

WEDNESDAY, SEPTEMBER 5TH 2012

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## 492. Bronchial challenge as a biomarker of respiratory disease

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**Baseline airway inflammation may be a determinant of ozone response in asthmatic patients**

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**Background:** It is well known that ozone (O<sub>3</sub>) exposure induces lung function decrease and airways neutrophilia, but a great variability in airways response has been observed among patients with asthma.

**Aim:** To find predictors of functional and biological airway response to O<sub>3</sub> exposure in mild to moderate asthmatic patients.

**Methods:** We studied 120 patients with mild-to-moderate asthma (FEV<sub>1</sub>% 89.4±14.3), randomly exposed to air or O<sub>3</sub> (0.3 ppm for 2 hrs) in a challenge chamber. Symptoms and Pulmonary Function Test were measured before and immediately after exposure. Six hours after exposure, induced sputum was collected. Patients were evaluated according to their functional ( $\Delta$ FEV<sub>1</sub>air-O<sub>3</sub>) and neutrophilic ( $\Delta$ neutro%air-O<sub>3</sub>) response to O<sub>3</sub>. Age, baseline FEV<sub>1</sub>%pred, inhaled corticosteroids (ICS) therapy, baseline sputum neutrophils/ml and eosinophils/ml counts, methacholine responsiveness, atopy and smoking habit were considered as possible predictors of functional and neutrophilic response.

**Results:** FEV<sub>1</sub> responders had a lower percentage of ICS-treated patients and lower baseline FEV<sub>1</sub> values in comparison with non-responders. Neutrophil responders were younger, more responsive to methacholine challenge and had lower baseline sputum inflammatory cell counts in comparison with non-responders.

**Conclusions:** Patients without ICS therapy and lower FEV1 are more susceptible to functional response to O3. Bronchial hyperresponsiveness and baseline sputum inflammation may predict a neutrophilic airway response to O3; high sputum neutrophil percentages are protective against a further neutrophilic response to O3. Therefore, determinants of functional and inflammatory responses to O3 are different.

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**Airway hyperresponsiveness to mannitol as a predictor of treatment response to ciclesonide in patients with suspected asthma: A double-blind, randomised, placebo-controlled trial**

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**Background:** Inhaled corticosteroids (ICS) are the basic treatment in patients with asthma. The aim of the study was to find predictors which detect subjects who clinically benefit from ICS using a combined endpoint (improvement in either FEV1 [ $>12\%$  and  $>200\text{ml}$ ], Juniper asthma control questionnaire [ACQ]  $>0.5$  or asthma quality of life questionnaire [AQLQ]  $>0.5$ ).

**Methods:** 70 treatment-naïve subjects (mean age 40.3 years (range 18-70); 21 male) with suspected asthma were randomised to inhale ciclesonide (320mcg/day,  $n=34$ ) or placebo ( $n=36$ ) in a double-blind manner for 1 month. Spirometry, airway hyperresponsiveness (AHR) to mannitol and to methacholine, exhaled nitric oxide, ACQ and AQLQ were assessed before and after treatment.

**Results:** An improvement in the combined endpoint was seen in 16 subjects (47%) in the ciclesonide group (1 FEV1, 9 ACQ, 14 AQLQ) and in 15 subjects (42%) in the placebo group (2 FEV1, 5 ACQ, 13 AQLQ). AHR to mannitol was found in 8/16 responders and only 3/18 non-responders in the ciclesonide group (sensitivity 50%, specificity 83%, PPV 72%, NPV 65%,  $p=0.038$ ). Using a logistic regression analysis, AHR to mannitol was the only significant predictor of a response to ciclesonide with an odds ratio of 5.0 (95%CI 1.03-24.28,  $p=0.046$ ).

**Conclusions:** In subjects with suspected asthma, AHR to mannitol is a predictor of subsequent response to ICS treatment.

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**Electromyography of the parasternal intercostal muscles in bronchial hyperresponsiveness testing**

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**Background:** Bronchial hyperresponsiveness testing (BHT) can be used to support or refute a diagnosis of asthma, but requires technically-acceptable spirometry to be performed. Parasternal intercostal muscle electromyography (EMGpara) provides a non-invasive, effort-independent method to assess load on the respiratory system. The relationship between EMGpara and spirometry in adults undergoing BHT is unknown.

