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Background: Eosinophilic airway inflammation is heterogeneous in asthma. We recently described a distinct subtype of asthma defined by the expression of genes inducible by Th2 cytokines in bronchial epithelium. This gene signature, which includes periostin, is present in approximately half of asthmatics, and correlates with eosinophilic airway inflammation. However, identification of this subtype depends on invasive airway sampling, hence non-invasive biomarkers of this phenotype are desirable.
Objective: Identify systemic biomarkers of eosinophilic airway inflammation.
Methods: We measured fractional exhaled nitric oxide (FeNO) and peripheral blood eosinophil, periostin, YKL-40, and IgE levels and compared these biomarkers to airway eosinophilia in 5 cohorts of asthmatics across a range of severity (N=150).
Results: We replicated our previous finding of a three-gene bronchial epithelial Th2 signature in a subset of asthmatics and found that peripheral blood periostin levels were highly correlated to the gene signature. Blood periostin is significantly elevated in asthmatics with evidence of eosinophilic airway inflammation relative to those with minimal eosinophilic airway inflammation despite inhaled corticosteroid (ICS) treatment across a range of disease severity. A logistic regression model including sex, age, body mass index (BMI), IgE, blood eosinophils, FeNO, and serum periostin in 59 severe asthmatics showed that, of these indices, serum periostin was the single best predictor of airway eosinophilia (p=0.007).
Conclusions: Periostin is a systemic biomarker of airway eosinophilia in asthma and has potential utility in patient selection for emerging asthma therapeutics targeting Th2 inflammation.

4715
Pathophysiology of airway hyperresponsiveness in patients with nasal polyposis
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It has been hypothesized that airway hyperresponsiveness (AHR) is characterized by sensitivity (strength of stimulus) and reactivity (responsiveness to stimulus); the latter could be the intrinsic characteristic of AHR. The underlying mechanisms leading to AHR could be 1) airway inflammation, 2) reduction of forces opposing bronchoconstriction, and 3) structural airway changes/geometric factors. Our main objective was to assess the relationships between reactivity and these three mechanisms using measurements of 1) bronchial and bronchiolar/alveolar NO, 2) bronchomotor response to deep inspiration, and 3) forced expiratory flows and an index of airway to lung size, i.e. FEF25-75%/FVC.
Patients with nasal polyposis underwent spirometry, multiple flow measurement of exhaled NO, assessment of bronchomotor response to DI by forced oscillation technique and methacholine challenge allowing the calculation of reactivity (slope of the dose-response curve) and sensitivity (PD10).
One hundred and thirty-two patients with nasal polyposis were prospectively enrolled of whom 71 exhibited AHR. Airway reactivity was correlated with alveolar NO concentration (rho= 0.35; p=0.017), with airflow limitation (FEF25-75%: rho= -0.40; p=0.003) and with an index of airway size to lung size (FEF25-75%/FVC: rho= -0.38; p=0.005), of which only alveolar NO remained the only independent factor in a stepwise multiple regression analysis. Airway sensitivity was not correlated with any pulmonary function or exhaled NO parameter.
Conclusion: In patients with nasal polyposis, the main determinant of reactivity is alveolar NO, suggesting that bronchiolar/alveolar lung inflammation constitutes one intrinsic characteristic of AHR.

4716
Steroid titration against mannitol in mild to moderate persistent asthma (STAMINA trial)
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Objectives: To compare titrating ICS against mannitol airway hyperresponsiveness (AHR) or British Thoracic Society (BTS) outcomes on asthma control over 1 year in the community.
Methods: After an ICS tapering phase, 157 persistent asthmatics were randomised (parallel) and followed for 1 year. Their subsequent ICS dose (as ciclesonide) was titrated against either mannitol PD_{10} (AHR strategy) or standard BTS outcomes. Study visits were performed in a primary care.
Results: Both groups prior to step down (n=80 AHR, n=77 BTS) were matched by age, sex, FEV_1%, mannitol PD_{10} and pre existing ICS dose. Significantly fewer cumulative episodes of loss of control occurred in the AHR rather than BTS group (84 vs 118, p=0.018), amounting to a 24% lower rate (1.32 v 1.73 episodes of loss of control/patient/year).
Significant improvements were seen in the AHR group for inflammatory markers including mannitol PD10, methacholine PC20, salivary eosinophil cationic protein (ECP), exhaled nitric oxide, symptoms and reliever use. Final mean inhaled Ciclesonide dose was higher (p<0.0001) in AHR group; 514 ug vs 208 ug (BTS), with no significant suppression of overnight urinary cortisol/creatinine.

