cases only and this is being used – with reference to a series of phantoms- to determine lung density and airway wall thickness as a measure of emphysema and airway disease, respectively. Bronchoscopy was done on 421 cases and 278 controls and here we obtained bronchial epithelium with sheathed brushes and alveolar macrophages by lavage with up to 150 mL of fluid. Fluid recovery in lavage in average was 43% for controls and 30% for cases, reflecting the more pronounced airway collapse during fluid retrieval in COPD. Recovery of brush material was similar between cases and controls, in that RNA obtained from the right upper lobe was 5.4 ug and 6.4 ug, respectively. The data show that in a multicentre study sizable amounts of biological material can be obtained from COPD lungs. This material is currently being analyzed for expression of markers associated with the emphysema-dominant and airway disease-dominant subphenotypes of COPD.
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Characterisation of patients with difficult-to-treat and treatment-resistant severe asthma

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Background: A recent World Health Organization (WHO) meeting proposed a uniform classification of severe asthma (SA) into 3 groups: untreated SA, difficult-to-treat SA, and treatment-resistant SA (JACI 2010).
Aim: To characterize and compare the demographic, functional and inflammatory parameters and the level of control of patients with difficult-to-treat and treatment-resistant SA.
Method: 12 patients with treatment-resistant SA and 24 patients with difficult-to-treat SA (defined according to WHO document) were enrolled in this study (mean age: 59 yrs). All patients underwent spirometry and methacholine test. Asthma control was evaluated according to GINA guidelines and also by ACT questionnaire, ACQ questionnaire and PEF diary. Sputum eosinophils percentage and exhaled nitric oxide (eNO) were measured as markers of airway inflammation. Furthermore, all patients underwent ENT visit to evaluate the presence of pathology of the upper airways (in particular rhinosinusitis and/or polyps).
Results: Patients with treatment-resistant SA had a higher sputum eosinophilia (57.1% (3.7-86.2) Vs 12.5 (0-95.6), $p< 0.01$) and a shorter asthma duration (11.5 (3-40) vs 25 (8-50), $p<0.01$) than patients with difficult-to-treat SA. The indices of asthma control, as well as FEV1, PD20 methacholine, eNO, and the level of treatment were similar in the two groups.
Conclusions: Patients with treatment-resistant SA have a greater eosinophilic inflammation of lower airway than patients with difficult-to-treat SA, but a similar level of control and pharmacologic therapy, suggesting the need for different treatment strategies in the two groups of patients.

P300

Endogenous hydrogen sulfide (H_2S) in serum and sputum as novel biomarker of asthma

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Background: Hydrogen sulfide (H_2S) is considered to be the third gasotransmitter

along with nitric oxide and carbon oxide. However little is known about the role. We examined whether H_2S in serum or sputum can be used as a biomarker of asthma.
Methods: Forty asthmatic subjects and 15 healthy volunteers were recruited in this study. Subjects were asked to answer asthma control questionnaire and take bloods, induced sputum, lung function tests, and exhaled nitric oxide (Fe_{NO}) measurements. H_2S concentrations in sputum and serum samples of patients with severe and non-severe asthma and of healthy subjects were measured using a sulfide-sensitive electrode and compared H_2S levels with other clinical parameters.
Results: H_2S levels in sputum from severe and non-severe asthmatic patients were significantly higher than those from healthy subjects but there was no difference between the severe and non-severe group. Serum H_2S levels were 10 times higher than in sputum and these were also higher in severe and non-severe asthmatic subjects compared to healthy subjects. There was a positive correlation between sputum and serum H_2S levels ($r=0.42$, $p<0.05$). Sputum H_2S levels were inversely correlated with FEV_1 %predicted ($r= -0.41$, $p=0.005$), and with reversibility to salbutamol ($r= -0.53$, $p<0.01$). There were significant correlations between sputum H_2S and sputum neutrophils and macrophages ($r= 0.464$ and $r= -0.456$, $p=0.001$, respectively), and a negative correlation between sputum H_2S and Fe_{NO} levels ($r=-0.510$, $p=0.003$).
Conclusions: Sputum H_2S levels may represent a novel biomarker of asthma, particularly useful as a marker of neutrophilic inflammation, chronic airflow obstruction and bronchodilator responsiveness.