**Methods:** 16 subjects (mean (SD) age 29.5 (7.4) years) underwent methacholine challenge testing, performed in accordance with ATS guidelines. Surface EMGpara was recorded bilaterally from the 2nd intercostal space, at baseline and between consecutive methacholine doses. EMGpara was converted to root-mean-square (RMS) and mean peak RMS EMGpara per breath calculated.

**Results:** Significant relationships were observed between methacholine concentration, EMGpara and FEV1 (all  $p<0.001$ ). Subjects with a positive test response ('responders', defined as change in FEV1  $\geq 20\%$ ,  $n=8$ ) showed a significant median (range) change in both EMGpara (45.2 (-0.65 - 157.4)%,  $p<0.05$ ) and FEV1 (-33.5 (-25.2 - -58.6)%,  $p<0.01$ ). EMGpara did not change significantly in subjects with  $<20\%$  drop in FEV1 ('non-responders',  $n=8$ ). The median (range) change in EMGpara at PC20 was 35.6 (2.6 - 57.6)%. Change in EMGpara was significantly correlated with change in FEV1 in the responder group ( $r=-0.883$ ,  $p=0.015$ ), but not in non-responders.

**Conclusions:** EMGpara can detect change in respiratory load during methacholine-induced bronchoconstriction. The change in EMGpara was greater than that in FEV1. EMGpara may provide an alternative outcome measure in BHT in patients unable to perform spirometry adequately.

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**Clara cell protein (CC16) in serum and urine after exercise challenge**

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Exercise is known to affect the airway epithelium, giving rise to a release of inflammatory mediators, such as Clara cell protein (CC16). The aim of this study was to investigate the epithelial involvement during exercise, by repeatedly measuring the level of CC16 in serum and urine in asthmatic subject and healthy controls. Asthmatic subjects ( $n=18$ ) and healthy controls ( $n=12$ ) performed an 8 min exer-

cise challenge on a treadmill. Serum was collected before and 1, 5, 10, 15, 20, 30, 45 and 60 min after the challenge. Urine was collected before and 30 and 60 after the challenge. CC16 was measured in serum and urine. Exhaled nitric oxide was measured at 50, 100, 200 and 300 ml/s.

The serum level of CC16 was significantly increased directly after exercise in all subjects with a further increase after an additional 15 minutes. The increase in serum CC16 was more pronounced in asthmatic subjects. In urine, there was a clear increase in CC16 levels in all subjects 30 min after exercise, returning back to baseline after 60 min. No difference between asthmatic subjects and healthy controls could be seen in urine levels of CC16. A decrease in alveolar NO concentration was seen after the exercise challenge.

The CC16 response measured in serum are more pronounced inter- and intra-individually than in urine. The increase in serum CC16 both directly after the test and at a later time point indicates that the CC16 increase in serum may be explained by a combination of leakage followed by active secretion. This could explain the different response between asthmatics and healthy controls with an assumed altered epithelial involvement seen in the asthmatic subjects after exercise provocation.

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**Alterations in static lung volumes during methacholine challenge (MCH) tests, assessed by whole-body plethysmography using the aerosol provocation system (APS)**

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**Rationale:** Airway hyperreactivity (AHR) is a characteristic feature of asthma, and methacholine challenge tests (MCT) are well established in asthma diagnostics and research, to quantitate AHR. Cut points have arbitrarily been selected, defining a fall of 20% in FEV1 as PC20. By this technical approach, however, changes in end-expiratory resting level cannot be detected. MCT using APS technology integrated in the Jaeger whole-body plethysmograph features the possibility to evaluate changes of airway dynamics concomitantly with changes of lung volumes [1].

**Objectives:** We aimed to assess changes of FRC<sub>pleth</sub>, IC, ERV and hence RV and TLC during MCT, and comparing changes of effective specific airway conductances ( $sG_{eff}$ ,  $sG_{0.5}$ ,  $sG_{tot}$ ) in relation to changes effected to FEV1 and MEF50.

