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ABSTRACTS
Soluble mediators derived from bronchial epithelium are able to drive Th2 differentiation in the context of rhinovirus infection

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Introduction: The majority of acute asthma exacerbations follow upper respiratory infections, and most are rhinovirus induced. The pathways by which a rhinovirus infection may lead to asthma development are still under scrutiny, but the role of bronchial epithelium in driving this mechanism has been considered of great importance. It has been shown that the epithelial derived cytokines IL25, IL33 and TSLP are upregulated in bronchial asthma and could be further induced by virus infection. We hypothesise that such cytokines might influence Th2 cell differentiation in the context of rhinovirus infection.

Methods: Beas2B cells were infected with rhinovirus 1b (1MOI) or incubated with uninfected Hela lysates and the supernatants were harvested 24 hours later. CD4+ T naive cells were isolated from CD Leukocyte Cone (buffy coat) and expanded for 12 days in the presence of anti-CD2, -CD3, -CD28 and IL2 stimulation. Th2 cells were also expanded out of the CD4+ T naive cells, in the presence of anti-CD2, -CD3, -CD28, -IL12 and IL4 stimulation. The already expanded Th0 cells were put into culture alone, in the presence of IL33, IL25 or TSLP (10 ng/ml) or with the supernatants from infected or uninfected cells above (4:1) for 12 more days. On Day 2, Day 6 and Day 12 of co-culture intracellular levels of IFNg, IL13, IL4 and FOXP3 were assessed by FACS.

Results: On Day 12 of culture, IL13 levels were elevated in virus infected supernatant, IL33 and IL25 conditions at 10.5, 33.2, and 21.5 per cent in comparison to 2.45, 1.76 and 1.45 per cent on Th0 cells alone or in the presence of uninfected supernatants or TSLP. IL4 levels followed similar patterns at lower levels. The positive control Th2 cells express IL13 at 76.6 per cent and IL4 at 67.4 per cent. In the same conditions (virus, IL33 & IL25) trends for reductions in IFNg positivity were observed, decreasing progressively from Day 2 through Day 6 to Day 12, while levels of expression of FOXP3 remained relatively constant. IL13, IFNg and FOXP3 expression was not greatly different between the three timepoints in any other condition studied.

Conclusion: Rhinovirus 1b infected bronchial epithelial cell supernatants, IL33 and IL25 promote Th0 cells to differentiate towards a Th2 phenotype and away from a Th1 phenotype during 12 days of co-culture. This model of co-culture might be helpful in investigating the role of the novel IL25, IL33 and TSLP in asthma development and exacerbation.
modulate inflammatory responses through TLR-mediated production of pro-inflammatory cytokines and inactivation of GSK3β (phosphorylated GSK3β) lead to a reduced IL-12 production, which was suggested to skew the balance towards a Th2 response.

**Aim:** To determine the degree of expression of TLRs 2, 4, 7 and 9 on monocytes as well phGSK3β using flow cytometry. We hypothesize that the co-existence of CRS in patients with asthma and especially patients with MSA has an increased TLR expression involved in the innate immune system.

**Method:** Participants were selected from an epidemiological cohort, the West Sweden Asthma Study. Clinical parameters as well as fresh peripheral blood cells were obtained from one group of non-asthmatic subjects with CRS (CRS) and four different groups of asthmatics: 7 subjects with MSA (MSA), 7 subjects with other asthma (OA), 7 subjects with MSA and CRS (MSA/CRS) and 9 subjects with OA and CRS (OA/CRS). These five groups were compared to a control group consisting of 10 healthy subjects without asthma or CRS.

**Result:** Individuals with MSA or CRS only, showed significantly increased TLR2, TLR7 and TLR9 expression (rMFI) on CD14+ monocytes compared to controls as well as the expression of phGSK3β and CD14. Individuals in the combined MSA/CRS group showed increased expression of CD14 and TLR2, but increased expression of TLR4 was only found in the CRS group.

**Conclusion:** The upregulated expression of TLRs in the MSA group compared to control group suggest a higher susceptibility towards an altered immune response that might reflect the degree of severity. Furthermore, the increased phosphorylation of GSK3β may indicate a switch in the immune response from a pro-inflammatory to a dysregulated Th2 response.

**Sputum “IL-5, IL-17A, IL-25-high” pattern is associated with uncontrolled asthma and worse lung function**

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**Background:** Asthma is a heterogeneous disease with various clinical, inflammatory and molecular phenotypes. Uncontrolled asthma is associated with an increased risk of asthma exacerbation. We evaluated sputum cytokine mRNA expression patterns in an unselected group of adults with stable asthma in comparison to healthy subjects in order to characterize patients’ specific airway cytokine profiles.

**Method:** Differential cell counts and cytokine mRNA (quantified by real-time PCR) were analyzed on sputum from 40 healthy and 66 asthmatic adults. A “cytokine-high” profile was defined if mRNA levels for that particular cytokine exceeded the 90th percentile value in the healthy population. Asthma control was assessed by Asthma Control Test (ACT) questionnaires.

**Results:** The “IL-5-high” asthma profile (n=13) merged with the “IL-25-high” (10/13) and surprisingly also with the “IL-17A-high” (11/13) profile. A sputum “IL-25-high” profile was even restricted to patients with an “IL-5-high” profile. Only a minority of “IL-5-high” patients were found to be “IL-4-high” (n=6), whereas 17 other patients had an ‘IL-4-high’ profile without being “IL-5-high”. Patients with an “IL-5-high”, IL-17A-high” and/or “IL-25 high” cytokine pattern had worse lung function parameters (FEV1%pred., PEF, FEF25-75%). Uncontrolled asthmas had significantly higher sputum IL-5, IL-17A and IL-25 mRNA levels.

**Conclusion:** Airway cytokine expression is highly heterogeneous amongst patients and their exact contribution to asthma pathogenesis is debated. Uncontrolled asthma was associated with higher levels of IL-5, IL-17A and IL-25 in the airways of asthmatics. Identifying patients’ aberrant cytokine expression in the airways by non-invasive techniques might help to define responders to current and future therapies.

**Activin-A is up-regulated in severe asthma, attenuates allergic responses and is associated with angiogenesis**

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Background: Activin-A (Act-A) is a pleiotropic cytokine belonging to the TGF-β superfamily. Recent studies from our group have shown that Act-A suppresses mouse allergic responses; however, its effects on human asthma remain unknown.

Objectives: Determine Act-A expression in healthy controls (CTRL) and asthmatics with mild-moderate (MMA) and severe asthma (SA), identify its cellular sources, examine its signaling mediators' expression and correlations with disease severity and airway remodeling, and investigate its effects on allergic inflammatory responses.

Methods: Act-A expression was quantified in the serum, BALF (by ELISA) and bronchial tissue samples (IHC) obtained from CTRL (n=41), MMA (n=46) and SA (n=26). Act-A signaling expression (ActRIIA, ALK4, pSMad2/3) and remodeling markers (basement membrane thickness, goblet cell hyperplasia and angiogenesis) were also assessed in bronchial tissue (IHC/IF/Confocal). Moreover, naive T cells from atopic CTRLs and MMA/SA were isolated and cultured ex vivo alone or in the presence of allergen and/or rAct-A and/or dexamethasone and further utilized in co-cultures with naive T cells and in adoptive transfer experiments using NOD/SCID mice in a humanized model of experimental asthma.

Results: Act-A levels were significantly increased in the serum, BALF and bronchial tissue of asthmatics, especially in the subepithelium in SA. Act-A was expressed by T cells, neutrophils, mast cells, macrophages and endothelial cells. ActRIIA, ALK4 and pSMad2/3 expression was downregulated especially in SA. Regarding remodeling, subepithelial Act-A expression correlated with tissue angiogenesis and Act-A/ALK4 were co-expressed in endothelial cells pointing to active signaling. Act-A attenuated allergic responses of human naive T cells (decreased IL4, IL5, IL13) through the induction of IL-10 producing regulatory-like T cells (Act-A-iTregs) and enhanced dexamethasone-induced T cell suppression. Adoptive transfer of Act-A-iTregs in the NOD/SCID mouse model suppressed experimental asthma.

Conclusions: Act-A expression is increased in severe asthma however its signaling pathways is downregulated. Act-A suppresses airway inflammation ex vivo through the induction of functional Act-A-iTregs. Our data suggest that Act-A plays a crucial role in the inflammatory and angiogenetic processes in asthma. Ongoing in vitro studies will further elucidate its specific role in angiogenesis.

Disruption of airway lymphatics as a novel cause of impairment of airway clearance in severe asthma

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Background: We previously reported the phenotypic distribution patterns of airway smooth muscles in severe asthmatics; with smooth muscle bundle thickening only in large airways and in whole airways (Am Rev Respir Dis, 1990), by different mechanisms, hypertrophy and/or hyperplasia (Am Rev Respir Dis, 1993), which was reexamined recently to fit in 55 fatal asthmatics by James AL, et al (Am J Respir Crit Care Med, 2012). We also revealed that pulmonary lymphatics distributed in interlobular septa and subpleural lesions were destroyed by increased fibrosis in patients with idiopathic pulmonary fibrosis which would impair alveolar clearance (Lymphat Res Biol, 2010). In this context, we were interested in the alteration of airway lymphatics in severe asthmatics and hypothesized that increased smooth muscle bundles and fibrosis in the airway walls would disrupt airway lymphatics and impair airway clearance in these patients.

Method: The autopsy lungs of severe asthmatics and controls were examined by immunohistochemistry to reveal the lymphatics and morphometry using an image analyzer system was applied to compare the distribution of airway lymphatics in the same level of airways among these asthmatics and controls. We also estimated the degree of airway smooth muscles and fibrosis around the airways which would interrupt or disrupt airway lymphatics.

Results: The total area of airway lymphatics in each lung was found to be positively correlated with the airway radius. The distribution areas of lymphatics in larger airways of both types of asthmatics were significantly decreased than controls, and the severe asthmatics with increased muscle layers only in larger airways were found to have less lymphatics in these airways than the other group of severe asthmatics with increased smooth muscles in whole airways. The lymphatics around smaller airways were also reduced in both phenotypes of asthmatics.
without statistic difference. The airway lymphatics of these severe asthmatics were interrupted by both thickened muscle bundle layers and fibrotic tissues developed in the airway walls.

**Conclusion:** These results indicate disrupted airway lymphatics as a novel cause of mucosal edema in severe asthmatics.

**Corticosteroid insensitivity in severe asthma: impaired nuclear translocation of glucocorticoid receptor in airway smooth muscle cells**

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**Background:** Patients with severe asthma are less responsive to the beneficial effects of corticosteroid (CS) therapy. CS mediate their therapeutic effects through activation of the glucocorticoid receptor (GR) and suppression of NF-κB activity.

**Objectives:** We investigated whether i) corticosteroid insensitivity was present in airway smooth muscle cells (ASMC) of patients with severe asthma and ii) there was a defect in the molecular action of the glucocorticoid receptor (GR) in ASMC of patients with severe asthma.

**Methods:** ASMC of the healthy (12), non-severe (NSA; 10) and severe asthma (SA; 10) obtained from endobronchial biopsies were pretreated with dexamethasone (Dex) at 10^{-10} to 10^{-6} M followed by stimulation with TNF-α and IFN-γ (10 ng/mL each), alone or in combination (T+I). CXCL8 and CCL11 release was determined by ELISA, GR and p65 protein was assessed by Western Blot, mRNA by RT-qPCR and p65 recruitment to the CCL11 promoter by ChIP assay.

**Results:** CCL11 release was higher in ASMC of non-severe but not severe asthmatics and non-asthmatic controls; CXCL8 release was similar in all groups. In severe asthmatics dex (10^{-6} M) caused less suppression of CCL11 (42% vs 14%, p<0.05) and CXCL8 (47.88% vs 26.7%, p<0.05) compared to non-severe asthmatics or healthy controls, respectively. TNFα-induced phospho-p38 mitogen-activated protein kinase was increased in severe asthmatic ASMC compared to non-severe and non-asthmatics while a p38 inhibitor increased the inhibitory effect of dex. GR expressed in severe and non-severe asthma was 49% of that in the healthy (p<0.01). Dexamethasone-induced GR nuclear translocation at 2h, in SA, was 60% of that in either the healthy or NSA (p<0.05). TNF-α induced greater p65 mRNA expression in SA whereas baseline and TNFα-induced nuclear abundance, and dexamethasone suppression of p65 expression, were similar between groups. While not modulating p65 nuclear translocation, dex attenuated p65 recruitment to the CCL11 promoter in the healthy and NSA, but this suppressive effect was impaired in SA.

**Conclusions:** Airway smooth muscle cells of patients with severe asthma are corticosteroid insensitive; this may be secondary to heightened p38 mitogen-activated protein kinase. Decreased GR expression with impaired nuclear translocation in ASMC, associated with reduced dexamethasone-mediated attenuation of p65 recruitment to gene promoters, may underlie the mechanism of CS insensitivity in severe asthma.

**Crowdsourcing to explore views about asthma severity among asthma experts**

**Lötvall, Jan¹; Papadopoulos, Nikos²; Custovic, Adnan³**

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Defining severe asthma and degrees of severity of asthma has been complicated, with classifications changing over time and also in different publications. The aim of the current investigation was therefore to start explore views about asthma severity among a wide group of asthma experts. We defined an asthma expert as an individual that since January 2005 has been an author for correspondence on a scientific publication on asthma (search term hit) in any of the top seven respiratory or allergy journals (impact factor). A brief questionnaire was distributed to 4791 e-mail addresses manually curated from the journals. Nine days after the first e-mail was circulated, and four days after a reminder e-mail, 725 individuals had responded. Of the respondents, 84.5% stated that they are either “very knowledgeable” or “knowledgeable” in asthma, and 14% state that they understand some aspects of asthma. A large majority (80.8%) of respondents suggested that reaching out to a large number of experts to crowdsourc on topics related to asthma is “reasonable”, whereas 8.9% did not think so. Respondents were also asked which crucial factors contribute to asthma severity, and the responses were: 1) Exacerbation frequency (84%), 2)
Exacerbation severity (78.4%, 3) Need of extensive medication (72.2%), 4) Low lung function (57.7%), 5) Night-time awakenings (50.1%), 6) Degree of drop in lung function when attacks occur (44.5%), 7) Number of asthma symptoms (40.3%), 8) Presence of any comorbidity (39.6%), 9) Degree of bronchial hyperresponsiveness (39.2%), 10) Lack of control according to brief questionnaire (36.8%), 11) Degree of variability of lung function (36%). A small majority (57%) voted that the definition of “severe asthma” should be given to those that are uncontrolled with current conventional therapy, but an important minority (38.3%) disagreed with this definition. Outreach to a wide group of experts in the field of asthma resulted in a significant response rate, where a vast majority acknowledge themselves as experts in the field, and also thought that this approach is reasonable. Crowdsourcing may be efficient to develop wide consensus on different aspects of asthma, including a definition of severe asthma. However, additional crowdsourcing surveys, involving comments from current respondents, will be needed. This work was financed by EAACI.