P301
Induced sputum substance P in children with difficult-to-treat bronchial asthma and gastroesophageal reflux: Effect of esomeprazole therapy
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Objectives: To assess the induced sputum substance P (ISSP) levels in children having difficult-to-treat asthma (DA) with and without gastroesophageal reflux (GER). We aimed also to evaluate the association of GER with childhood DA, relationship of GER severity with childhood asthma control test (C-ACT), FEV1, peak expiratory flow (PEF) variability, and ISSP. Finally, we tried to evaluate esomeprazole treatment effect on C-ACT and FEV1 in children with DA.
Methods: Spirometry, C-ACT, upper gastrointestinal endoscopy, and ISSP measurement were done for children with DA compared to healthy controls.
Results: ISSP was high in DA with higher levels in the group having associated GER. In the latter group, ISSP and C-ACT improved significantly after esomeprazole treatment while FEV1 and PEF variability did not improve. Reflux severity was positively correlated with ISSP and negatively correlated with FEV1.
Conclusions: GER was found in 49% of our patients with childhood DA. Very high ISSP levels in children with DA may be used as a marker for presence of GERD. Esomeprazole therapy improved asthma symptoms but did not improve lung function.

P302
Clara cell secretory protein (CC16) and surfactant protein D (SPD) in sputum supernatant in severe refractory asthma (SRA)
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Background: Severe refractory asthma (SRA) is characterized by amplified inflammatory/remodeling process. CC16 is associated with Th2 modulation. SPD plays an important role in surfactant homeostasis & eosinophil chemotaxis.
Aim: Measurement of CC16 & SPD levels in sputum supernatants of SRA patients, comparison with mild to moderate asthma and controls and investigation of inflammatory/remodelling mediator or cell associations.
Methods: 31 optimally treated SRA patients, 33 mild to moderate asthmatics and 12 controls were studied. All underwent lung function tests, bronchial hyperresponsiveness and Exhaled Nitric Oxide (FeNO) assessment, sputum induction for differential cell count and CC16, SPD, Il-8, IL-13, TGF-β & ECP measurement in sputum supernatants.
Results: CC16 [median (interquartile range) ng/ml] was significantly elevated in SRA compared to mild-moderate asthma and controls [214 (59-900) vs 58 (38-95) vs 33 (21-53), $p<0.001$].
SPD [ng/ml] was significantly elevated in SRA compared to mild to moderate asthma and controls [28.5 (18-45) vs 6.1 (4-9) vs 4.8 (3.4-7), $p<0.001$]. No difference was observed between mild to moderate asthmatics and controls for either CC16 or SPD. Regression analysis did not reveal significant associations between CC16 or SPD and sputum differential count, inflammatory/remodeling mediators, FeNO or lung function tests. A significant but moderate positive association was observed between CC16 and SPD [$r^2=0.2$].
Conclusions: SPD & CC16 levels are upregulated in SRA compared to mild to moderate asthmatics and controls, being consistent with the hypothesis that epithelium injury & repair play an important role in this phenotype.

P303
Increased levels of angiopoietin 1 & 2 in sputum supernatant in smoking asthma
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Background: Angiopoietin-1 (Ang-1), is an essential mediator of angiogenesis by establishing vascular integrity, whereas angiopoietin-2 (Ang-2) acts as its natural inhibitor.
Objective: We aimed to determine the levels of angiopoietins in sputum supernatants of patients with smoking asthma and to investigate possible associations with mediators and cells involved in both the inflammatory and the vascular remodeling process
Methods: Eighty-seven patients with asthma (42 smokers) and 28 healthy subjects (14 smokers) were studied. All subjects underwent lung function tests, bronchial hyperresponsiveness assessment and sputum induction for cell count identification and Ang-1, Ang-2, VEGF, TGF-β1, MMP-2, IL-13, ECP, and IL-8 measurement in supernatants. Airway vascular permeability (AVP) index was also assessed.
Results: Ang-1 (ng/ml) and Ang-2 (pg/ml) levels were significantly higher in patients with smoking asthma compared to patients with non-smoking asthma and both smoking and non-smoking healthy subjects [median, interquartile ranges 24 (13-37) vs. 10 (7-14) vs. 5.3 (3.7-6.5) vs 4.6 (3.8-5.7) respectively, $p<0.001$; and 168 (132-203) vs 124 (82-152) vs 94 (78-113) vs 100 (96-108) respectively, $p<0.001$]. Regression analysis showed a significant positive association for Ang-2 with AVP index, and VEGF in smoking asthma. A negative association was observed between Ang-1 and AVP index, MMP-2, sputum neutrophils and VEGF in smoking asthma.
Conclusions: Our results indicate that sputum Ang-1 and Ang-2 levels are higher in smoking asthma compared to non-smoking asthma and healthy subjects pointing towards a contribution of smoking through these mediators to the asthmatic angiogenesis process.

