441. Exhaled biomarkers to assess airway inflammation

4301

Determinants of exhaled NO in a population of subjects with different respiratory symptoms – Results from the Swedish GA²LEN survey

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Fraction of NO in exhaled air (FeNO) is a common method to assess airways inflammation. Determinants of FeNO have nevertheless mostly been studied in the healthy general population. Therefore, we studied determinants of FeNO in a large population of subjects with respiratory symptoms that may reflect the type of patients seen in clinical practice.

FeNO was measured in 1018 subjects with asthma, sinusitis, asthma and sinusitis, or symptoms suggestive of asthma but without fulfilling the asthma diagnosis criteria, aged 17-76 years (median 45 yrs).

Female gender, current smoking and having both parents smoking during childhood were related to lower FeNO while increased height, age, atopy and asthma diagnosis were related to higher FeNO, both before and after adjustments for variables given in Table.

In conclusion, constitutional factors, such as male gender, increased height and age, are related to increased FeNO in subjects with respiratory symptoms. They should be accounted for in clinical practice as their effect size is comparable to the effect of diagnosed asthma. Parental smoking during childhood was related to decreased FeNO and this warrants further studies.

	FeNO relative difference	Adjusted* FeNO relative difference
Female gender	–24% (–30, –19)	–11% (–20, –1)
Height (per 10cm)	15% (10, 19)	7% (2, 13)
Age (per 10yrs)	4% (1, 7)	6% (4, 9)
Atopy	22% (14, 32)	16% (8, 26)
Smoking	–42% (–47, –35)	–41% (–47, –34
Asthma	16% (7, 25)	19% (1, 40)
Both parents smoking during childhood	–18% (–25, –9)	–10% (–18, –1)

*for variables in the Table plus study center, rhinitis, sinusitis, unspecific symptoms, inhaled corticosteroid use, passive smoking and lung function.

4302

Increased exhaled nitric oxide levels predict uncontrolled asthma in children – Results from the MIDAS-study

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The fraction of exhaled nitric oxide (FeNO) is a marker of steroid-sensitive airways inflammation and is used in asthma management. The Asthma Control Test (ACT) is one of the most used instruments to assess control in asthmatic subjects. The evidence for a relation between airways inflammation and asthma control is inconclusive so far. The aim of the present study was to assess the relation between FeNO and ACT score in treated, stable asthma children.

Within the frame of an industry-academy collaboration on minimally-invasive diagnostics (MIDAS), measurements of FeNO and specific IgE against aeroallergen or food allergen mix were done in 165 asthmatic children (101 boys) aged 10-18 years. Among the children, 129 (79%) were positive against aeroallergens and 61 (40%) tested positive against food allergens. Uncontrolled asthma (ACT score <20) was confirmed in 53 children.

FeNO levels in subjects with uncontrolled asthma (n=53) were approximately 30% higher than in subjects with controlled asthma (n=112): 19.8 ppb (15.5, 25.3) vs 15.1 ppb (13.1, 17.4), p=0.04. FeNO was a determinant of asthma control in logistic regression models both before (p=0.01) and after adjustments for gender, age, BMI, FEV1, IgE sensitisation to aero- or food allergens (p=0.04). Furthermore, a significant relation between asthma control and FeNO could be found when using absolute values of the ACT score (r=-0.16, p=0.04).

In conclusion, increased FeNO in treated, stable asthmatic children relate to uncontrolled asthma. It has to be further studied if intensified anti-inflammatory therapy in these subjects would lead to improved asthma control.

4303

Modeling of exhaled nitric oxide in relation to smoking history – A population based study

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Smokers produce less NO in central airways compared to nonsmokers whereas the effect of smoking on NO concentration in the peripheral alveolar regions remain unclear, particularly if axial diffusion of NO is considered. It is also unclear to what extent exhaled NO recover among ex-smokers.

We have measured exhaled NO (FENO) at three flow rates in a population sample of 3968 subjects and the aim of the present analysis has been to try and clarify effects of current and previous smoking on exhaled NO and its central and alveolar origin and to provide reference equations for exhaled NO for healthy smokers.

The essential findings are 1) FENO of ex-smokers and nonsmokers are indistinguishable, 2) the apparent association between FENO and time since smoking cessation in ex-smokers disappears when age is taken into account, 3) smokers have higher NO concentration in the alveolar region than nonsmokers as detected when axial diffusion is considered, 4) the higher limit normal of healthy smokers is considerably lower than the corresponding limit of normal healthy nonsmokers.