Friday, 12 October 2012
13:00 – 15:00

High risk of adult asthma following severe wheeze in early life
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Background: We have previously reported on the outcome in childhood and adolescence in children with severe wheeze in early life. The aim of the present follow-up was to report on the asthma prevalence and risk factors for asthma in adult age.

Methods: We have prospectively studied asthma development in 101 children hospitalized due to wheezing before the age of two. The cohort was re-investigated at age 25-29 years and tested for bronchial hyper-responsiveness and allergic sensitization. The response rate at adult age was 81%. The results were compared to a population based aged matched control group (n=1210) recruited from the West Sweden Asthma Study.

Results: Current asthma was seen in 37% (30/82) and 50% of these had a moderate to severe asthma. In the control group 10% reported current asthma (OR 5.3, 95% CI 3.2-8.9; p<0.001) and 17% had wheezing during the last 12 months (p<0.001). Current use of asthma medication was reported in 31% of the cohort (of which 66% used inhaled corticosteroids and/or montelukast), compared to 8% in the control group (p<0.001). Current atopy was found in 54%, with 42% reporting doctor-diagnosed rhinitis, 11% current eczema and 16% food allergy. Among the controls rhinitis was reported in 29% (p=0.013) and eczema in 13% (ns). Smoking was reported in 30% of the cohort, compared to 16% in the control group (p=0.002).

In the cohort, current allergy (OR 9.7, 95% CI 3.0-31.1) and female gender (OR 3.2, 1.1-9.5) increased the risk of adult asthma independently of each other. Females with current allergy had the highest risk of adult asthma, compared to males without allergy (OR 29.4, 5.0-173.3).

This is illustrated in a stratified Cox regression analysis where the females with current allergy have the lowest chance of recovery (Hazard Ratio 0.2, 95% CI 0.06-0.5) compared to males without allergy.

Conclusion: Subjects with severe early wheeze have an increased risk of adult asthma. Females with current allergy had the highest risk of persistent asthma and the lowest chance of recovery.

Identification of inflammatory phenotypes of asthma by blood analysis and clinical parameters
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Background: Identification of an inflammatory asthma phenotype currently requires sputum induction. This technique is invasive, has a high variability, is time-consuming and a burden for patients. Therefore, there is a strong need for a routine blood test to establish the inflammatory phenotype of asthma. We designed a clinical cohort study (AIR-study, NCT01611012) in 115 asthma patients visiting the outpatient clinic to compare the results of such a blood test to sputum analysis. This abstracts focusses at the preliminary results in 21 patients.
**Objective:** To assess whether expression of active FcgammaRII(CD32) and MAC-1(CD11b) on neutrophils and eosinophils in peripheral blood enables the diagnosis of an inflammatory phenotype of asthma.

**Materials and Methods:** 21 asthma patients were recruited at the outpatient clinic of the University Medical Centre Utrecht. Clinical parameters were gathered and FENO(fractional exhaled Nitric Oxide), sputum induction and blood tests were performed. Eosinophils and neutrophils in whole blood were stained with a FITC labeled antibody against active FcgammaRII receptor (clone A17) and a PE-labeled antibody against CD11b in the absence and presence of the activator fMLP (1 microM). Subsequently, fluorescence intensity was measured by flowcytometry.

**Preliminary results:** Expression of Mac-1 (CD11b) and activeFcgammaRII (CD32) on eosinophils at basal level and after stimulation (fMLP) showed refractory cells (low responsiveness for fMLP) in case of granulocytic asthma (neutrophilic, eosinophilic and mixed phenotype). FENO values were overall lower in paucigranulocytic asthma compared to granulocytic asthma (p = 0.009, independent t-test).

**Conclusion:** Refractory blood eosinophils determined by a simple blood test were found in those patients that were more likely to suffer from granulocytic asthma (diagnosis by sputum analysis). Therefore, paucigranulocytic asthma can be distinguished from other phenotypes purely based on blood analysis and FENO measurement. A low expression of activation epitopes on eosinophils in peripheral blood might indicate that ‘activation prone’ eosinophils migrated to the lung.

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**Food allergy can be a major hidden cause of chronic asthma which can be uncovered or excluded by introducing a few foods diet**

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¹Independent allergist, UK; ²Independent allergist, none, UK

Case-histories of three severe chronic asthmatics are presented to draw attention to the possibility that even when there seems to be convincing evidence from history, skin tests, and immunology that dust mite is the major causative allergen, it is possible that food or foods can be the major cause. Difficulties in controlling their asthma over years in these cases suggested that the causative factors had still not been defined. A "Few foods diet" , containing only the foods which very rarely cause allergy, was introduced in addition to their usual regimen to be taken for two or three weeks to find out if foods were causing the asthma. Obvious subjective and objective improvement occurred within days, control of the asthma became easier than for years, Peak flow rates increased considerably, and quality of life improved greatly. Improvements were sustained while on the diet and while the causative food or foods were avoided. Blind provocation was not practical, but three exacerbations after re-introduction of specific foods, with continued well-being on avoidance, was accepted as proof. This experience suggests that a hidden allergy or intolerance to a common daily food can cause of chronic asthma, even when there is strong evidence that inhalant allergens are responsible. Enquiry regarding reflux, dyspepsia, or other gut disorders may have been omitted at the first consultation or at follow-up, and patients are most unlikely to associate gut symptoms with their asthma. These cases are presented because food allergy as a hidden cause of chronic asthma is rarely considered, the incidence is unknown, and skin tests and immunology unreliable. A trial of a few foods diet for two or three weeks can be very rewarding, and there is nothing to lose except some weight. A trial in a large group of severe asthmatics could define the true incidence of hidden food allergy in chronic asthma.

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**Assessment of primary care doctor´s diagnosis of difficult-to-treat asthma in school children**

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Asthma and Allergy Clinic, Denmark

**Rationale:** In primary care settings difficult-to-treat asthma may be interpreted as severe asthma. Little is known about diagnostic outcomes in children referred to secondary paediatric referral centres with an established primary care doctor's diagnosis of difficult-to-treat bronchial asthma.

**Objective:** To assess diagnostic outcome in school children referred to a secondary paediatric referral centre with an established primary care doctor's diagnosis of difficult-to-treat bronchial asthma.

**Methods:** 482 consecutively referred children aged 5-14 (mean 7.9) years, 99 girls (21%) and 383 boys (79%) with a primary care doctor's referral diagnosis of difficult-to-treat asthma were included from the prospective Asthma in a Secondary Pediatric Referral Centre Study (ASP 2002) in the present survey. At referral and during a 6 months evaluation period patient characteristics, history, symptoms, signs and results of type 1 allergy tests,
spirometry, post bronchial beta-2 agonist dilation tests, 4-weeks daily measurement of peak flow rates, corticosteroid reversibility trials and exercise challenge tests were entered into a pre-defined electronic form. The secondary referral centre (SRC) diagnosis of asthma was based on these data.

**Results:** A diagnosis of asthma was confirmed in 200 (41%), whereas it could not be confirmed in 282 (59%) of the children. Allergic rhinoconjunctivitis was diagnosed in 96 (48%) in the confirmed group, in 87 (31%) in the not confirmed group. A variety of differential diagnoses was made in the children in whom asthma was not confirmed.

**Conclusions:** In more than half of school age children with a primary care doctor’s diagnosis of difficult-to-treat asthma referred to a secondary paediatric referral centre the diagnosis may not be confirmed. Sensitivity and specificity of the diagnosis of asthma in school children made in primary care settings need further improvement.

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**Omalizumab in the treatment of severe allergic (IgE-mediated) asthma: an update on recent developments**

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Omalizumab (OMA), a humanized anti-immunoglobulin E (IgE) monoclonal antibody was approved in the EU in 2005 as an add-on therapy for patients with severe persistent allergic asthma (AA). We present an overview of recent developments in the use of OMA for this indication. Approval for paediatric use: Followed the completion of two clinical trials in children 6 to <12 years of age with moderate-to-severe AA, who were either well- or inadequately controlled with inhaled corticosteroids (CS). Pooled analysis of these data demonstrated the efficacy and safety of OMA and led to approval in the EU for use in this population. Expansion of the dosing table (DT): The initial DT applied to patients with IgE levels between 30–700 IU/mL and body weight up to 150 kg, with a maximum dose of 375 mg per administration. The DT was expanded to enable treatment of patients with levels up to 1,500 IU/mL, with a maximal dose of 600 mg per administration. Subsequent pharmacokinetic (PK) and pharmacodynamic (PD) modelling and simulation predicted that some doses could be doubled and given less frequently, while maintaining efficient suppression of free IgE without compromising safety. The DT was further revised and approved in the EU in 2012, simplifying the dosing schedule in a subset of patients (225–300 mg every 2 weeks) to receive treatment at double the dose every 4 weeks. Development of a pre-filled syringe: A more concentrated liquid formulation may eliminate a potential source of error during dosing and simplify drug administration. A PK/PD study demonstrated bioequivalence and a similar safety and tolerability profile between the liquid formulation and the lyophilized powder. OMA safety update: A pooled analysis of 32 trials showed no association between treatment and risk of malignancy (rate ratio 0.93 [95% CI, 0.39–2.27]). In an interim analysis of an observational study (N=8,023), the malignancy incidence was similar in both cohorts (+/-OMA). OMA ‘real-life’ 2-year registry (N=943): Efficacy results demonstrated an increase in the number of patients free from clinically significant exacerbations following OMA treatment (6.8% at baseline to 67.3% at 24 months). A reduction in maintenance oral CS use to 14.2% after 24 months compared with 28.6% at baseline and, improvements in asthma control and quality of life were also observed. Such advances in the use of OMA may benefit a wider range of patients with severe AA and address unmet needs in asthma treatment.

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**A Combined Phase I/IIa Study of the Safety, Bronchodilator and Bronchoprotective Effects of Nebulized RPL554, a Dual PDE3/4-inhibitor, in Healthy Subjects and Asthmatics**

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**Rationale:** RPL554 is a novel dual phosphodiesterase (PDE) 3 and 4 isoenzymes inhibitor that has been demonstrated to have both potent, long-acting bronchodilator and anti-inflammatory activity in preclinical models.
Objectives: To evaluate the safety, bronchodilator and bronchoprotective effects of single ascending doses of RPL554 in healthy subjects and asthmatics.

Methods: The safety of RPL554 0.003 and 0.009 mg/kg was evaluated in 18 healthy males (age 18-41 years; weight 58-90 kg; height 168-196 cm; BMI 18-29 kg/m²) in a randomised, double-blind study. For each dose group, 6 subjects inhaled RPL554 and 3 placebo. Study medication was administered by a calibrated electronic nebuliser and oro-nasal mask. Subsequently, RPL554 0.009 and 0.018 mg/kg were evaluated in 6 asthmatic males (22-29 years; 58-98 kg; 174-198 cm; BMI 19-27 kg/m², FEV1 75-118% pred.) using an open, adaptive design to select an effective dose. This dose (0.018 mg/kg) was further evaluated in a randomised, double-blind, placebo-controlled, cross-over study in 10 allergic asthmatics (20-50 years; 67-112 kg; 171-191 cm; BMI 21-33 kg/m², FEV1 82-112% pred.; baseline PC20(methacholine=MCh) 0.07-1.49 mg/mL; not on controller medication) to assess its bronchodilator effects and bronchoprotective activity against methacholine (MCh) challenge.

Results: Inhaled RPL554 0.003 to 0.018 mg/kg was well-tolerated in all subjects studied. In the asthmatics, RPL554 0.018 mg/kg produced a rapidly progressive, sustained bronchodilation with a mean maximal increase in FEV1 of 520 mL (95% CI: 320-720 mL; p=0.0061), or 15% increase from baseline. Furthermore, a single dose of RPL554 increased PC20(MCh) by a mean of 1.46 doubling doses (95% CI: 0.63 – 2.28; p=0.004) versus placebo. Adverse events were mild, transient and generally of equal frequency in RPL554 and placebo treated groups. No significant changes in heart rate or systemic blood pressure were noted with RPL554 when compared to placebo.

Conclusions: RPL554 was well-tolerated in the healthy subjects and asthmatics studied. In patients with mild asthma, a rapid, potent and long-lasting bronchodilator response was produced. The maximum bronchodilator effect appeared at least comparable to published values of inhaled salbutamol. RPL554 has the potential to become a first-in-class bronchodilator for airways obstruction.

Poster Abstract Sessions

Thursday, 11 October 2012
10:30 – 12:00

1 From an opium drugs to the endogenous opioid system: is there a need for research in pulmonology and asthmatic childhood?
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Background: In pulmonology about a hundred years ago the treatment of coughs, bronchitis, asthma a. o. was quite widespread with the using of opium drugs (Morphini hydrochlorici, Apomorphini hydrochlorici, Heroini, Tinct. opii simplicis) [Landessmann, E. und Marburg, O. Die Therapie an den Wiener Kliniken, Franz Deuticke, 1904; 5-14]. But in modern pulmonology positive effect of opium on the respiratory system can be obtain indirectly by methods of stimulation impact on the endogenous opioid system, such as electroacupuncture [Kapustin, A.V. et al., European Respiratory Journal, 2003; Vol. 22, 45:137s].

Methods: We used electroacupuncture (apparatus Lasper-504 , Japan) in children with mild bronchial asthma and radio-receptor analysis of opioid peptide in blood plasma.

Results: Radio-receptor analysis has showed that electroacupuncture results in pronounced increasing level of opioid peptide δ-type ligand activity from 54,0 + 4,03 to 77,0 + 5,3 (p<0,05) ng/ml DADLE [D-Ala2, D-Leu5] Enkephalin, but doesn't change activity of μ-type ligand in blood plasma. Reliable increasing correlation coefficient (r=0,88, p=0,00001) between the activity of ligands both opioid peptide μ-type and δ-type evidences about the electroacupuncture stimulation impact on the endogenous opioid system state as well. Children had no symptoms of bronchial asthma attacks during treatment of electroacupuncture.