P304
Short-term and long-term reproducibility of non-invasive clinical and inflammatory parameters in asthma
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Several non-invasive parameters have been proposed to monitor bronchial inflammation in asthma. Aim of this study was to assess the reproducibility of established parameters in asthma within 7 days and after 6 months.
In 83 medically treated asthma patients (51 female, mean±SEM 44±1.5 yrs, FEV1% pred. 85±2, 20 pts. controlled, 30 partly controlled, 33 uncontrolled) with a constant level of asthma control (GINA) and unchanged medication the following tests were performed at baseline, after 7 days (n=76) and after 6 months (n=37): asthma control questionnaire (ACQ-5), exhaled nitric oxide (eNO), lung function tests, methacholine provocation (PC20), measurement of total serum IgE and sputum induction.
Within 7 days ACQ-5, eNO, FEV1 and total IgE were stable with a Cronbach's alpha between 0.946 (eNO) and 0.996 (ACQ5) and intraclass correlation coefficients between 0.898 (eNO) and 0.993 (IgE). Reproducibility of PC20 and sputum eosinophilia was low. In patients with a stable level of asthma control ACQ-5 (mean±SEM baseline – month 6: 0.1±0.1), eNO (0.1±2.3ppb), FEV1 (0.02±0.04L) and total IgE (13.6±13.7 IU/ml) were equally stable after 6 months. Reproducibility was good with Cronbach's alpha [intraclass correlation coefficients] between 0.873 [0.775] (ACQ-5) and 0.997 [0.994] (IgE).
In patients with a constant level of asthma control and unchanged medical treatment clinical parameters are reproducible in a short- and long-term interval.

P305
Increased levels of osteopontin in sputum supernatant in patients with COPD
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Background: Osteopontin (OPN) is a glycoprotein that has been associated with inflammation and fibrosis. Recently published data supports that OPN is upregulated in surgical lung tissue samples of patients with COPD (Schneider F et al FASEB 2010).

Aim: The aim of this study was to determine the levels of OPN in sputum supernatants of patients with COPD, and compare them with healthy subjects and to investigate their possible association with mediators and cells involved in the inflammatory and remodeling process as well as with the extension of emphysema as defined by HRCT.
Methods: Seventy-seven patients with COPD and 40 healthy subjects (20 smokers) were studied. All subjects underwent lung function tests, sputum induction for cell count identification and OPN, TGF-β1, MMP-2, IL-8, LTB4 measurement in sputum supernatants. A HRCT was performed for quantification of emphysema
Measurements and Main results: OPN levels [median (interquartile range) pg/ml] were significantly higher in patients with COPD compared to both healthy smokers and non-smokers [1340 (601-6227) vs 101 (77-109) vs 69 (50-89) respectively, $p<0.001$]. Regression analysis showed a significant association between OPN and sputum neutrophils, IL-8, MMP-2 and the extent of emphysema. The above associations were not observed in healthy subjects.
Conclusions: Our results indicate that OPN levels are higher in patients with COPD compared to both smoking and non-smoking healthy subjects. Moreover, the association of OPN with sputum neutrophils, IL-8 and MMP-2 indicates a role of OPN in neutrophilic inflammation while its association with the extent of emphysema shows a role in the pathogenesis of this particular COPD phenotype.