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nNO is a good but not perfect screening test for PCD

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Introduction: Nasal NO (nNO) is used to screen for Primary Ciliary Dyskinesia (PCD). nNO and exhaled NO (FENO) are low in PCD and cystic fibrosis (CF), but nothing is known about nNO in patients with humoral immunodeficiencies (HID). We want to study the discriminate validity of nNO and FENO or composite scores for the diagnosis of PCD.

Methods: 157 patients between 5 and 25 years old performed nNO and FENO measurements using the chemiluminescence analyzer Spiroware 3.0® (Eco Medics): 27 with PCD, 28 with CF, 32 with asthma (A), 30 with HID and 40 healthy controls

(C). 6 nNO measurements were performed during slow exhalation against a fixed resistance. For FENO, 2 measurements were done conform ATS/ERS guidelines.
Results: For nNO, median coefficient of variation (CV) was 7.2% (P25 4.1%, P75 19.7%, range 1 to 92%), despite perfect test performance.
nNO differed significantly between PCD and no PCD and between PCD and CF, A, HID and C individually. nNO < 270 ppb had a sensitivity of 92.6% and a specificity of 89.2% to diagnose PCD. Area under the curve (AUC) for the ROC curve was 0.978. However, some overlap exists between PCD and CF, HID and A. FENO was significantly lower in PCD versus no PCD and in PCD versus CF, A, HID and C. FENO < 7.1 ppb had a sensitivity of 92%, but only a specificity of 71.6% (AUC 0.833).
The composite scores nNO + FENO, nNO * FENO, nNO * $FENO^2$ all had a lower AUC for the ROC curve, only the composite score nNO^2 * FENO had an equal AUC and a slightly higher specificity (93.1% for a sensitivity of 92.0%) than nNO measurement alone.
Conclusion: Both nNO and FENO are useful screening tests for PCD: using the composite score nNO^2 * FENO increases the specificity with stable sensitivity.

4305
Expiratory flow rate and breath hold affect exhaled volatile organic compounds (VOC) in healthy subjects
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Introduction: Exhaled breath volatile organic compound (VOC) analysis for airway disease monitoring is promising; however, contrary to nitric oxide the method for exhaled breath collection has not been standardized and the effects of expiratory flow rate and breath hold have not been studied. These manoeuvres may also reveal the origin of exhaled compounds.
Methods: 12 healthy volunteers (34±7 years) participated in the study. Subjects inhaled through their nose and exhaled immediately at two different flow rates (5 L/min and 10 L/min) into methylated polyethylene bags. In addition, the effect of 15 s breath hold following inhalation to total lung capacity was studied. The samples were analyzed for ethanol and acetone levels immediately using proton-transfer-reaction mass-spectrometer (PTR-MS, Logan Research).
Results: Ethanol levels were negatively affected by expiratory flow rate (250±38 vs. 204±31 ppb at 5 L/min and 10 L/min, respectively, $p<0.01$), but remained unchanged following the breath hold (205±43 vs. 179±41 ppb, without and with breath hold, respectively, p=0.11). On the contrary, acetone levels were increased following breath hold (963±272 ppb vs. 1,155±306 ppb, without vs. with breath hold, respectively, p=0.02), but were not affected by expiratory flow rates (1,196±208 vs. 1,058±217 ppb, 5 L/min vs. 10 L/min, respectively, p=0.14).
Conclusions: Exhalation parameters such as expiratory flow rate and breath hold may affect VOC levels significantly; therefore standardisation of exhaled VOC measurements is mandatory. Our preliminary results suggest a different origin in the respiratory tract for these two gasses.
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Acute effect of inhaled corticosteroid on exhaled breath temperature in asthmatic patients
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Background: Measurement of exhaled breath temperature (EBT) with a portable hand held device is applicable as a non-invasive method to monitor the control of asthma. While inhaled corticosteroids (ICS) have proven suppressive effect on airway inflammation decreasing EBT in the long run, there is no data on the immediate effect of inhaling them. The aim of this study was to explore the acute effect of ICS on EBT.
Methods: We recruited 21 subjects (mean age 47 years, 12 women) from among the patients of the Clinic of Allergy and Asthma in Sofia, Bulgaria: 12 were with controlled; 9 with partly controlled asthma. Patients were randomized to inhale 2 puffs of either ciclesonide or placebo from identical looking inhalers in the morning of consecutive days. Spirometry and assessment of EBT by means of PHD (X-halo, Delmedica, Singapore) were performed before and at 4 time points (5, 30 60 and 120 min.) after inhalation.
Results: Compared to baseline EBT (mean 33.78 ± s.e.m.0.10°C, ±), there was a significant increase in EBT 5 min. (34.17±0.10, P=0.001), 30 min. (34.07±0.10, P=0.002), and 60 minutes (33.90±0.10, P=0.046) post ICS inhalation. The pre-/post- inhalation differences between ICS and placebo differed significantly (P=0.001). Inhalation of ICS and placebo did not bring about any significant changes in the spirometry of the patients. There was no difference between the controlled and the partly controlled asthmatics in EBT changes.
Conclusions: EBT increases 1 hour after inhalation of ICS in a dose, prescribed for maintenance treatment of asthma. This needs to be taken into consideration if EBT is applied for monitoring of the disease.