Conclusion: Thus, we have shown that the using of electroacupuncture produces the opioid peptides, which may be one of the mechanisms of the positive therapeutic effect in asthmatic children. We are sure that this fact may be the basis for further investigations.
2 Healthcare utilization and indirect cost of treatment associated with severe allergic asthma in a real-world setting

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¹St. Franciscus Gasthuis, Department of Pulmonary Medicine, India; ²Flevoziekenhuis, Department of Pulmonology, Netherlands; ³Novartis Pharmaceuticals UK Limited, Clinical Development, UK; ⁴Novartis Pharmaceuticals Corporation, Clinical Development, USA; ⁵Novartis Pharma AG, Clinical Development, Switzerland

Background: With an estimated 300 million individuals affected worldwide, asthma is associated with substantial social and economic burden. The cost of treating uncontrolled severe allergic asthma (SAA) is high encompassing a variety of direct medical costs and indirect costs. We present data on real-world healthcare utilization (direct) and school/work absence (indirect) in uncontrolled SAA patients receiving omalizumab in the eXpeRience registry.

Methods: eXpeRience was a 2-year, global, single-arm, observational registry. Data were collected on real-world effectiveness, safety and use of omalizumab in patients with uncontrolled SAA. Asthma-related healthcare utilization (hospitalizations, emergency room visits or unscheduled doctor visits) and number of days missed from school/work were recorded.

Results: The intent-to-treat population comprised 916 (97.1%) patients. Compared with the pre-treatment period, there were reductions in healthcare utilization and school/work absence after 12 and 24 months of omalizumab treatment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-treatment * (N=916)</th>
<th>12 months (N=734)</th>
<th>24 months (N=643)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma-related hospitalizations</td>
<td>0.7 (1.32); 882</td>
<td>0.1 (0.43); 702</td>
<td>0.1 (0.41); 628</td>
</tr>
<tr>
<td>Duration of hospitalization stay due to asthma, days</td>
<td>5.3 (11.05); 852</td>
<td>0.7 (3.84); 703</td>
<td>0.5 (3.39); 628</td>
</tr>
<tr>
<td>Asthma-related emergency room visits</td>
<td>1.8 (2.87); 867</td>
<td>0.7 (0.64); 700</td>
<td>0.1 (0.33); 627</td>
</tr>
<tr>
<td>Unscheduled asthma-related doctor visits</td>
<td>3.8 (4.79); 823</td>
<td>0.7 (1.43); 684</td>
<td>0.4 (0.99); 619</td>
</tr>
<tr>
<td>Asthma-related medical healthcare uses*</td>
<td>6.2 (6.97); 811</td>
<td>1.0 (1.96); 684</td>
<td>0.5 (1.28); 618</td>
</tr>
<tr>
<td>Absence from work due to asthma#, days</td>
<td>26.4 (49.61); 347</td>
<td>3.5 (17.28); 295</td>
<td>1.0 (4.66); 296</td>
</tr>
<tr>
<td>Absence from school due to asthma#, days</td>
<td>20.7 (27.49); 57</td>
<td>1.6 (4.28); 59</td>
<td>1.9 (5.46); 58</td>
</tr>
</tbody>
</table>

Table shows annualized data (12 month combined 16 weeks, 8 and 12 month data; 24 month combined 18 and 24 month data). n – number of patients with data recorded. *Within 12 months prior to start of omalizumab treatment.
*Total number of asthma-related healthcare uses was calculated if data for asthma-related hospitalizations, emergency room visits and unscheduled doctor visits were available. Excluded those patients for whom this category was not applicable.

Conclusion: Results from the eXpeRience registry showed that omalizumab reduced healthcare utilization and the number of days missed from school or work by asthma patients in a real-world setting. Thus, omalizumab treatment was associated with a positive and substantial impact on the direct and indirect costs linked with uncontrolled SAA.

3 Restoring AMs-HDAC1 expression in Allergic Asthma Mice Model by Novel Medicinal Plant

El-housseiny, Lamia¹; Hoaallah, Essam²
¹Medical University of Vienna, Universitätsklinik für Chirurgie; kardiovaskuläre, Austria; ²National Research Center, Agriculture Microbiology Department, Egypt

Worldwide ~235 million people currently suffer from asthma. We searched the integrative medicine and the ancient cultures in the Middle East, Africa, and China for new anti-asthma treatment. LE25-0712 is an herbal extract (formula under patent). Here, we used it to validate the old knowledge known, to treat acute allergic asthma mice model. We also identify the effect of our novel extract on the expression of AMs-HDAC1 (alveolar macrophages-histone deacetylase1). We had proven in a previous study, deletion of HDAC1 had increased allergic airway inflammation and enhanced Th2 cytokine production in asthma mice model. In asthmatic and COPD patients, PBMC-HDAC level was reduced compared to healthy individual. In this study, we evaluated the effect of LE25-0712 on the expression level of AMs-HDAC1 in treated asthmatic mice (TAM) model compared to asthmatic control (AC) group.

Methods: we used the acute asthma model of C57BL/6 mice, i.p. sensitized and i.n. challenged on d28, 29 with (OVA). LE25-0712 (0.5 mg/kg) was i.n. administered b.i.d (d25-29). WE assayed bronchoalveolar lavage fluid (BALF) for the total and differential types of inflammatory cells, and the expression of HDAC1 by IF (d31). Náive
and AC groups were included. As LE25-712 is a safe herbal supplement, allergic asthma patients were treated b.i.d by it combined with corticosteroid, until complete replacement of steroid.

**Results:** LE25-0712 treatment of TAM group resulted in significant anti-inflammatory and anti-allergic activity as shown by reduced BAL total leukocytes (P < 0.03), and reduced eosinophils (P < 0.002) compared to the AC group. The expression of HDAC1 in AMs-naïve was (87%), which was decreased in CA group to (8%). LE25-0712 restores HDAC1 expression in AMs of treated asthma model (45%, P < 0.0001). In 15 chronic asthmatic patients, LE25-0712 exerts a dramatic improvement in the general condition in 100% of patients, as it relieves the obstructive airway condition, which leads to complete spare of corticosteroids administration.

**Conclusion:** The novel LE25-0712 is an effective natural safe medicinal extract for the prophylaxis of acute asthma disease in mice, as well as controlling the recurrent asthmatic attacks in chronic patients. The effectiveness and safety administration of this extract give it the upper hand over the traditional corticosteroid treatment. Moreover it restores the HDAC1 expression in TAM groups, which can be used as a follow up marker.

### 4 Sputum and serum hydrogen sulfide (H2S) as novel biomarker of asthma

**Junpei Saito; Pankaj Bhavsar; Qingling Zhang; Christopher Hui; Andrew Menzies-Gow; Kian Fan Chung**

**Experimental Studies, National Heart and Lung Institute, Imperial College London & Biomedical Research Unit, Royal Brompton Hospital, London, UK.**

**Background:** Hydrogen sulfide (H2S) is a gas produced by respiratory cells including smooth muscle cells and may play a role as a gasotransmitter. We determined whether H2S levels in serum or sputum supernatants could represent a biomaker of asthma.

**Methods:** We measured H2S in induced sputum and serum samples of patients with severe and non-severe asthma and of healthy subjects. H2S concentrations were measured using a sulfide-sensitive electrode.

**Results:** H2S levels in induced 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 H2S 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 blood H2S levels (r=0.42, p<0.05). Sputum H2S levels were negatively correlated with FEV1 %predicted (r= -0.42, p=0.003), and with reversibility to salbutamol (r= -0.54, p<0.01). There was a correlation between sputum H2S and sputum neutrophils and macrophages, and a negative correlation between sputum H2S and FeNO levels.

**Conclusions:** Endogenous H2S, measured in induced sputum, may be a marker of neutrophilic inflammation and bronchial narrowing.

### 5 Influence of physical activity in asthmatic children

**Tancredi, Giancarlo¹; Ernesti, Ilaria²; di Coste, Annalisa³; Schiavi, Laura⁴; Tubili, Flavia⁵; Bardanzellu, Flaminia⁶; De Vittori, Valentina³; Duse, Marzia³**

¹Sapienza Università di Roma, Policlinico Umberto I, Italy; ²Sapienza Università di Roma, Policlinico Umberto I, Dipartimento di Immuno-Allergologia pediatrica, Italy; ³Sapienza Università di Roma, Policlinico Umberto I, Dipartimento di Immuno-Allergologia Pediatrica, Italy; ⁴Sapienza Università di Roma, Policlinico Umberto, Dipartimento di Immuno-Allergologia Pediatrica, Italy; ⁵Sapienza Università di Roma, Policlinico Umberto I, Dipartimento di Immuno-Allergologia Pediatrica, Italy; ⁶Sapienza Università di Roma, Dipartimento di Immuno-Allergologia Pediatrica, Italy

**Aim of the study:** This preliminary study investigated the influence of physical activity levels on lung function tests, exercise test and differences of preparticipation screening (noncompetitive or competitive) in asthmatic children and controls.

**Methods:** We compared functional respiratory testing in 72 asthmatic children (mean age 11.4±2.6 years) and in 70 healthy subjects (mean age 12.5±2.5 years). Each group was divided in 2 subgroups using a physical activity level cut-off (< 2 or > 3 hours spent for week), that way generating 4 study groups: asthmatic trained children, non asthmatic trained children, trained controls, non trained controls. We investigated type of preparticipation screening (noncompetitive or competitive) and functional characterizes. All subjects underwent a maximal treadmill exercise test, determining maximal oxygen uptake by indirect method, metabolic equivalents and exercise time, and performed spirometry pre and post exercise.
Results: No significant differences were found on spirometry between asthmatic children and controls. Exercise testing showed, respectively for controls and asthmatic subjects, metabolic equivalents 15.2±2.7; 13.0±2.7 (p<0.0001); maximal oxygen uptake 52.2±9.9; 44.6±8.7 ml/kg/min (p<0.0001); exercise time 12.1±2.2; 10.5±2.4 min (p<0.0001). Competitive screening preparticipation was performed in 45.7% of controls and 22.2% of asthmatic subjects, noncompetitive screening in 54.3% of controls and 77.8% of asthmatic children (p<0.003).

Conclusions: Physical activity influences exercise parameters (exercise time, METS, VO2max) in asthmatic children: asthmatic trained children, with asthma controlled, have functional parameters better than non trained controls and similar to trained controls.

6 Steroid responsiveness of peripheral blood t cells derived from steroid sensitive, steroid dependent, and steroid resistant asthmatics, and induction of steroid resistance by costimulatory signal

Mori, Akio¹; Abe, Akemi¹; Koyama, Satoshi¹; Yamaguchi, Miyako¹; Iijima, Yo¹; Mitsui, Chihiro¹; Oshikata, Chiyako¹; Tanimoto, Hidenori¹; Sekiya, Kiyoshi¹; Tsuburai, Takahiro¹; Taniguchi, Masami¹; Ohtomo, Mamoru¹; Maeda, Yuji¹; Hasegawa, Maki¹; Akiyama, Kazuo¹; Ohtomo, Takayuki²; Kaminuma, Osamu³

¹National Hospital Organization, Sagamihara National Hospital, Clinical Research Center, Japan; ²Tokyo University of Pharmacy and Life Science, Department of Pharmacotherapeutics, Japan; ³The Tokyo Metropolitan Institute of Medical Science, Department of Allergy and Immunology, Japan

Background: Severe asthmatics are characterized by low responsiveness to inhaled corticosteroid (ICS) compared to mild asthmatics. Steroid resistance has been ascribed to various cell types including T cells, mononuclear cells, bronchial smooth muscle cells, etc.

Method: Peripheral blood mononuclear cells (PBMC) obtained from mild (steroid sensitive, SS), steroid dependent (SD), and steroid resistant (SR) asthmatics were stimulated with either mitogens or allergens. Effects of glucocorticoids (GCs) on the proliferation and cytokine synthesis were analyzed. Der f 2-specific Th clones were established from PBMC by the limiting dilution.

Result: IL-5 production by PBMC of SS asthmatics was significantly reduced after ICS administration, but that of SD asthma remained high. IC50 values of dexamethasone suppression on cytokine synthesis and proliferation was not statistically different among SS, SD, or SR asthmatics. Addition of CD28 signal made proliferation of anti-CD3-stimulated Th clones steroid-resistant. The induction of steroid resistance was dependent on IL-2 receptor signal and PI-3 kinase activity.

Conclusion: Besides T cell intrinsic mechanisms, steroid responsiveness of T cells seems to be determined by the microenvironment, costimulatory signals and cytokines. Costimulatory signal might be involved in the induction of steroid resistance in T cells of SD asthma. The notion is consistent with our recent finding that administration of CTLA4-Ig made SR asthma model SS.

7 Multi-symptom asthma as an indication of disease severity in epidemiology

Ekerljung, Linda¹; Bossios, Apostolos²; Lötvall, Jan²; Olin, Anna-Carin³; Rönmark, Eva⁴; Wennergren, Göran⁵; Torén, Kjell³; Lundbäck, Bo²

¹University of Gothenburg, Sweden; ²University of Gothenburg, Institute of Medicine/Krefting Research Centre, Sweden; ³University of Gothenburg, Institute of Medicine/Dept of Occup & Envir Med, Sweden; ⁴University of Umeå, Dept of Public Health & Clinical Medicine, Sweden; ⁵University of Gothenburg, Dept of Pediatrics, Sweden

Epidemiological questionnaires have failed to identify individuals with severe asthma. The extent of symptoms of asthma can, however, easily be established in epidemiological studies by identification of multiple symptoms. We hypothesized that reporting of multiple symptoms of asthma reflects uncontrolled disease and can be a sign of more severe asthma. The aims of the current study were, therefore, to determine the prevalence and determinants of multi-symptom asthma. In this paper we report our definition of multi-symptom asthma and its clinical characteristics. A postal questionnaire was mailed to 30,000 randomly selected subjects aged 16–75 yrs. A subgroup underwent detailed clinical examinations including lung function test, exhaled NO, methacholine test in addition to a detailed clinical history by using structured interview. Multi-symptom asthma was defined as
questionnaire reported physician-diagnosed asthma, use of asthma medication, recurrent wheeze, attacks of shortness of breath, and at least one additional respiratory symptom. The overall prevalence of physician-diagnosed asthma was 8.3%, while of multi-symptom asthma the prevalence was 2.0% (women 2.4%, men 1.5%, p < 0.001). Multi-symptom asthma versus other asthma was associated with lower FEV1 (88.8% pred vs. 98.8% pred), higher FeNO (29.3 ppb vs. 23.2 ppb), a greater proportion having PD20 < 1.96 mg methacholine chloride (82.9% vs. 58.7%), all statistically highly significant. The same pattern was found for asthma exacerbations, emergency department visits and hospitalizations. All respiratory symptoms were more common in multi-symptom asthma compared with other asthma, and that was true also for symptoms of bronchitis, rhinitis and rhino-sinusitis. In contrast, allergic rhinitis and allergic sensitization were not more common in multi-symptom asthma than in other asthma. Multi-symptom asthma cannot be used for defining severe asthma. We conclude, however, that multi-symptom asthma, as we defined the condition, is related to signs of more severe disease and could be used as an epidemiological marker of asthma severity.