P306
Reference equation and upper limits of fraction of exhaled nitric oxide (FENO) in a Chinese population
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Background: Measurement of FeNO has been proposed as a biomarker for monitoring and management of airway diseases. Limiting information is available in regard to the reference levels of FeNO levels in adult Chinese.
Aim: To investigate the reference equations and upper limits of FeNO in Chinese adults.
Method: 1093 (577males) healthy non-smoking subjects with age 18-90yrs were recruited. FeNO was measured online using a chemiluminescence analyser (NOA 280i, Sievers Instruments, Boulder, CO, USA). Other assessments included spirometry, skin prick test, total serum immunoglobin E (IgE) levels and eosinophil count in peripheral blood.
Results: The geometric mean FeNO was 32.6 (95% reference interval [RI] 29.0-36.1)ppb (RI=geometric mean±1.96SD) for all subjects. FeNO value was higher in males than females (geometric mean[95%RI]:38.3[34.8-41.8] vs 27.1[23.6-30.6]ppb,$p<0.0001$); and in atopic when compared with non-atopic subjects (34.6[31.0-38.3] vs 29.8[26.4-33.2]ppb, $p<0.0001$). FeNO correlated with age ($r^2=0.23$), height ($r^2= 0.20$), IgE level ($r^2=0.18$), % eosinophil counts ($r^2=0.18$) with p value all <0.0001, but not with spirometric parameters. Based on multiple regression modeling, the reference equation of F_ENO value was as follows: log(FeNO)=0.781 + 0.104(sex [1=male, 0=female]) + 0.004(age in years) + 0.084(atopy [1=atopic, 0=non-atopic]) + 0.003(height in cm). Example of a non-atopic man and woman with age 41-50 and height 160-170cm would have their 95% upper limits of FeNO being 32.6 and 25.7ppb respectively.
Conclusion: It appears that the FeNO level of Chinese adults is higher than the Caucasian population.
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P307
An alternative sampling support for collection of particles in exhaled air
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We have previously reported a method to collect material from the airways in the form of exhaled particles (PEx)[1]. In the original set-up, PEx were sampled on silicon wafers to fulfill the conditions for downstream TOF SIMS analysis. Application of analytical techniques, such as immunoassay or LC-MS, involves extraction of material from the sampling support. PEx extraction from silicon wafers requires large volumes and generates unwanted silica debris.
The aim of this study was to identify PEx sampling supports suitable for use of wet chemistry on the collected material.
Five individuals exhaled 60L onto different sample supports. All sample supports were commercially available filters (Millipore). Samples were extracted in 200 µl of PBS/0.13%TWEEN and analyzed using albumin ELISA. Lipid was extracted using chloroform/methanol/water, 3:6:2 v/v/v, and analyzed by MALDI TOFMS.
The six tested sample supports were: (1) LCR Membrane; (2) glass fiber; (3) mixed cellulose membrane; (4) Omnipore Membrane; (5) Durapore Membrane; (6) Isopore Membrane. Silica wafers were included for comparison. The extraction efficiency was evaluated based on the albumin amount extracted from the filters. The best results were obtained for Filter1, which gave the highest amount of albumin/PEx, w/w. The filter showed good linearity for observed albumin recovery as function of PEx mass, $R^2=0.98$. The suitability of the filter for lipid analysis was confirmed by MALDI TOFMS of dipalmitoylphosphatidylcholine.
We conclude that hydrophilic LCR Membrane is a good solid support for sampling PEx, suitable for collection of samples when wet chemistry is involved in the followup analysis.
Reference:
[1] Almstrand, et al., Anal. Chem., 2009;81:662.

P308
Endogenous particles in exhaled air – Variability of particle number in healthy individuals
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Endogenous particles in exhaled air (PEx) are formed during airway reopening following airway closure. Analysis of PEx may be used for monitoring biochemical alterations in respiratory tract lining fluid in the small airways. The number of exhaled particles is strongly dependent on breathing pattern but there is also a substantial difference between individuals.
The aim of the study was to assess intra-individual variability of PEx number using a standardized breathing maneuver involving airway closure.
10 healthy subjects participated in the study. They were instructed to exhale slowly and completely, inhale to total lung capacity and exhale at 500 ml/s until functional residual capacity. Particles were counted in the final exhalation of the maneuver using an in-house developed instrument. The maneuver was repeated until 80 liters of exhaled air had been sampled. Sampling was performed 3 times (two mornings and one afternoon) for each subject.
The average total number of PEx varied from 9700 to 93 000 particles/exhalation among individuals. The coefficient of variation of total PEx number/exhalation within individuals was 3.9-36%, median 21%. The coefficient of variation between individuals was 65%. There was no intra-individual difference in particle numbers between morning and afternoon samplings.
The variability of PEx number was substantially higher between individuals than within individuals. The high variability between individuals may be due to differences in lung architecture and possibly to differences in the chemical composition of airway lining fluid. Our results support the fact that it is important to control for mass of PEx for any given concentration of non-volatile analyte.