488. Phenotyping airway diseases

4714
Periostin is a systemic biomarker of eosinophilic airway inflammation in asthma
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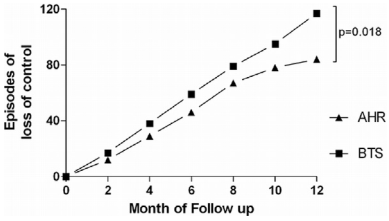

Conclusions: Managing patients in primary care using mannitol to guide ICS therapy resulted in significant reductions in episodes of loss of control, symptoms and reliever use, along with suppression of inflammatory markers but not adrenal function.

4717

Preparing asthmatic patients to climb to extreme high altitude (asthma Aconcagua expedition)

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A group of 18 asthmatics were evaluated in preparation for an expedition to climb the Aconcagua mountain (6900m).
Patients were evaluated at different time points before expedition: screening visit, hypoxia chamber and cold air exposition (in an attempt to simulate conditions at extreme altitude) as well as before/after the expedition. During the hypoxia simulating experiment, patients stayed for 30 min in a chamber filled with 11% of oxygen followed by a maximal exercise test. During the cold air exposition, patients resided for 24 hours in an indoor ski resort (mean temperature of -8 °C). All patients except one (score of 18/25) had an ACT well controlled asthma (ACT > 20). During one year of preparation, FeNO values significantly reduced (p=0.01) while lung function parameters remained stable. FEV1 and FeNO values were slightly but significantly lower (mean change in%pred FEV1 of 2.7%, p=0.01 and mean change in FeNO of 2.2 ppb, p=0.03) after a maximal exercise protocol under hypoxic conditions (FiO2=11%). A significant decrease in FEV1 was also observed after a 24h stay at -8 °C (mean change of 6.6%, p=0.009). This was accompanied by an increase in sputum neutrophils (13% pre versus 48.6% post, p<0.01) but was not associated with a change in FeNO levels (p=0.07).
During one year of preparation prior to climbing the Aconcagua mountain, there was an improvement in airway inflammation despite adequate asthma control at baseline. Exercising in hypoxic conditions (60 min, FiO2 11%) induced a minor decrease in FEV1 and FENO in asthmatics. Exposure of asthmatic patients to cold air for 24hr resulted in a larger decrease in FEV1, which was associated with a neutrophilic airway inflammation.

4718

Inflammatory subtypes of non-atopic asthma

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Introduction: In asthma, several types of airway inflammation have been described (Simpson Respirology 2006), which may represent clinically different subtypes of asthma. Whether non-atopic asthma can also be divided into different subtypes according to airway inflammation is unknown.
Aim: To investigate whether different inflammatory profiles correspond with distinct clinical and functional characteristics in patients with non-atopic asthma.
Methods: In a cross-sectional study we included outpatients with non-atopic asthma and divided them into 4 types of airway inflammation. All patients filled out questionnaires (co-morbidity, ACQ, AQLQ, Sino-Nasal outcome Test (SNOT)) and underwent spirometry, blood tests, sputum induction and nasal endoscopy.
Results: 62 patients (90% adult onset) were included. We found no differences in BMI, questionnaires or spirometry. Differences between groups: see Table 1.

Table 1

	Eosinophils >3% (n=14)	Neutrophils >64% (n=23)	Both eo/neutro (n=3)	Non eo/neutro (n=22)	p-value
Male%	77	30	0	27	0,01
Age (SD)	59 (10,3)	53 (15,2)	57 (11,2)	49 (14,3)	0,24
Auto-immune, %	8	44	67	18	0,03
IgE median, range	180 (23–2110)	13 (1–243)	30 (5–133)	15 (3–88)	<0,001
PC20 median, range	2,5 (0,3–>9,8)	6,7 (0,8–>9,8)	0,02 (0,2–>9,8)	3,6 (0,2–>9,8)	0,07
Nasal polyps, %	39	11	0	5	0,04

Auto-immune = DM, thyroid dysfunction, reumatoid arthritis and other.

Conclusion: Almost all adult non-atopic asthma patients have their onset of asthma in adulthood. There are at least 2 distinct phenotypes: an eosinophilic phenotype, characterized by male gender, high levels of IgE and nasal polyps, and a neutrophilic phenotype associated with extra-pulmonary auto-immune diseases.
Implication: Fenotype specific therapies, that might improve outcome of non-atopic asthma, should be further evaluated.

4719

The role of food allergy in adult onset asthma

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Background: Adult onset asthma is a poorly characterized phenotype of asthma. Whereas in childhood-onset asthma food allergies are closely related to the development of asthma (Zeiger JACI 1995), this relationship has never been studied in adult onset asthma. We hypothesize that food allergies are present in adult onset asthma, in particular in patients with high levels of total IgE.
Aim: To investigate the presence of specific IgE antibodies against a panel of common food and aeroallergens in patients with adult onset asthma, and to relate this to clinical, lung function and inflammatory markers.
Methods: In 150 patients (65% female; age 52.3 (±13.5)yr) specific IgE against food allergens (milk, soy, cod, peanut, ovalbumin, wheat), aeroallergens (house dust mite, cat, dog, tree, grass, herbs, mould), total IgE, FeNO and spirometry were cross-sectionally compared between patients with and without (food) allergies.
Results:

	Non-atopic (n=81)	Atopic (n=67)		P-value
		No food allergy (n=51)	Food allergy (n=16)	
Age*	56.1 (11.8)†	51.9 (10.5)	51 (12)	0.3
Male gender%	36	36	63#	0.04
Asthma severity%	51	36	56	0.4
Nasal polyps%	52†	32	19	0.053
Total IgE‡	50 (2–1068)†	226 (17–2076)	700 (105–4567)#	<0.01
Blood eosinophils‡	0.19 (0.02–0.92)	0.26 (0.05–1.17)	0.2 (0.03–0.21)	0.053
Exhaled NO‡	48.5 (55.6)	44.9 (40.5)	38.8 (34.9)	0.5
pbFEV1	90.8 (21.4)	91.8 (20.3)	90.6 (17.2)	0.9

*Mean (SD); ‡Median (range); pb = post bronchodilator. #p<0.05 atopic with food allergy vs non-atopic, †p<0.05 non-atopic vs atopic.

Conclusions: 10% of patients with adult onset asthma have food allergies. These patients have higher levels of total IgE and are more often males. This implies that screening for food allergies in patients with adult onset asthma and high levels of IgE is warranted.

4720

Expression of prohibitin 1 mitochondrial protein in non-COPD and COPD smokers

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Introduction: Prohibitin 1 (PHB1) is a versatile protein that is located in the inner mitochondrial membrane, maintaining normal mitochondrial function and morphology. Prohibitin interacts with NADH dehydrogenase, a protein complex essential for the oxidoreductase activity within cells. However, its expression in lung epithelium, especially in patients with inflammatory lung diseases associated with increased oxidative stress, such as COPD, is unknown.
Aim: To study PHB1 expression in lung tissue of non-smokers, non-COPD smokers and COPD patients.
Methodology: Lung tissue specimens from 30 male subjects were studied: 15 COPD patients [age: 65.9±6.2 years, smoking: 88.9±35.2 pack-years, FEV1 (% pred): 58.4±16.4, FEV1/FVC (%): 66.2±8.6], 10 non-COPD smokers [age: 57.0±11.7 years, smoking: 67.1±39.9 pack-years, FEV1 (% pred): 84.0±15.9, FEV1/FVC (%): 80.0±3.8] and 5 non-smokers. Quantitative Real-Time PCR and Western Blot experiments were carried out for PHB1, using beta-actin as internal control.
Results: Non-COPD smokers exhibited lower prohibitin levels when compared to non-smokers (0.55±0.06 vs. 0.90±0.06, p=0.011), while PHB1 mRNA levels were even further decreased in COPD patients (0.32±0.02), a finding statistically significant vs. both non-COPD smokers (p=0.012) and non-smokers (p=0.009). Western blot analysis verified the above results (non-smokers: 1.77±0.10; non-COPD smokers: 0.97±0.08; COPD patients: 0.62±0.09, p=0.028).
Conclusion: The significantly downregulated prohibitin levels in non-COPD and COPD smokers in comparison with non-smokers possibly reflects a distorted mitochondrial function, resulting in decreased anti-oxidant activity, especially in the mitochondria of COPD patients.

4721

Biomarker discovery in chronic obstructive pulmonary disease (COPD) using epithelial lining fluid: A proteomic approach

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Aim: To discover proteins that change in abundance in ELF (Epithelial Lining Fluid) from COPD patients versus healthy controls using a quantitative proteomics approach.

Methods: The ELF proteome from COPD patients and healthy controls was studied by 1D polyacrylamide gel electrophoresis followed by in-gel tryptic digestion to assess the feasibility of such an approach. 40 gel slices were obtained from each lane of the gel (corresponding to one patient). Digested samples were analyzed by nanoChip-LC-MS/MS using an ion trap.

We performed a quantitative pilot study of ELF from 4 COPD patients and 4 healthy controls [table 1] to test for statistically significant differences in protein levels. ELF samples were digested by trypsin, labeled with stable isotope-containing reagents (iTRAQ®, 8-plex) and processed by strong cation-exchange chromatography followed by nanoLC-MS/MS. In order to validate the results, a second quantitative analysis of an independent sample set (4 COPD vs 4 healthy) using the same methodological approach was done.

Results: The 1D electrophoretic approach resulted in more than 300 identified proteins. Most of the identified proteins were present in both COPD and healthy samples, although some proteins were only identified either in healthy control or in COPD samples.

The quantitative studies showed a number of proteins significantly different between ELF of COPD patients and controls, including 4 up-regulated proteins in common in both studies.

Conclusions: The obtained results show the possibility to discover proteins differentially expressed in ELF of COPD patients and controls. We are currently validating these proteins by western blot and immunohistochemistry.