8 Circulating eosinophil progenitors express major trafficking related molecules and are more activated compared to mature eosinophils in patients with asthma

Lu, You; Malmhäll, Carina; Sjöstrand, Margareta; Rådinger, Madeleine; Lundbäck, Bo; Lötvall, Jan; Bossios, Apostolos
University of Gothenburg, Sahlgrenska Academy, Institute of Medicine, Kreft ing Research Centre, Sweden

Background: Eosinophilic inflammation represents a hallmark for allergic asthma. Eosinophils differentiate in the bone marrow from CD34+ cells and are released into the blood and traffic to the lung tissue. To date, the majority of studies originate from animal models or from humans after allergen exposure. Thus, it is unclear if mature eosinophils and eosinophil progenitors express similar levels of trafficking related molecules. Therefore, we characterized the expression of trafficking related molecules on circulating eosinophil progenitors in patients with stable asthma.

Methods: Participants, 13 patients with stable asthma; 7 at the high end (>0.3x10^9/L) and 6 at the low end (≤0.2x10^9/L) of normal range of blood eosinophils, and 5 healthy controls were selected from the West Sweden Asthma Study. Airway eosinophils were studied in induced sputum. Mature (CD45+IL-5Rα+SSC^high^) and progenitors (CD45+CD34+IL-5Rα+SSC^low^) eosinophils and their expression of selectin (PSGL-1), integrins (VLA-4:CD49d+CD29+, Mac-1:CD11b+CD18+), eotaxin(s) receptor (CCR3+), activation (CD69+, CD25+) and apoptosis (active-caspase 3, CD95+) were quantified in fresh blood by flow cytometry.

Results: Asthma patients with high blood eosinophils had increased sputum eosinophils and blood eosinophil progenitors compared to the healthy controls (p<0.05). Mature eosinophils and eosinophil progenitors expressed similar levels of PSGL-1 and VLA-4. Mac-1 was expressed in all mature eosinophils but was reduced in progenitors, in all groups (<0.01). Mature eosinophils expressed higher levels of CCR3 compared to progenitors (<0.05). However, the CCR3+ eosinophil progenitors showed increased expression of CD25 and CD69 i.e. were more activated in all groups (<0.01) compared to mature eosinophils. No differences in the expression of apoptosis related markers were found between CCR3+ eosinophil progenitors and CCR3+ mature eosinophils.

Conclusion: Circulating eosinophil progenitors in patients with stable asthma express major trafficking related receptors found in mature eosinophils, thus suggest their capacity for migrating to the lung tissue. Notably, the eosinophil progenitors primed to eotaxin(s) (CCR3+) are highly activated compared to mature eosinophils.

9 Provocation test in patients with different phenotypes of aspirin hypersensitivity

Specjalski, Krzysztof¹; Gorska, Lucyna²; Jassem, Ewa²
¹Medical University of Gdansk, Poland; ²Medical University of Gdansk, Department of Allergology, Poland

Background: Aspirin (ASA) hypersensitivity has a complex clinical presentation with three major phenotypes: aspirin-exacerbated respiratory disease (AERD; asthma and/or nasal polyps), chronic urticaria and aspirin-induced anaphylaxis. In patients with AERD asthma is often severe and difficult to control. Moreover, NSAID ingestion may provoke life-threatening attack. Thus, diagnosing of ASA hypersensitivity is an important issue. In everyday practice ASA hypersensitivity is confirmed by oral (OPT), nasal (NPT) or bronchial provocation tests. However their protocols
vary in terms of maximum cumulative dose. Besides, phenotype of hypersensitivity is not taken into consideration when choosing the protocol.

**Aim of the study:** The aim of this study was to compare responsiveness to ASA in patients with different phenotypes of ASA hypersensitivity (AERD, chronic urticaria, anaphylaxis).

**Patients and methods:** 238 patients (179 women, 59 men) aged 19-71 with the history suggesting hypersensitivity to NSAIDS were provoked with aspirin. The group comprised 92 patients with AERD, 83 with chronic urticaria and 63 with suspicion of anaphylaxis caused by ASA. Single-blind oral provocation test was performed according to two-day protocol (day 1- placebo, day 2- increasing doses of ASA: 50, 100, 150, 300, 400mg). Anterior rhinomanometry, spirometry and clinical monitoring were used to assess the reaction. The test was considered positive in case of clinical symptoms of hypersensitivity, significant decline of FEV1 or positive nasal response in anterior rhinomanometry.

**Results:** Provocation test was positive in 81 (34%) patients, including 26 with chronic urticaria, 45 with asthma and/or nasal polyps and 10 with history of anaphylaxis. Proportions of positive results in the subgroups were 31%, 49% and 16%, respectively. The mean dose provoking reaction was significantly lower in AERD group compared to patients with chronic urticaria (212mg vs. 604mg, p<0.05). 36% of positive responders with asthma reacted to doses ≤200mg. 19 subjects (8%) responded to placebo making the provocation impossible to evaluate.

**Conclusions:** Patients with AERD usually respond to smaller doses of ASA compared to patients with chronic urticaria. As an oral provocation test is associated with the risk of anaphylactic reaction and its severity is dose-dependent it may be suggested to introduce new protocol for patients with severe asthma, based on lower first doses of ASA.

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**10 Design and assessment of an adherence monitoring device for inhalers**

Costello, Richard¹; Costello, Richard²; Reilly, Richard³

¹RCSI, Ireland; ²RCSI, Medicine, Ireland; ³Trinity College, Dublin, Engineering, Ireland

**Background:** Inhalers are widely used in the treatment of asthma. Non-adherence to inhalers is considered to be a contributing factor to poor control of this condition. Non-adherence with inhaled medications may arise because the drug is taken at incorrect times or when the correct steps in the use of the inhaler are not followed.

**Methods:** We designed a device with a high fidelity microphone and small sized acoustic storage to make time stamped acoustic recordings of an individuals use of an inhaler. We related the acoustic features to the various steps involved in the correct use of an inhaler. We established a minimum acoustic profile required to use the inhaler correctly, thereby objectively quantifying technique. A cohort of subjects with moderate/severe asthma (n=44) used a salmeterol/fluticasone discus inhaler with the device attached for a month. The subjects were instructed in the use of the inhaler.

**Results:** There was almost a full correlation between the number of audio files in which drug priming occurred (n=1674) compared to the number of doses administered (n=1687). Analysis of the recordings indicated that 6 (18%) had missed more than 20% of doses and 7 (21%) had more than 20% of doses with an error. The most common error was that subjects blew into the device, after the drug was deployed and with sufficient force that the drug was dispersed (n=184). Overall, 19 (57%) had more than 20% doses with an error in timing or technique. **Conclusion:** These studies indicate that this device and associated processing may be useful for the management of conditions such as severe asthma.

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**11 Is the measurement of delivered dose a good performance measure? a characterization of delivered and respirable delivered dose in two brands of jet nebulizer**

Rehman, Mariam; Metcalf, Adam; Hatley, Ross

Respironics Respiratory Drug Delivery (UK) Ltd, Philips Home Healthcare Solutions, UK

**Background:** Use of a nebulizer facilitates rapid administration of high doses of drug, which is desirable for patients presenting with acute severe asthma. Breath-enhanced jet nebulizers direct the patient's air flow through the nebulizer handset during inhalation producing aerosol output with a more favorable respirable profile. SideStream Plus (SS+; Philips Respironics) and Nebutech (NB; Salter Labs) are breath-enhanced jet nebulizers, designed to produce a high delivered dose in a short treatment time. A previous in vitro characterization of delivered dose and
droplet size distribution of arformoterol nebulized via the SS+ and the NB indicated that the SS+ performed favorably, compared with the NB. We present results of an \textit{in vitro} characterization of salbutamol sulphate (albuterol sulfate) delivery via the SS+ nebulizer and the NB nebulizer.

**Method:** Each nebulizer was filled with 3 mL salbutamol sulphate (5 mg/2.5 mL) and run with a driving flow of 8 L/min into a CEN adult tidal breathing pattern (Vt: 500mL, 15 BPM, I:E ratio: 1:1). The nebulizers were run in triplicate to sputter point and sputter point plus 60 s. Dose delivered to a filter (delivered dose; DD), placed between the nebulizer and breathing emulator, was quantified using high performance liquid chromatography and expressed as a µL solution equivalent. A laser diffractor (Malvern Spraytec) was used to assess fine particle fraction (FPF) and mass median diameter (MMD) after 60 s nebulization time. Fine particle dose (FPD) was calculated (FPF x delivered dose).

**Results:**

<table>
<thead>
<tr>
<th>Experimental parameter</th>
<th>SS+</th>
<th>NB</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMD (µm)</td>
<td>3.2</td>
<td>5.6</td>
</tr>
<tr>
<td>FPF (%&lt;5 µm)</td>
<td>74.2</td>
<td>43.7</td>
</tr>
<tr>
<td>DD; Sputter (µL solution)</td>
<td>432</td>
<td>424</td>
</tr>
<tr>
<td>FPD; Sputter (µL solution)</td>
<td>321</td>
<td>185</td>
</tr>
<tr>
<td>DD; Sputter + 60 s (µL solution)</td>
<td>542</td>
<td>594</td>
</tr>
<tr>
<td>FPD; Sputter + 60 s (µL solution)</td>
<td>402</td>
<td>260</td>
</tr>
</tbody>
</table>

**Conclusion:** The total DD from the 2 nebulizers was comparable. Aerosol delivered via the SS+ nebulizer had a lower MMD and higher FPF, compared with that delivered via the NB nebulizer. This resulted in a 74% higher FPD delivered to sputter, and a 55% higher FPD delivered to sputter plus 60 s from the SS+ nebulizer compared with the NB nebulizer. The FPD represents respirable aerosol, and is therefore more clinically relevant than the DD. These results are consistent with previous work, and indicate that a high respirable dose of aerosol is produced by the SS+ nebulizer.


12 \textbf{In vitro assessment of inter and intra batch variability of a breath-enhanced jet nebulizer}

Rehman, Mariam; Hurren, Antony; Metcalf, Adam; Hatley, Ross

Respironics Respiratory Drug Delivery (UK) Ltd, Philips Home Healthcare Solutions, UK

**Background:** Guidelines recommend that patients presenting with acute severe asthma with life-threatening features receive the necessary high doses of β2-agonists via the nebulized route. The SideStream Plus (SS+; Philips Respironics) is a breath-enhanced nebulizer designed to maximize respirable output, while minimizing treatment time. Prior to the introduction of a new version of nebulizer, various in vitro assessments, including inter and intra batch variability, are performed using a limited number of devices. We present results of an analysis of inter and intra batch variability of SS+ nebulizers.

**Method:** SS+ nebulizers (3 batches; n=30 per batch) were assessed in terms of 5 experimental parameters; nebulization time, residual volume, particle size distribution (mass median diameter (MMD), fine particle fraction (FPF)), and emitted dose. Each batch was provided with a driving flow from a different Porta-neb compressor (Philips Respironics). Measurement of particle size distribution was achieved using a laser diffractor (Malvern Spraytec) with an extraction flow of 15 L/min. Each nebulizer was weighed and filled with 2.5 mL salbutamol sulphate (albuterol sulfate) solution (1 mg/mL). To assess nebulization duration the nebulizers were run continuously until 'end of treatment', defined as when the obscuration level of the sample in the laser diffractor fell below 5% for 5 s. At this point the SS+ were re-weighed to calculate residual volume. The dose delivered to a filter placed between the laser diffractor and extraction air flow was quantified using high performance liquid chromatography. The coefficient of variation (CV) for each experimental parameter was calculated for each batch.
Results:

<table>
<thead>
<tr>
<th></th>
<th>Batch A CV (%)</th>
<th>Batch A Mean</th>
<th>Batch B CV (%)</th>
<th>Batch B Mean</th>
<th>Batch C CV (%)</th>
<th>Batch C Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nebulization time (s)</td>
<td>13.1</td>
<td>274</td>
<td>9.3</td>
<td>258</td>
<td>9.6</td>
<td>270</td>
</tr>
<tr>
<td>Residual volume (mg solution)</td>
<td>6.6</td>
<td>1035</td>
<td>8.1</td>
<td>1100</td>
<td>9.9</td>
<td>1086</td>
</tr>
<tr>
<td>MMD (µm)</td>
<td>2.9</td>
<td>3.8</td>
<td>3.1</td>
<td>3.9</td>
<td>3.9</td>
<td>3.8</td>
</tr>
<tr>
<td>FPF (%&lt;5 µm)</td>
<td>2.1</td>
<td>63.2</td>
<td>2.3</td>
<td>62.6</td>
<td>3.0</td>
<td>63.4</td>
</tr>
<tr>
<td>Emitted dose (µg salbutamol)</td>
<td>6.9</td>
<td>1127</td>
<td>9.2</td>
<td>1125</td>
<td>8.8</td>
<td>1101</td>
</tr>
</tbody>
</table>

Conclusion: The intra batch coefficient of variation indicates that variability in each of the 5 experimental parameters did not differ substantially across the 3 batches tested. The close similarity between mean values for each of the experimental parameters indicates that inter batch variability was low. Taken together, these results suggest that different batches of SS+ nebulizers should perform similarly in terms of nebulization time, particle size distribution, and emitted dose.


13 Ultrafine pMDI Beclometasone/Formoterol HFA with or without spacer in non-controlled asthmatic patients on Budeosnide/Formoterol DPI. A randomized study with blind end-point evaluation

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Background: Asthmatics with non-controlled asthma on medium dose steroid plus LABA should have their steroid dose increased or an extra controller added. Beclometasone/Formoterol HFA pMDI ultrafine formulation (BD/F – Chiesi Farmaceutici - Italy) improves total lung deposition and could be effective at the same dosage.

Objective: to evaluate the response of equivalent doses of BD/F-pMDI (200µg/12µg B.I.D.) with or without spacer in patients with non-controlled asthma already on treatment with budesonide/formoterol-DPI (Bud/F400µg/12µg B.I.D.), preliminary results.

Methods: Design: randomized pragmatic study with blind end-point evaluation. After 2-weeks run-in period patients still non-controlled on Bud/F were randomized to receive BD/F with (G1) or without (G2) a spacer(Vortex™). Patients were evaluated before(V1) and 4(V2) and 8(V3) weeks after treatment by ACT and FEV1. Statistical analysis used mixed linear model for longitudinal data with group and time as explanatory variables.