P309
Microsatellite alterations at 3p and 19q in EBC/DNA of smokers: Are they reversible after smoking cessation?
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Microsatellite alterations (MAs) at 3p and 19q are early targets for cigarette smoke and markers of genetic susceptibility to lung cancer. Several susceptible genes modulated by smoking have been found to return to baseline years after smoking cessation. Although great interest has been devoted to the classification of smoke-related genetic alterations as reversible or irreversible, as it seems to influence the different biological functions, none have yet focused on MAs at 3p and 19q.
The aim of this study was to analyse MAs at 3p and 19q in exhaled breath condensate (EBC)/DNA and WB/DNA of smokers after 12 months from smoking cessation.
The 63 smokers enrolled in the study participated in a multidisciplinary smoking cessation program with a genetic study. All the subjects enrolled underwent EBC and whole blood (WB) collection at baseline. The 28 smokers (20 M, 53±8.5 yrs) who stopped smoking followed up and repeated sample collection after 12 months. All subjects had allelotyping analysis of DNA from EBC and WB from of a selected panel of seven microsatellites located in 3p and 9q.
MAs at 3p and 19q resulted higher in EBC/DNA than in WB/DNA and dose-dependent from cigarette smoking. These somatic alterations both in EBC/DNA and in WB/DNA resulted as being not modificable after 12 months from smoking cessation.
In conclusion we demonstrated for the first time that MAs at 3p and 19q are not modificable in short term from smoking cessation, although a longer follow-up is needed to better classify MA at these loci. Furthermore, we supported the usefulness of smoking cessation programs based on the information on genotype for its potential ethical consideration.

P310
Exhaled MMP-9 in lung cancer
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Background: MMP-9 has been recognized in several types of tumour development and progression, including lung cancer, for its role in the degradation and remodelling of lung tissue. Furthermore, increased MMP-9 has been commonly described in the serum and airways of non small cell lung cancer (NSCLC) patients.
Objective: The aim of this study was to investigate, for the first time, MMP-9 in the exhaled breath condensate (EBC) of NSCLC patients.

Participants: We enrolled 40 NSCLC patients and 40 controls affected by transudative pleural effusion.
Measurements: MMP-9 concentrations were measured in the EBC, whole blood (WB) and pleural effusion (PE) of all the subjects under study using EIA kits.
Results: MMP-9 levels were found to be significantly higher in EBC, WB and PE of NSCLC patients compared with controls. A positive correlation was observed between MMP-9 in EBC, cigarettes smoked and stage of cancer.
Conclusion: Exhaled MMP-9 was elevated in NSCLC patients, especially during tumour progression, and could represent a suitable non-invasive marker in the diagnosis and monitoring of lung cancer.