**Methods:** We retrospectively analysed data from our hospital database including 140 test persons (asthmatics and controls; 59 males; 81 females; age: 11 to 82 y), in whom MCT have been performed. Methacholine was administered during 3 consecutive cumulative challenge levels (P1:0.2 mg; P2:1.0 mg; P3:2.2 mg) computerized by the APS system.

**Results:** During MCT not only airway mechanics, but also static lung volumes (FRC<sub>pleth</sub>, IC) changed consistently.

**Conclusions:** In the assessment of AHR by MCT  $sG_{eff}$  and  $sG_{tot}$  qualified as most sensitive target parameter, taking into account changes of airway dynamics concomitantly with changes of lung volumes. Comparison between volume-time and flow-volume parameters with plethysmographically assessed  $sG_{eff}$ ,  $sG_{0.5}$  during MCT is presented in abstract 850930.

[1]. Matthys H, et al.: Respiration: 1975;32(2):121-134.

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**Bronchial hyperresponsiveness using mannitol in morbid obesity before and after bariatric surgery**

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**Introduction:** The association between bronchial hyperresponsiveness (BHR) and morbid obesity (MO) is common.

**Objective:** To investigate the prevalence of BHR using mannitol (MAN) in patients with MO before and one year after bariatric surgery (BS).

**Methods:** We determined BHR in 70 patients with MO (46 $\pm$ 11 yr; BMI, 46 $\pm$ 6 kg/m<sup>2</sup>; 53 females; smokers, [33 $\pm$ 26 pack-yr] 59%). BHR(+) was defined as a PD15 $<$ 635mg. Severe OSA (apnea/hypopnea Index [AHI]  $\geq$  30 events/h) was present in 31 patients.

**Results:** Before BS, patients had FEV1, 93 $\pm$ 15%; FEV1/FVC, 0.83 $\pm$ 0.05; bronchodilator test, +4.9 $\pm$ 0.7%. Twenty two had BHR(+) (PD15=78 mg) with an FEV1 fall of -20 $\pm$ 2%. BMI and central obesity (abdominal circumference and waist-hip-ratio) were higher in patients with BHR(+) than in those BHR(-) ( $p<0.05$  each). Likewise, the AHI was higher (53 $\pm$ 10 vs 29 $\pm$ 5 events/h) in patients with BHR(+) than in those BHR(-) ( $p<0.05$ ).

The ratio-dose-response (RDR) to MAN was positively associated with BMI ( $r=0.30$ ,  $p<0.05$ ). There were also associations between AHI and TNF- $\alpha$ , and between the abdominal circumference and TNF- $\alpha$  ( $r=0.40$ ,  $p<0.05$  each).

After BS, all but one BHR(+) patient, reverted their BHR (PD15  $\geq$  635 mg) with an FEV1 fall of -8 $\pm$ 2%, and IL-8 levels decreased in all patients ( $p<0.05$ ).

**Conclusions:** In MO the prevalence of BHR with MAN is elevated, which is associated with increased BMI, central obesity and AHI. Our findings suggest that

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obstructive sleep apnea and central obesity can in MO share a similar pathogenic mechanism related to development of BHR.

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#### Sensitivity and specificity of airway hyperreactivity (AHR) based on methacholine challenge (MCH) tests – Comparison of $sG_{eff}$ with $FEV_1$ and $MEF_{50}$ as target parameter

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**Rationale:** Airway hyperreactivity (AHR) is a characteristic feature of asthma, and methacholine challenge tests (MCT) are well established, mostly defining a fall of 20% in  $FEV_1$  as  $PC_{20}$ . In contrast to this spirometric technique, whole-body plethysmography features the possibility to evaluate changes of airway dynamics concomitantly with changes of lung volumes (see also abstract 850929).

**Objectives:** Assessment of sensitivity and specificity of MCT, comparing changes of effective specific airway conductances ( $sG_{eff}$ ,  $sG_{0.5}$ ,  $sG_{tot}$ ) with changes effected to  $FEV_1$  and  $MEF_{50}$ .

**Methods:** Data from 140 test persons (asthmatics and controls; 59 males; 81 females; age: 11 to 82 y), in whom MCH was administered during 3 consecutive cumulative challenge levels (P1:0.2 mg; P2:1.0 mg; P3:2.2 mg) computerized by the APS system, were retrospectively evaluated.