Results: Were included 37 patients, 20 in G1 and 17 in G2. Basal characteristics were similar. G1 mean ACT scores (CI95%) were 12.5 (11,51-13,99), 19,5(17,83-21,2) and 18,9(16,79-21,01) for V1, V2 and V3 respectively. For G2, values were 13,2(11,34-15,06), 21,1(19,34-22,6) and 22,5(21,92-23,08) . There were no differences between groups (p=0,12) but within groups there was a statistically significant difference between V1 and V2 and V3 (p<0,001). G1 mean FEV1 (CI95%) expressed as predicted percentage were 49,2%(41,6-56,8), 57,8%(49,9-65,6) and 61,8%(52,3-71,3) for V1, V2 and V3 respectively. For G2 values were 54,4%(44,5-64,3), 61,0%(51,1-70,9) and 60,6%(50,7-70,5). There were no differences between groups (p=0,23) but within groups there was a statistically significant difference between V1 and V2 and V3 (p<0,01).

Conclusion: Our preliminary results show that the combination Beclometasone200µg/Formoterol12 µg Modulite B.I.D with or without spacers is effective in this group of patients with severe uncontrolled asthma on Budesonide 400µg/Formoterol12 µg B.I.D. Clinica trials registration # NCT01453881
Thursday, 11 October 2012  
14:30 – 15:30  

14 Bronchodilatation increases number of particles in exhaled air in subjects with asthma  
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¹Occupational and Environmental Medicine, Sweden; ²Gothenburg University, Occupational and Environmental Medicine, Sweden; ³Gothenburg University, Dept Respiratory Medicine and Allergology, Sweden  

**Background:** Particles in exhaled air (PEx) are derived from the small airways and are formed during airway closure and re-opening. They mainly contain surfactant; both phospholipid and protein composition in PEx resemble that of BAL. Measurements of surfactant protein A in PEx from 100 L exhaled air were shown to be highly reproducible, making the PEx a promising tool in the monitoring of asthma. The number of exhaled particles varies substantially, mainly among subjects, but also within subjects. To enable a correct interpretation of the results using PEx it is crucial to examine how airway constriction affects the number of exhaled particles.  

**Aim:** To examine the effect of bronchodilatation on exhaled PEx concentration.  

**Method:** 16 subjects with pollen-asthma and 14 healthy non-atopic subjects (all non-smokers) were examined before and after bronchodilation during the pollen season and outside the pollen-season. PEx, spirometry, blood-samples and answers to a questionnaire were obtained. The subjects performed a breathing maneuvers allowing for airway closure and re-opening and PEx concentrations in about 60 L of exhaled air were measured with an in-house developed instrument based on particle impaction.  

**Results:** PEx concentrations were not significantly different between asthmatics and controls but asthmatics showed lower PEx concentrations during pollen season compared to outside pollen season (3.46 v s 4.32 p=0.01) whereas controls showed non-significant differences between seasons (6.86 v s 4.54 p=0.15). PEx concentrations increased after bronchodilatation in asthmatics (median 4.05*10³ to 4.92*10³, p=0.02), but not in controls (median 4.47*10³ v s 4.50*10³ p=0.12). The change in PEx concentration (%) was associated with the change in FVC (%) (rp=0.51, p=0.001) and FEV1 (rp=0.46, p=0.003) among subjects with asthma whereas there were no significant correlations among controls.  

**Conclusion:** In the present study the subjects had mild symptoms and rather low reversibility also during pollen-season. Nevertheless, PEx concentrations were apparently influenced by bronchomotor tone and increased after bronchodilatation, presumably reflecting increased airway opening following bronchodilatation in asthmatics with ongoing airway inflammation.

15 Reduction in oral corticosteroid use in patients with severe allergic (IgE-mediated) asthma receiving omalizumab in a real-world setting  
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**Background:** Patients with severe allergic asthma (SAA) are often inadequately controlled despite available treatments including high-dose inhaled corticosteroids and long-acting β2-agonists. Use of oral corticosteroids (OCS) in SAA patients may not achieve full asthma control, and leads to significant long-term side effects. Omalizumab is a recombinant humanized monoclonal anti-immunoglobulin E (IgE) antibody approved in the European Union as an add-on therapy for patients with SAA. In clinical studies, omalizumab has been shown to reduce OCS use. Here we report the effect of omalizumab treatment on OCS maintenance use for up to 24 months in patients with SAA in the real-world eXpeRience registry.  

**Methods:** eXpeRience was a 2-year, multicentre, non-interventional, single-arm, observational registry initiated to collect data from patients receiving omalizumab for uncontrolled SAA. Data were collected on OCS maintenance use
at baseline, Month 12, and Month 24. Parameters assessed were incidence of OCS maintenance use, total daily OCS dose and change from baseline, and time to reduction in OCS dose or stopping therapy.

**Results:** At Month 24, 49% of the patients on OCS had discontinued their use and 20% had reduced their OCS dosage, this was incremental from Month 12. OCS maintenance use at baseline, Month 12 and Month 24 is summarized in Table 1.

<table>
<thead>
<tr>
<th>Patients on OCS maintenance monotherapy, n (%)</th>
<th>Baseline N=916</th>
<th>12 months N=734</th>
<th>24 months N=643</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD) total daily OCS dose*, mg</td>
<td>15.49 (14.01)</td>
<td>7.68 (10.94)</td>
<td>5.77 (8.89)</td>
</tr>
<tr>
<td>Mean (SD) reduction from baseline in total daily dose, mg</td>
<td>-</td>
<td>7.89 (13.77)</td>
<td>9.95 (15.58)</td>
</tr>
</tbody>
</table>

* OCS dose was reported in prednisolone equivalent dose as mg per day. OCS – oral corticosteroid; SD - standard deviation; n – number of patients who received OCS at baseline and who provided OCS information at 12 months and 24 months (*n=246; *n=189; *n=168; *n=108; *n=116).

**Conclusion:** Omalizumab reduced the need for maintenance OCS use in patients with severe allergic (IgE-mediated) asthma in a real-world setting. Reduction in OCS maintenance use may reflect better asthma control and decreases the risk of long-term morbidity of corticosteroid exposure.

### 16 Polymorphism of multidrug resistance gene are markers of therapy-resistant bronchial asthma in russian patients

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**Background:** Bronchial asthma (BA) belongs to the group of multifactorial diseases with different functionally related genes being involved in its pathogenesis. Genetic polymorphisms influence BA progression and severity as well as response to BA therapy. Polymorphism Ñ3435Ò in exone 26 of MDR1 gene is associated with different levels of Pgp activity and glucocorticosteroids (GCS) metabolism changes and associated with resistance to anti-inflammatory treatment, including GCS in the patients.

**Aim:** To investigate the frequency and association of C3435T MDR1 gene with severity of BA and effectiveness of BA pharmacotherapy in therapeutic resistant BA (TRBA) patients and healthy patients (control group).

**Methods:** Genomic DNA was extracted from peripheral leukocytes. The genotypes were detected by PCR and analysis of the length of restriction fragments. 122 asthmatics were included: 46 - of them had TRBA, 76 - with therapeutic sensitive asthma (TSBA) and 103 healthy controls. Surveyed group did not include the patients with steroid-resistant asthma.

**Results:** Duration of BA, GCS treatment duration, daily dose of oral and inhaled GCS, GCS treatment complications rate was higher in patients with TRBA. Distribution of MDR1 genotypes in control group was differ to other European populations: CC-8,7% (n=9), CT-43,7% (n=45), TT-47,6% (n=49). Distribution of MDR1 genotypes in TRBA were CC-37% (n=17), CT-39,1% (n=18), TT-23,9% (n=11), in TSBA were CC-21,3% (n=16), CT-52% (n=39), TT-26,7% (n=20). Rates of C allele (“aggressive allele”) and Ò allele of MDR1 gene in TRBA group were 0,56 and 0,44, in controls 0,30 and 0,70. We revealed associations of genetic variants with increased risk (IR): 3435CC with IR of BA (OR=3.92, 95%CI 1.74-8.79); 3435CC with IR of GCS doses more than 20 mg of prednisolon (OR=20.89, 95%CI 5.10-85.53); 3435CC with IR of therapy-resistant BA (OR=6.12, 95%CI 2.42-15.48).

**Conclusion:** For the first time, we identified significant association of MDR1 (Ñ3435 Ô) gene polymorphism with TRBA. Analysis of MDR1 polymorphisms is useful for both preventive care (revealing subjects with increased predisposition to BA) and pharmacotherapy optimization due to prediction of BA severity and risk of TRBA. Pharmacogenetic approach in evaluation of BA patients gives a possibility to predict the response to
glucocorticosteroids as well as it can help to improve individual anti-inflammatory treatment principles with optimization of "efficacy-safety" ratio.

17 Ace polymorphism in asthmatic patients
Cortez, Margarida¹; Matos, Andreia²; Ferreira, Joana²; Lopes, Leonor²; Gil, Angela²; Bicho, Manuel²
¹CHLN-HSM, Portugal; ²Lisbon Medical School, Genetic Department, Portugal

Background: The aim of the this study was to analyse if there is an association between angiotensin converting enzyme (ACE) insertion/deletion (I/D) polymorphism (287 base pairs, on chromosome 17q23, intron 16(rs4340)) with asthma severity. ACE plays a vital role in the renin-angiotensin-system (RAS) which regulates blood pressure by converting angiotensin I into a powerful vasoconstrictor angiotensin II, that also has an important role in airway remodeling and inactivation of bradykinin and tachykinins. The signaling pathways, related with this polymorphism regulating the cytokine production by T cells, could induce a different Th profile modulating the immune response in asthma.

Methods: Asthmatic patients:n=68;were compared with a control group of n=204 healthy blood donors. The insertion/deletion (I/D) polymorphism was determined by PCR- Polymerase chain reaction. Control of asthma assessed by validated instrument (ACQ7 and PAQLQ).Statistical analysis was performed with PASW 18, establishing a significance level of p< 0.05.

Results: The mean age of the 68 asthmatics was 39.95 ± 18.9 years; 42 females and 26 males; 66 caucasians and 2 noncaucasians; 57 atopic and 11 nonatopic. The mean age of the control-group (n=204) was 40.97 ± 12.08 years; 69 females and 135 males .In asthmatics the frequencies of the D- Allele (ACE-D) is 0.647 and of the I-Allele (ACE-I) is 0.353; in controls: 0.868 and 0.132 respectively. There is statistical difference between these groups (p=0.008). Genotypes in the asthmatics- DD: 44.1%; ID: 20.6%; II: 35.3%; in control group- DD:48%; ID:38.8%; II: 13.2%. There is statistical difference between these groups(p=0,000). The II genotype was more frequent in the asthmatics when compared with controls being the risk associated 3.576 (CI 95% [1.883 – 6.799],( p =0.000). In asthmatics, there is no statistical differences in genotype frequencies (p>0.05) between : atopics and non atopics; controlled and uncontrolled asthma; males and females; and in the different age-groups.

Conclusions: The role of ACE insertion/deletion (I/D) polymorphism ,in asthmatic patients is a controversy risk factor to the severity of asthma, but we concluded that he II genotype is more prevalent in the asthmatics from this hospital – based population.

18 The clinical and genetic factors for predicting the response to inhaled corticosteroids
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¹Teikyo University School of Medicine, Department of Medicine, Japan; ²National Hospital Organization Sagamihara National Hospital, Clinical Research Center, Japan; ³Gifu University Graduate School of Medicine, Department of Pediatrics, Japan; ⁴National Hospital Organization Tokyo National Hospital, Department of Respiratory Diseases, Japan

Background: It has been reported that a part of asthma patients does not respond well to inhaled corticosteroids (ICS) and the benefit by long term treatment by high-dose ICS is limited in such patients. But the clinical or genetic factors for predicting ICS response have not been fully established especially in adults. The aim of this study is to establish the factor for predicting the response to ICS treatment.

Method: Patients treated only by ICS for more than 6 months were enrolled and classified into responder group (R group, n=30) if FEV1 improvement ≥ 5% and non -responder group (NR group, n =40, FEV1 improvement<5%). The relationship between ICS response and pre-treatment clinical indices and 21 single nucleotide polymorphism (SNP) were retrospectively analyzed.

Results: In R group, peripheral blood eosinophil% (R: NR = 7.5: 4.3%) and serum total IgE level (550.4: 497.1 U/ml) was significantly higher as compared to NR group. R group also showed significantly lower %VC (97.5: 114.8%), %FEV1.0 (77.9: 101.3%), FEV1.0% (67.5: 76.4%) and higher bronchial hyperresponsiveness. Bronchial reversibility test was available in 30 patients (R: n=11, NR: n=19) and reversibility was significantly higher in R group (15.9: 5.4%). By logistic multivariate analysis for those 30 patients, bronchial reversibility was significantly related to ICS response. SNP in IFN-γ R1 L467P was also related to ICS response. Post /Pre FEV1 ratio was significantly higher in homo/hetero group as compared to wild group (140.2 vs 108.8%).
Conclusion: Pre-treatment bronchial reversibility was identified as a prediction factor for ICS response. It has been reported that SNP in IFN-γR is associated with allergic diseases and serum IgE level and the SNP might be potentially related to ICS response.

19 On-line italain register for severe/non-controlled asthma
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¹Consiglio Nazionale delle Ricerche, Istituto di Fisiologia Clinica, Italy; ²Università degli Studi di Palermo, Malattie dell’Apparato Respiratorio, Italy; ³Azienda Ospedaliera Universitaria Ospedali Riuniti di Ancona, Dipart. Malattie Respiratorie e Immunologiche, Italy; ⁴Università di Verona, Unità di Epidemiologia e Statistica Medica, Italy; ⁵Regione Lazio, Dipartimento di Epidemiologia, Italy; ⁶Università di Pisa, Dipartimento Cardio Toracico e Vascolare, Italy; ⁷Università di Genova, Dip. Medicina, Malattie Allergiche e Respiratorie, Italy; ⁸Università di Perugia, Dipartimento di Medicina Clinica e Sperimentale, Italy

Background: Severe/Non-controlled asthma (SNCA) is a crucial challenge for physicians and a socio-economic burden for National Health Services (NHS). In Italy more than 50% of costs for asthma (1-2% of total NHS expenditure) are due to SNCA and moreover, within the European Community Respiratory Health Survey, Italy was the country with the lowest % of ICS daily use (29%) and with the highest % of subjects with uncontrolled asthma despite treatment (67% vs an overall European mean of 47%). Despite few data from very selected centres, in our country a precise estimate of the epidemiological figures and the disease related costs for SNCA is not available. Thus, we aimed at instituting of an on-line Italian register for SNCA (Registro Italiano Asma Grave e Non Controllata) published on the website of the Italian Health Agency (Istituto Superiore di Sanità -ISS) and financed by the Italian Drug Agency (Agenzia Italiana per il Farmaco – AIFA).

Objectives: To assess in general population and in clinical settings the effectiveness of therapeutic strategies for SNCA, defined in accordance with GINA guidelines. Secondary objectives are: obtain clinical indicators of diagnostic and therapeutic appropriateness for SNCA, evaluate direct and indirect costs of this disease, obtain continuous monitoring of SNCA patients, some epidemiological figures and finally disseminate the use of the register among Italian clinical centres.