P311
Influence of oral steroid use in difficult-to-control asthma patients on metabolomic profile of oxidative stress in exhaled breath condensate (EXAIR project)
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Difficult-to-control asthma (DCA) remains a pending clinical problem despite recent advances in therapy. It is supposed that intensity of inflammation and oxidative stress is higher in DCA.
Aims: Our hypothesis was that oxidative stress metabolomics in exhaled breath condensate (EBC) differs in oral corticosteroid (CS) dependent DCA patients (group OCS, n=10) versus DCA treated by inhaled CS (group ICS, n=10) from severe asthma center and versus healthy controls.
Methods: We have used metabolomic analysis of EBC using liquid chromatography and mass spectrometry to detect concentrations of 22 markers of oxidative stress (e.g. malondialdehyde, leukotrienes, 8-isoprostane, o-tyrosine). EBC was taken by a standardized protocol. Results were analyzed together with FEV1, eNO50, blood eosinophils and differences in OCS and ICS subgroups were statistically evaluated.
Results: OCS ad ICS did not differ in gender, age (54 vs 53), asthma control test (12 vs 15), FeNO50 (36.5 vs 50) and FEV1 (53 vs 57% predicted) (all $p>0.05$). Peripheral blood eosinophils were higher in ICS group (0.46 vs 0.22 x10/9L, $p=0.03$). 8-isoprostane was significantly higher in ICS group (101.7 vs 54.5 pg/ml, $p=0.002$) only. All other measured markers in EBC did not differ between OCS and ICS, however all markers in EBC of OCS and ICS were higher in comparison to control group (all $p<0.001$).
Conclusion: Our data shows increased lipoperoxidation and blood eosinophilia in ICS DCA patients. We speculate that ICS DCA would benefit from earlier chronic oral CS therapy to prevent consequences of oxidative stress, however further data are needed.
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Ethnic difference and diagnostic value of exhaled nitric oxide levels for predicting asthma in children
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Exhaled nitric oxide (eNO) has been use as non-invasive marker of airway inflammation but normal reference data in children are limited. Levels of eNO in a random sample of 1099 Chinese children aged 11-18 years recruited from Hong Kong were measured online by a chemiluminescence analyzer according to ERS/ATS standard. Each student also completed an ISAAC questionnaire. Children who had never been diagnosed to have asthma and did not have any symptoms of wheeze, rhinitis or eczema were considered as normal controls. Ninety-four steroid naïve asthmatics were recruited from the Asthma Clinic for eNO measurement for comparison. Among the control children, there were 369 boys and 373 girls. In control children, the eNO levels (median, interquantile range) were significantly higher ($P<0.001$) in males (11.4 ppb; 7.7-21.6) than in females (9.0 ppb; 5.9-13.4). For asthmatic males, the median eNO (interquartile range) was 45.5 ppb (28.3-74.1); for asthmatic females, 47.2 (28.8-69.5). Using a cutoff of 15 ppb for girls, the sensitivity and specificity for differentiating asthma from controls are 86% and 78% (AUC of ROC curve =0.89); for boys, the sensitivity and specificity are 80.4% and 79.4% using a cutoff of 25 ppb (AUC of ROC curve = 0.84). This is one of the largest studies of eNO in children and demonstrates a gender difference of eNO levels in Chinese schoolchildren and their levels are higher than the reported values for Caucasians. Different cutoff vales for boys and girls are needed when eNO is used for supporting the diagnosis of asthma in children.
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Classification ability of two electronic noses in asthma and COPD
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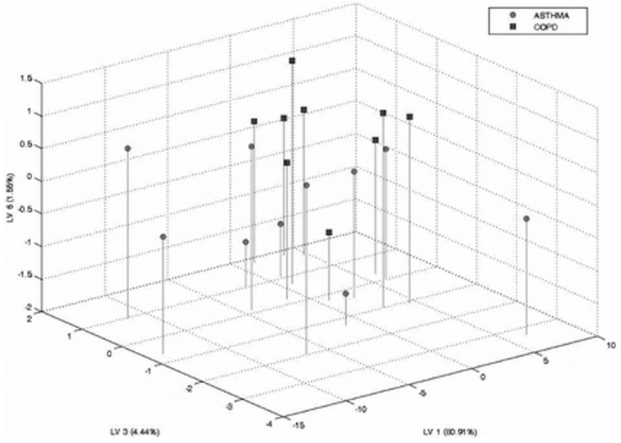
We compared the classification ability of two e-noses based on different technologies in asthma and COPD. Ten patients with severe asthma (3/7, males/females, age 67±2.4 yrs, FEV_1 51.7±7.8% pred, FVC 82.2±7.6% pred, $P<0.001$; 9 non-smokers, 1 current smoker), 9 COPD patients (7/2, males/females, age 69±3.4 yrs, FEV_1 68.9±5.1% pred, FVC 82.6±5.3% pred, $P<0.001$; ex-smokers) and 6 healthy non-smokers (4/2, males/females, age 49±6.7 yrs, FEV_1 109.6±3.6% pred, FVC 109.4±4.4% pred) were studied in a cross-sectional pilot study. After 5 min of tidal breathing with volatile organic compound-free air, two breath samples were collected from each subject and immediately analyzed with Cyranose 320 (Smiths Detection, Pasadena, USA) and Ten 2010 (University of Rome Tor Vergata, Italy). Data were analyzed by partial least square discriminant analysis with leave-one-out cross-validation. E-noses were initially used for classifying healthy subjects and patients with pulmonary disease and, then, asthma and COPD patients. Classification capacity between patients with respiratory disease and healthy subjects was as follow: Cyranose 320, 88%; Ten 2010, 88%; e-nose combination, 92%. Classification rate between asthma and COPD patients was as follow: Cyranose 320, 92%; Ten 2010, 86%, e-nose combination, 94%.



These preliminary results suggest that a combination of e-noses slightly increases classification capacity in patients with severe asthma and COPD.