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Calibration of a (semi)-automatic measurement and control platform for centralized, simultaneous electronic nose (eNose) analyses in multi-centre trials
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Rationale: Breath analysis by electronic nose (eNose) technology represents a promising diagnostic tool in lung disease. A critical step in making this technology suitable for multi-centre trials, such as the U-BIOPRED Study, is to facilitate centralized measurements on multiple eNoses simultaneously. This can be accomplished with a (semi)-automatic measurement and control platform.
Aim: To calibrate and analyze repeatability of multiple sensors in an eNose platform (5 eNoses, 4 brands).
Methods: Ethanol was chosen as one of the calibration gases. Different concentrations (500 ppb-8 ppm) were generated by a permeation system. Measurements at all concentrations were done in duplicate. Total number of sensors in the platform was 81. The obtained data were processed by averaging duplicate measurements after normalisation (scale 0-1).
Results: The platform (not all individual sensors) was sensitive to ethanol at used concentrations (Fig. 1). The difference in normalized sensor deflections between duplicate measurements at 2 ppm was (mean [SD], range): 0.09 [0.1], 0.55-0.0004.

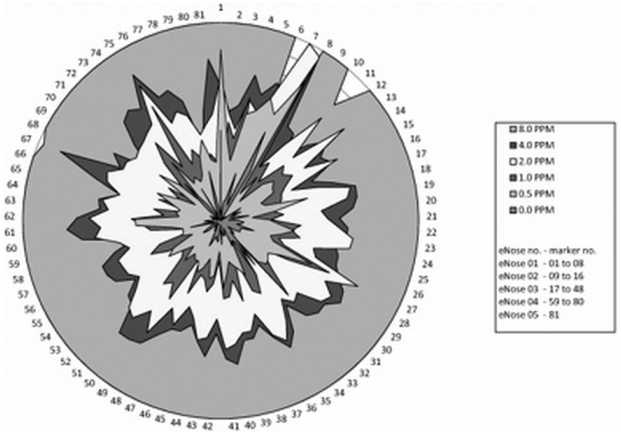

Figure 1. Normalized data from 5 eNoses based on different concentrations of ethanol.

Conclusion: The eNose platform is capable of detecting ethanol at concentrations from 500 ppb to 8 ppm level with acceptable repeatability.
Implication: This method of platform calibration with standard gases is feasible and mandatory for quality control of eNose assessments in a multi-centre setting.

4308
Do volatile organic compounds discriminate between eosinophils and neutrophils *in vitro*?
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Inflammation associated oxidative stress leads to peroxidation of polyunsaturated fatty acids thereby generating volatile organic compounds (VOCs) excreted in exhaled air. The purpose of the present study is to examine whether specific VOCs are associated with eosinophilic and neutrophilic inflammation, and thus offers the possibility of noninvasive monitoring of both asthma inflammatory phenotypes.
Methods: Eosinophils and neutrophils were isolated from 27ml blood of 16 healthy non-smokers by gradient centrifugation using lymphoprep. Eosinophils were isolated from neutrophils by immunomagnetic cell separation (MACS) using anti-CD16. The average absolute number of eosinophils and neutrophils upon isolation was 3.5 x 10^6 and 19.4 x 10^6 respectively. Cells were incubated in RPMI at 37°C and activated with phorbol 12-myristate 13-acetate (100ng/ml). Headspace air was sampled at time 0', 30', 60' and 90' by introduction of ultra-pure nitrogen in closed flasks at a flow rate of 200 ml/min during 10 min. The air was pushed out onto a carbon tube and the total amount of trapped VOCs (volatome) was analysed by time-of-flight GC-MS.
Results: From the 2005 compounds present in the volatome, those present in at least 8% of the samples (1123 compounds) were used for further analysis. Discriminant analysis (SPSS statistics19) showed that 5 VOCs were able to distinguish between both culturing types with 100% and 96% correct classification in original

and cross-validated set respectively. Chemical identification of the compounds is ongoing and these are potential candidates to check in asthmatic patients for their possible diagnostic value in asthma phenotyping.