Methods: The register is composed of 9 different sections and a follow-up section accessible, at present, only to the 8 centers participating in the AGAVE (Severe asthma: epidemiological and clinical cohorts follow up; therapeutic appropriateness and outcome assessment) project, financed by AIFA. These centres will use the register to enter clinical data from their SNCA patients. All patients with a diagnosis of severe asthma since at least one year and all uncontrolled asthma despite regular treatment according to GINA step 3 or 4 are eligible. Patients will be selected from prospective and retrospective longitudinal studies of pre-existing cohorts of different age groups and different geographical areas. Follow-up will be performed every 6 months for 24 months. The estimated number of recruited patients from the clinical setting is 375, while 500 patients are expected from the epidemiological setting.

Conclusions: Our goal is to collect more data on SNCA patients and disseminate the on-line register for use by National Health Service physicians.

20 The role of type 1 angiotensin 2 receptor polymorphism in asthmatic patients
Cortez, Margarida¹; Matos, Andreia²; Ferreira, Joana²; Lopes, Leonor²; Gil, Angela²; Bicho, Manuel²
¹CHLN-HSM, Portugal; ²Lisbon Medical School, Genetic Department, Portugal

Background: It is known that type 1 angiotensin II (Ang II) receptor (AGTR1) could be related with the pathogenesis of bronchial asthma. It is involved in Th polarization, through different signaling pathways modulating allergic airway inflammation, and also may participate in airway remodeling and bronchoconstriction, that could be related with AGTR1 polymorphism. The purpose of this study is to analyze the association between AGTR1 1166A/C (rs5186) gene polymorphism with asthma severity.

Methods: Asthmatic patients : n=37; were compared with a control group of n=32 healthy blood donors. The AGTR1 1166A/C (rs5186) gene polymorphism was determined by PCR-RFLP(Polymerase chain reaction- restriction fragment length polymorphism). Control of asthma assessed by validated instrument (ACQ7 and PAQLQ) .Statistical analysis was performed with PASW version 18 establishing a significance level of p< 0.05.
Results: The mean age of the 37 asthmatics was 40 ± 18 years; minimum 7 and maximum 71; 24 females and 13 males; 35 caucasians and 2 non-caucasians; 34 atopics and 3 non-atopics; 22 with controlled and 15 with uncontrolled asthma. The control group for this polymorphism is in Hardy-Weinberg equilibrium (p > 0.05). In asthmatics the frequencies of the allele A is 53% and the allele C is 47%; in controls: 64% and 36% respectively. There is no statistical difference between these groups (p>0.05). Genotypes in the asthmatics- AA: 29.7%; AC: 46%; CC: 24.3%; in control group-AA: 34.4%;AC: 59.4%; CC: 6.2%. There is no statistical difference between these groups(p>0.05). When we associate AGTR1 genotypes, there was a tendency, but not significant, between asthmatics and controls (CC vs AC+AA ; p = 0.086) being CC genotype more frequent in the asthmatic group. In asthmatics, there is no statistical difference(p>0.05) in genotypes: between atopics and non atopics; controlled and uncontrolled asthma; males and females; by ethnic-group; and in the different age-groups.

Conclusions: Our findings provided some evidence that there is a trend, although not significant, that AGTR1 gene A1166C polymorphism might be a genetic marker for the pathophysiology of allergic airway inflammation, remodeling and bronchoconstriction in asthmatic disease.

21 Severe asthma is really uncommon
Zicari, Anna Maria¹; Cutrera, Renato²; Lollobrigida, Valeria³; Ernesti, Ilaria³; Ceson Marcelli, Azzurra³; Celani, Camilla³; De Vittori, Valentina³; Duse, Marzia³
¹Sapienza Università di Roma, Policlinico Umberto I, Italy; ²Rome, Hospital, Broncopneumologia Ospedale Bambino Gesù, Roma, Italy; ³Sapienza Università di Roma, Policlinico Umberto I, Immuno-allergologia pediatrica, Italy

We describe the case of a 10-year-old girl with a history of severe persistent asthma and exercise-induced-asthma, controlled using an appropriate treatment with inhaled corticosteroid-long-acting beta-2 adrenergic agonists (ICS+LABA) and leukotriene receptor antagonists. She was healthy until the age of 8 years, when she presented two episodes of radiologically diagnosed pneumonia. After that, she began to present persistent cough, also nocturnal, stridor, dyspnea and respiratory distress and she was sent by pediatrician to our hospital. She performed a global spirometry which shows an obstructive and restrictive phenotype (FEV1: 75,3% and MEF50: 57,6%), without a significantly dilatation after inhaled salbutamol (400 mcg). She underwent to a systemic therapy with oral corticosteroid, with not benefit. She had no fever neither upper respiratory tract infections. We excluded gastro-oesophageal reflux disease, cystic fibrosis, Mycoplasma and Chlamydia pneumonia. Cardiological examination was negative. During hospitalization, she spontaneously expectorated a thick fibrinous mucoid formation. A chest X ray and a computed tomography (CT) scan showed atelectasis of both lung, widespread hyperlucency, and occlusion of the right main bronchus, compatible with a diagnosis of plastic bronchitis. Plastic bronchitis is a rare disease characterized by the formation of large gelatinous or rigid branching airway casts. The prevalence and etiology of plastic bronchitis are still unknown and the symptoms may also overlap with those of other diseases such as severe asthma, in the severe mucus plugging sometimes seen in allergic bronchopulmonary Aspergillosis (ABPA) or in middle lobe syndrome. In the pathogenesis of the disease the inflammation is usually present and initiates cast formation. Treatment includes bronchodilators, inhaled and oral corticosteroids, mucolytics, airway clearance therapy and antibiotics. Other therapies can include inhaled heparin, urokinase, tissue plasminogen activator (TPA), dornase alfa and oral macrolide antibiotics as mucoregulatory therapy.

Conclusions: The presence of asthmatic symptoms without clinical improvement after appropriate therapy is not always suggestive of severe asthma. Therefore, for the appropriate diagnosis, we have to exclude the other lung diseases and, among the differential diagnoses, is possible to consider also plastic bronchitis.

Friday, 12 October 2012
10:30 - 12:00

22 Determinants of health outcome in individuals with asthma
Axelsson, Malin; Ekerljung, Linda; Lundbäck, Bo; Lötvall, Jan
Gothenburg University, Sahlgrenska Academy, Institute of Medicine, Krefting Research Centre, Sweden
Background: For many individuals, asthma has great impact on their everyday life, not least in terms of medication treatment and disease management. Therefore, it is important to evaluate recommended treatments on an individual level. In that respect, estimations of health-related quality of life (HRQL) function as an essential health outcome, as they capture personal perspectives and experiences of everyday life with a chronic disease such as asthma. The aim was to identify determinants of health-related quality of life in adult individuals with asthma.

Method: Participants with asthma (n=487) derived from a population-based study completed questionnaires on asthma control, emotional status, adherence and HRQL. Additionally, data on the Five-factor model personality traits: Neuroticism, Extraversion, Openness, Agreeableness, Conscientiousness were gathered. Data were statistically analyzed using t-tests, bivariate correlations and multiple regressions.

Results: Participants, who perceived their asthma as being poorly controlled (n=126, 26.3%), reported both poorer emotional status and HRQL. Participants with poor physical HRQL seemed to have both poorer emotional status and asthma control as well as poorer asthma control. Moreover, participants with low levels of Extraversion were more likely to perceive their physical HRQL as worse. Participants with poor mental HRQL reported poorer emotional status and adherence to asthma medication as well as poorer asthma control. Additionally, participants with high levels of Neuroticism and low levels of Extraversion, Agreeableness and/or Conscientiousness perceived their mental HRQL as worse. Asthma control was identified as predictor for physical HRQL. Emotional status, adherence to asthma medication, asthma control, Neuroticism and Agreeableness were identified as predictors for mental HRQL.

Conclusion: The current findings argue that in addition to medical examinations, we also need to consider asthmatics emotional status, adherence behaviour, asthma control but also personality characteristics in evaluations of their health outcome.

23 Asthma control, emergency visits, lung function and FENO in asthma and non-asthma in the West Sweden Asthma Study (WSAS)
Lötvall, Jan¹; Bjerg, Anders²; Ekerljung, Linda²; Lundback, Bo²
¹Krefting Research Centre, Sweden; ²Krefting Research Centre, University of Gothenburg, Sweden

WSAS is a clinical epidemiological study, based on a random population of 30000 individuals living in West Sweden. After having answered a questionnaire, a random population was invited to extensive clinical phenotyping. We here report lung function FENO data from 954 asthmatics and 1030 non-asthmatics. Asthma was defined as “doctor’s diagnosis of asthma” and “ever asthma” + “current asthma medication or symptoms common in asthma”. According to GINA criteria, 57.6% of asthmatics had controlled asthma, 29.3% partly controlled asthma and, 13.1% uncontrolled asthma. Weekly night-time asthma awakenings was reported by 12.6% of asthmatics. The prevalence of emergency visits over the last year due to asthma was 13.9% in non-smokers, 17.1% in ex-smokers, and 31.6 in current smokers (p=0.006 for trend). Mean %pred FEV1 was 105.3% in non-asthma and 96.9 in asthma. In the non-asthma group, 29.4% of individuals had FEV1 <100% predicted, whereas 52.6% of asthmatics had <100% predicted FEV1. In the non-asthma group, 4.6% of individuals had FEV1 <80% predicted, whereas 14.8% of asthmatics had <80% predicted FEV1. Mean FENO was 25.2 in asthma, and 19.9 in non-asthma (P<0.001). Low FENO (<20ppb) was observed in 62.8% of individuals in the non-asthma group, and 54% in the asthma group. High FENO (>50ppb) was observed in 3% of the non-asthma group and 9.6% of the asthma group. Asthma with significant severity, leading to emergency visits, is common in the WSAS, and previous or current smoking increase that risk. Furthermore, close to every other individual with asthma report uncontrolled or partly controlled disease. Despite significant disease severity in this epidemiological setting, very few asthmatics express low lung function or very high exhaled FENO. Neither FEV1, nor FENO may be appropriate markers of disease severity in an epidemiological setting. However, WSAS may be utilized to further explore determinants of asthma severity and their risk factors, which will be reported at the meeting. This work was financed by the VBG Foundation against Asthma/Allergy.

24 Deficiency of regulatory b cells in a house dust mite model of asthma
Braza, Faouzi¹; Chesne, Julie²; Mahay, Guillaume³; Cheminant, Marie-Aude³; Lair, David³; Magnan, Antoine³; Brouard, Sophie⁴
Background: Asthma is a chronic disorder leading to bronchial obstruction in response to inhaled allergen. It is associated with immune deregulation with specific expansion of Th2 and Th17 CD4+ T cells. Both T cell populations support B cell response by stimulating their proliferation, survival and IgE secretion. B cells are described for their effector functions but recent reports have described their regulatory role in autoimmune and inflammatory disorders. However, definitive identification has been challenging because regulatory B cells (Breg) are rare, do not have a specific marker, and express detectable IL-10 or TGF-B only upon ex vivo stimulation. In asthma models local inhalation tolerance and helminth infection induce the generation of regulatory B cells. But no physiological role of this population in the development of asthma has been described yet.

Methods: Mice were sensitized on days 0, 7, 14 and 21 by percutaneous administration of HDM onto the ears. Intra-nasal challenges were performed on day 27 and 34 with 250 μg HDM. One day after each challenge, we realized by flow cytometry a complete B cell phenotyping in spleen and lungs. Splenocytes and lung cells were isolated and stimulated ex vivo with LPS and PMA, ionomycin to induce IL-10 secretion by B cells.

Results: No differential frequency was observed for all B cell populations in the spleen of HDM allergic mice, suggesting a normal B cell development. In contrast, HDM allergic mice exhibit a strong infiltration of CD19+ B cells in lungs and broncho-alveolar lavage after the second challenge. We found an increase of CD19 IgDhi IgMlow mature and CD19 IgD- IgM- switched memory B cells in the lung of HDM allergic compared to control mice. We looked at CD19+ IL-10+ CD1dhi CD5+ CD21+ CD24hi IgMhi B cell population that has been shown to display regulatory properties in other situations. Whereas this population is present in spleen and lungs of HDM allergic mice, it produce less IL-10 than control after the first (vs control, p<0.001) and the second challenge (vs control, p<0.05) both in lung and spleen (vs control, p<0.05).

Conclusion: These results suggest a potential defect of B cell regulation in asthma. Future investigations will focus on their regulatory capacities in vitro and in vivo.

25 The content of natural killer T cells in the peripheral blood of patients with mild and severe atopic asthma

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Asthma is a chronic inflammatory disease of the airways, involving various immunopathogenetic mechanisms and multiple cell types, some of which are natural killer T (NKT) cells. Recent studies demonstrate an important role of NKT cells in asthma pathogenesis. NKT cells represent a unique subset of T lymphocytes with features of both T and natural killer (NK) cells. The aim of our study was to evaluate the content of NKT-cells in peripheral blood obtained from patients with mild and severe atopic asthma (ABA).

Methods: We examined 40 patients with ABA 19-45 years of age. The 3 groups were formed: the I group - 20 patients with the symptoms of mild BA, the II group - 20 patients with severe persistent ABA, the III group (control) - 15 healthy volunteers 20-45 years of age. Diagnosis and severity of BA were verified according to the Global Initiative for Asthma guidelines. All patients and controls underwent clinical examination and specific allergy tests. Lymphocyte populations were measured with flow cytometry. Two-tone staining of peripheral blood cells was performed using monoclonal antibodies containing antibodies to CD3 / CD16 +56 +.

Results: Breathlessness, wheezing and cough were the leading symptoms in all patients. All the patients were sensitized to household allergens. Reduced lung function were recorded in all asthmatics, with more significant changes in patients with severe ABA (p < 0,05).The total number of CD3 + T cells in all groups were not statistically different (p > 0,1). A significant increase of NK-cells were revealed in the I group vs the II (14 ± 5,76 and 6 ±1,95 respectively). Whereas content analysis of NKT-cells showed their significant increase in patients with severe asthma (4,059 ± 2,34) compared to other groups (1,48 ± 1,008 in group with mild ABA and 1,41 ± 0,625 in the controls). In groups with mild asthma and controls, the number of blood NKT cells showed no significant differences.

Conclusion: In our study NKT cells in the peripheral blood were found in all the groups. Their number was significantly higher in patients with severe ABA compared to other groups. These results may indicate that NKT-cells...
can play one of the key role in the pathogenesis of severe asthma. Identification and content analysis of blood NKT-cells may help in understanding the mechanisms that drive the development of asthma, particularly in the cases of severe, poorly controlled and steroid-resistant forms of the disease, define new therapeutic approaches in asthma management.