**Results:** Highest response rates were found for  $sG_{eff}PD_{50}$  (82.1%) and  $sG_{tot}PD_{50}$  (77.1%), lowest for  $FEV_1PD_{20}$  (34.3%), and intermediate for  $MEF_{50}PD_{50}$  (45%).  $sG_{eff}$  reached its  $PD_{50}$  at  $0.56 \pm 0.48$  mg,  $FEV_1$  its  $PD_{20}$  only at  $0.94 \pm 0.57$  mg MCH. Sensitivity of MCH-tests (s) and specificity (f) were obtained as follows:  $sG_{eff}$  (s: 95.7%; f: 80.0%),  $sG_{tot}$  (s: 91.3%; f: 88.0%),  $FEV_1$  (s: 38.3%; f: 84.0%) and  $MEF_{50}$  (s: 48.7%; f: 72.0%).

**Conclusions:** In comparison to  $FEV_1$ , assessment of AHR by  $sG_{eff}$  and  $sG_{tot}$  reached their PD at much lower MCH doses, in a much higher percentage of tests. The advantage of MCT by plethysmography, measuring changes of airway dynamics in relation to changes of the volume history, features the advantage to perform MCT with better safety for the patients.

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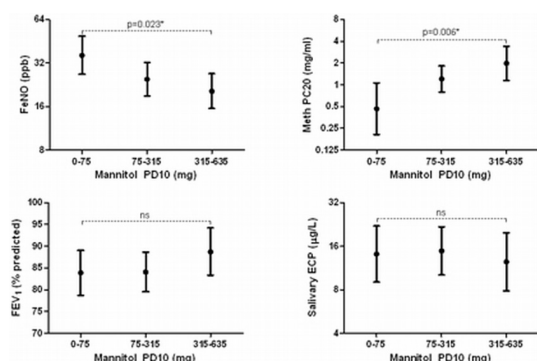
#### Relationships of mannitol challenge to methacholine challenge and inflammatory markers in asthmatics receiving inhaled corticosteroids

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**Background:** Mannitol is a novel osmotic indirect bronchial challenge agent thought to reflect underlying inflammation in asthma. We explored relationships of mannitol airway hyper-responsiveness (AHR) to other measures of airway inflammation and to methacholine challenge.

**Methods:** Screening data were analysed of 143 mild to moderate asthmatics, all taking inhaled corticosteroids (ICS), who had mannitol and/or methacholine challenges, fractional exhaled nitric oxide (FeNO) and salivary eosinophilic cationic protein (ECP) measured together. Mannitol AHR was grouped by  $PD_{10}$ : mild (315-635mg); moderate (75-315mg); severe (0-75mg).

**Results:** Mannitol  $PD_{10}$  groups were significantly different overall for FeNO ( $p=0.023$ ): 43% higher in the severe vs. mild group. There was a significant overall difference for methacholine  $PC_{20}$  ( $p=0.006$ ): a 2.1 doubling dilution difference between severe vs. mild mannitol groups.



Mannitol  $PD_{10}$  airway hyper-responsiveness groups: vs. FeNO (top left), Methacholine  $PC_{20}$  (top right), Forced expiratory volume in 1 second ( $FEV_1$ ) as % of predicted (bottom left), and salivary ECP (bottom right). Data presented as geometric means with 95% confidence error bars. \*ANOVA comparisons with significance at  $p < 0.05$ .  $PD_{10}$  = provocative dose (of mannitol) causing 10% fall in  $FEV_1$ . FeNO (fractional exhaled nitric oxide). Meth  $PC_{20}$  = provocative concentration (of methacholine) causing 20% fall in  $FEV_1$ . ECP = eosinophilic cationic protein. ppb = parts per billion.

No significant differences were found across groups for: salivary ECP,  $FEV_1\%$ , or ICS dose. Mannitol  $PD_{10}$ , methacholine  $PC_{20}$  and FeNO as continuous variables all correlated with each other.

**Conclusions:** Mannitol challenge reflects both underlying inflammation using FeNO and direct AHR using methacholine. Thus mannitol may be a useful screening tool for the assessment of asthmatic patients receiving ICS.