26 Features of severe asthma in young children from Romania
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¹Institute for Mother and Child Care, Carol Davila Medical University, MedLife Children’s Hospital, Romania; ²Institute for Mother and Child Care, Carol Davila Medical University, Respiratory Diseases, Romania

Background and aim: Asthma is one of the most important chronic disease in children, due to high prevalence and increased direct and indirect costs. A complex effort [GA2LEN, Me-DALL and ARIA groups] was organized to address the area of severe allergic diseases, including severe asthma. The concept of “problematic-severe-asthma” has risen and two major groups of asthmatic children were identified: difficult-to-treat asthma and therapy resistant asthma. There were published few papers on asthmatic children from Romania, only one paper addressing the area of severe asthma in school-age children. We describe features of severe asthma in young and preschool children from Romania.

Material and method: Cross-sectional study of patients in tertiary-referral asthma clinic: were included children with previously diagnosed asthma or recurrent-wheezing phenotype, up-to 10 years of age. All these children were referred by GP or pediatrician because of severe/uncontrolled disease. A complex evaluation was performed to exclude alternative diagnostics. At first visit, an extensive training for device use, inhalation technique and troubleshooting was implemented.

Results: 313 referrals (between Oct 2011-Mar 2012) were evaluated. 233 children were evaluated 1-5 times. 216 children were included. They were 56.7 months old (4-125mo), 153(70.83%) were boys, 182(84.26%) were inner-city children. For 202(93.53%) evaluation per-protocol was completed. 153(75.74%) had identifiable factors for not achieving control. 49(24.26%) had severe disease that generated multiple visits or exacerbations. In 6 children (12.24%) asthma was excluded. The remaining 43(87.76%) had uncontrolled asthma, but really difficult-to-treat or refractory asthma was documented only in 8(3.7% of included patients, 16.33% of children with more severe disease and no identifiable factors). They didn’t present male dominance (50% girls) were significantly older (83.6 months) had frequently severe rhino-conjunctivitis (75%) and atopic dermatitis (62.5%). In 2cases severe side effects of medication were documented (severe depression associated with LTRA). High frequency of exacerbations (87.5% with 1-4 episodes) was noted.

Conclusion: 1. Problematic-severe-asthma is more frequent than previously described in romanian children (3.7% vs 1.5%). 2. They are older, more often girls, have severe allergic associated-diseases and exacerbate more frequent than other asthmatic children in spite of aggressive treatment.

27 Tracheobronchomalacia in the patients with treatment-resistant severe asthma: case reports
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Pramongkutklao Hospital, Department of medicine, Thailand

Background: Tracheobronchomalacia is defined as the condition that the airway lumen is 50 percent narrower than normal. The acquired tracheobronchomalacia usually occurs in adults, however this condition is not usually found in asthmatic patients.

Purpose: We report two cases of the elderly patients who have severe persistent asthma that cannot be controlled with full asthma medication.

Case reports: Case 1 – 70 years old woman, with history of severe persistent asthma for 10 years was referred to our allergy clinic. She could not control her asthma and asthmatic attack always happened at night, so it was worse when she slept. She was treated with fluticasone/salmeterol accuhaler (250/50 mcg) 2 puffs twice daily, montelukast (10 mg), theophylline (200 mg), procaterol (50 mcg) and salbutamal evohaler 2-3 times daily. Pulmonary function test showed moderate restrictive lung disease, FEV1 59%. Additionally, chest CT scans detected
collapse of trachea at posterior wall. Afterwards we treated tracheomalacia by continuous positive airway pressure at night. As a result, her asthma symptoms have been improving. Case 2 – 72 years old woman, with history of severe persistent asthma for 15 years, was partly controlled (asthma controlled test score 20 and peak expiratory flow rate 180 L/min) with asthma medication such as fluticasone/salmeterol accuhaler (500/50 mcg) 2 puffs twice daily, montelukast (10 mg), theophylline (200 mg), tiotropium (18 mcg), salbutamal evohaler 4-5 times and omalizumab (300 mg) every two weeks. 3 months before investigating this case, her asthma could not be controlled (ACT score 7 and PEFR 100 L/min) despite she was treated by the oral corticosteroid (20 - 30 mg) every day. Her chest CT scans were normal, but the bronchoscope found bronchomalacia at her right and left bronchus of lower lungs.

**Conclusion:** We reported 2 cases of tracheobronchomalacia that found in the patients with severe persistent asthma. Before diagnosis of refractory asthma, it is important to consider and exclude other diseases such as tracheobronchomalacia particularly in aged.

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**28 When bronchial obstruction is not only asthma: a case of congenital cystic adenomatoid malformation type 0**

Indinnimeo, Luciana¹; Lollobrigida, Valeria²; Melengu, Taulant²; Carbone, Maria Palma²; Cesoni Marcelli, Azzurra²; Occasi, Francesca²; De Vittori, Valentina²; Duse, Marzia²

¹Università La Sapienza, Policlinico Umberto 1, Italy; ²Università La Sapienza, Policlinico Umberto 1, Servizio di Allergologia ed Immunologia Pediatrica, Italy

Congenital cystic adenomatoid malformation (CCAM) is one of the most common congenital lung anomalies. It is a rare pulmonary alteration, characterized by lung tissue dysplastic or hamartomatous, mixed with normal tissue. The injury is likely related to an insult during embryological development with altered terminal bronchiolar structures. We describe an unusual case of a boy came to our observation at the age of 12 years, for mild persistent bronchial asthma with exercise induced asthma, allergic to dust mites, pollens of Grasses, Cypress and to epithelium of the cat. At birth, respiratory distress treated with oxygen therapy, which resolved on the second day of life. For the first two years of life, he had recurrent episodes of wheezing requiring bronchodilator therapy and oral corticosteroids; subsequently, he presented rare episodes of bronchospasm, and asthma induced from intensive exercise. He practiced martial arts at a competitive level. The routine spirometry showed FEV1 58.5% and MEF50 24.7%, values that were not reversible after salbutamol, despite therapy with CSI + LABA. Chest x-ray showed thickening of the right parietal pleura and of the apical one and evidence of fibrotic striae. The chest HRCT revealed mosaic bilateral parenchymal destruction, bronchiectasis and multiple formations nodules like, poly-lobed with blurred margins, the larger of a diameter of 12 mm. The bronchioalveolar lavage was normal. Inflammatory, autoimmune, infectious and other congenital diseases were excluded. The histology of the lung biopsy obtained by thoracotomy surgery, suggested the diagnosis of CCAM type 0 without malignancy.

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**29 Comparison of the prevalence of asthma related symptoms in two ISAAC surveys in Birjand Iran**

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¹Asthma, Allergy & Immunology Research Center, Birjand University of Medical Sciences, Iran; ²Birjand University of Medical Sciences, Asthma, Allergy & Immunology Research Center, Iran

**Background:** The prevalence of asthma and other allergic diseases has been increasing significantly during the past decades. several studies have confirmed this finding not only in modern countries but also in developing and under developed countries. Many factors contribute to this increase but the main factors are different in different societies and areas. Data about the change in prevalence and risk factors in each society is primary step for prevention and management of allergic diseases. The aim of this study was to evaluate the changes in prevalence of asthma and asthma related symptoms in two different ISAAC surveys 16 years apart in Birjand city.

**Methods:** In a cross-sectional study, validated Persian version of ISAAC written questionnaire was used to evaluate prevalence of asthma related symptoms among 6-7 and 13-14 school children in 2011. The same questionnaire and...
protocol has been used in 1994 ISAAC survey. In total, 3320 and 2829 children in 13-14 years group and 2970 and 2623 children in 6-7 years age group were participated in 2011 and 1994 surveys respectively.

**Results:** Table one show the prevalence of asthma related symptoms in both age groups in 1994 and 2011. There was no significant difference in prevalence of asthma ever in two surveys but the prevalence of wheeze ever and current wheeze increased significantly from 1994 toward 2011 in both age groups. Table 1: prevalence of asthma and wheezing in 6-7 and 13-14 years children in 1994 and 2011

<table>
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<tr>
<td></td>
<td>6-7 years</td>
<td>13-14 years</td>
<td>6-7 years</td>
<td>13-14 years</td>
</tr>
<tr>
<td>Asthma ever</td>
<td>2.6%</td>
<td>3.8%</td>
<td>2.3%</td>
<td>3.7%</td>
</tr>
<tr>
<td>Wheeze ever</td>
<td>8%</td>
<td>12.2%</td>
<td>16%</td>
<td>21.6%</td>
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<tr>
<td>Current wheeze</td>
<td>7.8%</td>
<td>5.1%</td>
<td>9.7%</td>
<td>14.2%</td>
</tr>
<tr>
<td>Current exercise wheeze</td>
<td>3.9%</td>
<td>14.2%</td>
<td>3.2%</td>
<td>23.6%</td>
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**Conclusion:** The result of this study confirmed that the prevalence of asthma has not been changed during 16 years and is still very low but prevalence of wheeze increased significantly during this period. Further studies need to reveal the underlying factors for this increase.

**30 Good outcomes of pregnancy in two cases of women treated with omalizumab due to the severe asthma**

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Medical University of Lodz, Dept. of Internal Medicine, Asthma and Allergy, Poland

The prevalence of asthma during pregnancy is estimated on 4%. Uncontrolled, severe asthma is a risk for both a mother and an unborn child. Therefore, optimization of asthma treatment during pregnancy is vital in achieving good outcomes. Physicians and their patients may have some doubts about safety of anti-asthmatic medications, especially if drugs are new. Omalizumab is a humanized recombinant anti-IgE monoclonal antibody recommended for the treatment of chronic severe IgE-mediated asthma. Clinical and observational studies confirmed its effectiveness in improving asthma control, reducing severe exacerbations and improving quality of life. The safety data concerning pregnancy come from the experimental studies on animals and they show no teratogenic effect. We present case reports of two women who became pregnant during the treatment with omalizumab. Both with very severe asthma treated chronically with all available medication including systemic steroids (first – 20 mg prednisolone/day, second – 40 mg prednisolone/day). Both started their regular treatment with omalizumab in 2007 and have significant improvement (withdrawal of oral steroids or significant reduction of their dose, better asthma control). First, 32-year old woman became pregnant in 2010 and gave birth in Oct 2010 - it was her 3rd pregnancy, and 3rd labor, second 31-year old – also became pregnant in 2010 and gave birth in Jan 2011 - it was her 5th pregnancy and 2nd labor. Both had complications during previous pregnancies and labors and decided to continue therapy with omalizumab. First woman, besides omalizumab, was treated with high doses of ICS and LABA, second - high doses of ICS, LABA and 5 mg prednisone/day. The pregnancies proceeded without asthma exacerbations and other complications. First woman delivered healthy girl (Apgar 9, weight 3200g, length 56 cm) in 40 week of pregnancy by caesarean section due to the narrow pelvis, second - health boy (Apgar 9, weight 3800g, length 56 cm) in 40 week by caesarean section due to the aggravating obstetrical history. In both cases treatment with omalizumab did not affect pregnancies and newborns.

**31 Metabolomic applied to omalizumab effect in severe asthmatics – A preliminary result**

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**Background:** Omalizumab is a recombinant monoclonal anti-immunoglobulin (IgE) antibody (Ab), approved for treatment of severe asthma. The mechanisms by which is effective in asthma control are not yet fully understood but is known that it has an anti-inflammatory effect conducting to important clinical benefits. Urine sample collection
is the least invasive form for biofluid sampling. The analysis of metabolomic urine profile in asthmatic patients undergoing omalizumab may add important data to the current knowledge.

**Method - Case report:** We present the case of a 48 year old woman, with severe persistent allergic asthma, despite level 4 (GINA) medical treatment, who initiated omalizumab in order to control her nocturnal symptoms and her frequent unscheduled medical visits. Before treatment and at 12 weeks: clinical evaluation with ACT was registered; lung function, FeNO, IgE and eosinophils were measured. Two-dimensional gas chromatography (GC × GC-ToFMS) combined with headspace solid phase microextraction (HS-SPME) was applied to the untargeted study of the volatile metabolomic urine profile.

**Results:** Patient showed a good clinical response: ACT improved from 16 to 22, with nocturnal and effort symptoms control, without any unscheduled medical visit, showing a stable lung function, despite an imprudent auto stepped-down inhaler treatment. Regarding metabolomic urine profile, the present work was focused on aldehydes and alkanes (metabolites possibly linked to oxidative stress and/or inflammation processes). Previously to treatment, the urine profile was mainly characterized by alkanes; after treatment aldehydes had a major importance in the characterization of urine composition.

**Conclusions:** Being alkanes end-compounds in the sequence of oxidation reactions, it can indicate that oxidative state is at a higher extent before treatment, when compared to urine profile after treatment. In spite of being a case-study, the results suggest that the urinary volatile profiles obtained by HS-SPME/GC × GC-ToFMS may be useful for differentiating subjects with different physiological conditions, thus making it worth to further explore its diagnostic potential and follow-up therapy effects.

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**32 Switching from systemic steroids to high doses of ciclesonide restores the hypothalamic pituitary-adrenal axis**

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¹Medical University of Lodz, Poland, Poland; ²Medical University of Lodz, Poland, Department of Pneumonology and Allergy, Poland

**Question:** Treatment of difficult asthma with oral corticosteroids (OCS) may suppress hypothalamic-pituitary-adrenal axis. In this study we checked if high doses of ciclesonide instead of OCS may restore the adrenal function without loss of the disease control.

**Methods:** In five asthmatics with poor control of the disease despite treatment with systemic corticosteroids OCS were replaced with high doses of ciclesonide (1600-2400 µg/day). The pulmonary function tests (PFTs), asthma control test and the morning levels of cortisol and ACTH were measured at baseline and in 28 and 56 day of treatment.

**Results:** All patients improved in asthma control scores from mean value 9,4 to, 19,8 in 70 days. In four subjects FEV1 improved significantly with mean increase of up to 585 mL in 70 days ACTH levels were normalized in 3 patients after 28 days of observation and in all patients after 56 days. Cortisol level was normalized in 3 patients after 28 days and in next two subjects after 56 days.

**Conclusions:** In patients with difficult to treat asthma switching from the prednisone to high doses of ciclesonide may normalize hypothalamic pituitary adrenal axis function and improves the disease control and PFTs.

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**33 Itraconazole as “bridge therapy” to anti-IgE in patients with severe asthma with fungal sensitization**

Pizzimenti, Stefano; Heffler, Enrico; Badiu, Iuliana; Bussolino, Claudia; Raie, Alberto; Rolla, Giovanni

University of Torino - AO Mauriziano Umberto I, Allergy and Clinical Immunology, Italy

**Background:** Sensitization to fungi has been reported to play an important role in a particular phenotype of severe asthma, the so called severe asthma with fungal sensitization (SAFS), characterized by high levels of total IgE, which may be an obstacle to anti-IgE therapy. A few studies showed the benefit of antifungal therapy in improving the quality of life of patients with SAFS associated to a decrease of total IgE serum concentration. We describe here the role of antifungal therapy as “bridge therapy”, which provided us the opportunity to start anti IgE therapy in one polysensitized patient with severe asthma, who had very high levels of total IgE, beyond the upper limits recommended for proper prescription of omalizumab.
Method: A 59-year-old woman with uncontrolled severe asthma and frequent exacerbations, which required oral steroids courses, while on therapy with LABA / high dose inhaled fluticasone and montelukast, was evaluated for possible allergic broncho-pulmonary aspergillosis (ABPA).

Results: The patient was sensitized to Aspergillus Fumigatus, House Dust mites and Grass pollen. She did not have Aspergillus precipitins and lung HRCT did not show bronchiectases or lung infiltrates, while she had very high levels of total IgE (1793 kUA/L), with specific grass, D. Pter and A. fumigatus IgE (respectively 15.3, 8.4 and 11.3 kUA/L). The patient received the diagnosis of SAFS and started itraconazole therapy (200 mg b.i.d.) as add-on therapy for 12 weeks. After treatment, a significant decrease of total IgE (1043 kUA/L) was found, associated to a mild improvement in asthma control (ACT from 16 to 20). At that time, omalizumab was started at recommended doses (300 mg every 2 weeks). During the six months after anti-IgE therapy the patient did not report any asthma exacerbation and ACT score (23) showed the good control of asthma.

Conclusion: Antifungal therapy, as add-on treatment in patients with SAFS, may provide the opportunity to start anti-IgE therapy at usual recommended doses.

Friday, 12 October 2012
15:00 - 16:00

34 Omalizumab: clinical use for the treatment of an adolescent with difficult asthma
De Castro, Giovanna¹; Melengu, Taulant¹; Carbone, Maria Palma¹; Di Coste, Annalisa²; Giancane, Gabriella¹; Pansa, Paola¹; De Vittori, Valentina¹; Duse, Marzia¹
¹Università La Sapienza, Policlinico Umberto 1, Servizio di Allergologia ed Immunologia Pediatrica, Italy; ²Università La Sapienza, Policlinicoi Umberto 1, Servizio di Allergologia ed Immunologia Pediatrica, Italy

We describe the case of a 14 year old girl, followed by our Pediatric Allergology and Immunology Service for persistent rhinitis and asthma. The child suffered from asthma since the age of three years with a worsening of symptoms during the winter months. At 4 years of life, she was hospitalized for the first severe asthma episode. The SPT were positive for dust mites, Alternaria, pollens of Grasses, Cypress, Birch, Plane, epithelium of dog and cat, fish and soy. She started a fish and soy free diet and therapy with ICS (50 mcg daily for 2), antihistamine and antileukotriene. At six years of life she performed spirometry, that showed moderate airflow obstruction (FEV1 75.8%) and significant dilatation after salbutamol (+16%). For persistence of asthma despite the therapy, at the age of 8 years she added LABA to the ICS (25/125 mcg daily for 2), with significant improvement for the next two years; spirometry normalized (FEV1 88.2%). When ten, she started with almost daily wheezing that required the use of OCS; spirometry showed severe bronchial obstruction and restriction (FEV1 58.8%). Chest x-ray was performed, showing peribronchial infiltration and air trapping; ph-metry, sweat test and Mantoux were negative; HRCT showed areas of thickening with appearance "ground glass" air trapping and bronchiectasis predominantly in the upper lobes. Immunological and autoimmune evaluation were negative. Monitoring with the Peak Flow Meter showed the persistence of frequent and severe symptoms; FEV1 was 48.5% with expansion of 28.6% after salbutamol. The girl was considered a candidate for therapy with Omalizumab and this was started in Autumn 2011 (575 mg / 2 weeks) with significant improvement (FEV1 100.5%). To date she is still treated with Omalizumab, plus therapy with CSI + LABA, antileukotriene and antihistamine, with good control of asthma. On the basis of our experience, the use of omalizumab is an effective treatment for asthma resistant to common therapies, to reduce bronchial reactivity, symptoms and use of OCS.

35 Antibodies and sensitization of granulocytes to fungal allergens in patients with bronchial asthma
Titova, Nadezhda¹; Novikov, Pavel²
¹Belarusian Medical Academy of Postgraduate Education, Belarus; ²Vitebk state medical university, Allergy department, Belarus
Background: In the development and recurrence of asthma, fungal sensitization plays an important role. The purpose of this study was to examine the role of IgE-dependent and IgE-independent allergic reactions to fungal allergens.

Method: Clinically and laboratory examined 80 patients with bronchial asthma at the age of 18 to 60 years and 20 people without allergic diseases. Determined IgE, IgG, IgA antibodies to fungal allergens - Penicillium, Alternaria tenuis, Candida albicans, Aspergilla fumigates, Rhizopus nigricans. To identify sensitization of granulocytes to fungal allergens used reaction the release of myeloperoxidase under the influence of the allergen: after the incubation of granulocytes with fungal allergens granulocytes are activated and secrete the enzyme myeloperoxidase. Increase its activity in the supernatant was assessed by the intensity of the color of substrate - chromogenic mixture (tetramethylbenzidine and hydrogen peroxide).

Results: Prick-test was positive in 17.5% of patients, IgE-antibodies were present in the blood serum of 40.0%, IgG-antibodies - in 42.5%, IgA-antibodies - in 50.0%, sensitization of granulocytes was at 52.5 %. The correlation between skin test and sensitization of granulocytes was high for Candida albicans $r = 0.704$, moderate to Rhizopus nigricans $r = 0.627$, for Alternaria $r = 0.607$, for Penicillium $r = 0.576$ and for Aspergilla fumigates $r = 0.515$ ($p < 0.05$). Between sensitization of granulocytes and IgE-IgG-antibodies to all fungal allergens revealed a moderate correlation. In 46.7% patients response to fungal allergens has been with the participation of IgE-antibodies (IgE-dependent variant) and 53.3% showed IgE-independent (non-IgE-antibodies in serum) variant of allergic reactions to fungal allergens.

Conclusion: Patients with asthma are developing various types and combinations of allergic reactions with the participation of IgE-, IgA-, IgG-antibodies and sensitized granulocytes to fungal allergens. People with asthma need to use complex methods to eliminate sensitization to fungal allergens.

36 Aspergillus nidulans - a source of aeroallergens causing Ige mediated sensitization in asthma patients

Sircar, Gaurab¹; Pandey, Naren²; Gupta Bhattacharya, Swati³

¹Bose Institute, India; ²B M Birla Medical Research Centre, Allergy and Asthma, India; ³Bose Institute, Division of Plant Biology, India

Background: Airborne molds are significant constituents of atmospheric bioaerosol, are well-known source of allergens and can cause allergic rhinitis and bronchial asthma in sensitive subjects. Aspergillus nidulans is a widely distributed filamentous ascomyceteous mold. Although a significant exposure risk is assumable in indoor environment, the role of this fungus in provoking allergic symptoms in pre-sensitized individuals, however, was poorly investigated. We conducted this study to monitor airborne A. nidulans and to evaluate its potential as an aeroallergen causing nasobronchial allergy in sensitized individuals for the first time.

Methods: Seasonal periodicity of A. nidulans was studied for three years (May, 2010-April, 2012) by Andersen air sampler. The relationships between meteorological parameters and airborne A. nidulans concentration were explored by linear regression models. The allergic potential of A. nidulans extract was studied on 289 respiratory allergic patients by performing skin prick tests (SPT) and measuring the allergen-specific IgE levels in SPT positive patient’s sera by ELISA, SDS-PAGE and immunoblotting with ten individual asthma patient sera were performed to identify its IgE-binding components.

Results: Airborne A. nidulans concentration range was 6-57 CFU/m3 around the year and reached the peak concentration in December. Relative humidity and rainfall were found to be a significant predictor for occurrence of A. nidulans in air. Positive skin reaction was observed in 105 patients (27%) including 10 (9.5%) showing markedly high (2+ to 3+) skin sensitization. Crude antigenic extract of A. nidulans was resolved in 38 protein bands in the molecular weight range of 12 to 97 kDa on SDS-PAGE (12% gel). Three IgE-reactive bands (52, 46, 48, 55 kDa) were revealed by immunoblot analysis.

Conclusions: Exposures to A. nidulans in environments where it naturally occurs may confer risk of IgE-mediated sensitization in sensitive individuals.

37 Safety of a dust mite extract in severe allergic asthma

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¹Hospital del Tórax-Ofra, Spain; ²Hospital del Tórax-Ofra, Allergy, Spain
**Background:** The main goal of the current study was to evaluate the clinical tolerance of a modified dust mite subcutaneous extract in a small subset of severe extrinsic asthmatic subjects.

**Methods:** We selected 3 adult patients with a confirmed diagnosis of severe persistent allergic asthma (ATS criteria for severe asthma) for at least five years. Mean average topical steroid (inhaled) daily dose was above 1000 mcg of fluticasone propionate or 1600 mcg of budesonide. All patients included in the current trial were clinically relevant sensitized to dust mites (Dermatophagoides spp.) as shown by skin prick test and/or specific IgE. Inclusion criteria required no hospital or emergency admissions for the last 2 months with no changes in their daily medication in the last four weeks prior to the administration of immunotherapy. No pre-treatment with systemic steroids and/or antihistaminics were used. Modified standarized specific dust mites immunotherapy extracts were subcutaneously administered according to a validated protocol to achieve a final dose of 100 DDP/ml in a two-week cluster schedule. None of the subjects in both groups have been previously treated with omalizumab. Clinical observation and lung function was strictly monitored in all subjects until the maintainance dose of immunotherapy was reached.

**Results:** The three patients could reach the proposed allergen dose according to the immunotherapy schedule in two weeks. No significant adverse reactions were recorded, with no changes in the lung function. Minor immediate local reactions at the injections site were only observed at the maximal allergen dose in 2 patients showing a good response to oral antihistaminics in all cases. No late adverse reactions were present.

**Conclusion:** The standarized specific allergen immunotherapy dose was successfully reached in a small group of severe persistent allergic asthmatics. Further studies are needed to evaluate the role of specific immunotherapy in controlled severe allergic asthma.

**38 Immuno-proteomic identification of major aeroallergens from a common indoor mold Rhizopus oryzae went & prins.geerl**

Sircar, Gaurab¹; Pandey, Naren²; Gupta Bhattacharya, Swati³

¹Bose Institute, India; ²B M Birla Medical Research Centre, Allergy and Asthma, India; ³Bose Institute, Division of Plant Biology, India

**Background:** The objective of the present study is to identify IgE reactive molecules from Rhizopus oryzae (RO) a predominant airborne mold, using immuno-proteomics and to evaluate its potential as a cross reactive aeroallergen causing asthma and nasobronchial allergy.

**Methods:** Total 132 asthma patients were tested with antigenic extract by SPT. Specific IgE was examined by ELISA using sera of RO +ve patients. Total protein was resolved in 12% SDS-PAGE, two Dimensional SDS-PAGE & immunoblotted with ten individual and pooled patient sera. Periodic Acid Schiff's staining was done to detect the glycoproteins. IgE reactive spots were trypsin digested and identified by Tandem Mass Spectrometry (MALDI-TOF-TOF). Periodate modification of blot was performed to study the antigenicity of sugar moiety. Several bioinformatic tools and comprehensive allergen databases like Allermatch, T-cofee alignment, Full FASTA alignment with SDAP-were used for assessing the allergenicity of the major allergen. Subcellular localization and signal peptide was predicted by in-silico analysis. ELISA and immunoblot inhibition was performed to study its cross reactivity with Aspergillus fumigates.

**Results:** 64% patients were found RO positive and 21 of them with high titre of specific IgE. Immunoblots confronted with sera of atopic individuals revealed seven IgE reactive zones of which 44 KDa was observed in majority of sera and considered as major allergen. In 2D immunoblot, 14 IgE reactive zones were identified. Some of these spots were identified as proteins already reported as allergen from other molds and others as novel allergens. The major 44 kDa protein was identified as non-cytosolic Aspartyl endopeptidase containing twenty amino acids long signal peptide. This is a glycoprotein but with an epitope of peptidic nature. It showed significant sequence homology and hit identity with Bla g 2 (cockroach) and Asp f 10 (mold). It also showed in-vitro cross reactivity with Aspergillus fumigatus. A 41 kDa immunoreactive band of Aspergillus fumigatus was inhibited in immunoblot by RO protein.

**Conclusions:** Exposures to R. oryzae in environments where it naturally occurs may confer risk of IgE-mediated sensitization in atopic individuals and the major & minor allergens, if purified, can be used for successful asthma diagnosis and possible immunotherapy.
**Case report:** A 31-year old man, tenor, present with a history of chronic cough of two years duration. The cough was present day and night, much more during singing, with little mucus production and some associated feeling of shortness of breath and choking sensation, especially at night. Additional complaints included postnasal drip, runny nose, sensation of mucus in the throat and symptoms of heartburn and acid indigestion. The patient was referred in our clinic, after inadequate response to previous treatments prescribed by previous specialists, including antihistamines, nasal steroid sprays, oral prednisone, antibiotics, combine therapy and proton-pump inhibitors. Physical examination was unremarkable. Spirometric values were normal. Results of chest radiography, computed tomographic scans of the sinuses, and high-resolution computed tomographic scans of the chest were all normal. The patient underwent a methacholine inhalation challenge. FEV1 was reduced 7% at the highest concentration (25 mg/mL). Bronchoscopy was normal. The Esophageal pH monitoring was resulted positive. Skin prick test and RASTs resulted positive for cat dander. As the methacoline test was negative, patient was challenged in Italy, with a well-characterized cat-exposure model. Challenge result was considered positive for the upper respiratory symptoms, but there was no significant decline in FEV1 at 6 or 23 hours after cat exposure, the maximum fall was 9%. The patient was treated with cat-immunotherapy 15 μg of Fel d 1 at maintenance dose, and conservative therapy with combine therapy for 1 year, with no clinical improvement. He performed surgical intervention of the Nissen fundoplication, laparoscopically. There was significant improvement in quality of life only at 3 months after surgery. Because the patient's symptoms did not improve, we advised him to continue cat-immunotherapy, nasal steroid sprays, treatment with combine therapy and to stay away from the work and home environment for a period of time. Some months later the symptoms were significantly improved.

Cat and dog allergens are commonly present in homes without pets, as well as in a variety of public buildings, and these are carried from one home to another on the clothing of pet owners. Non-catowners can collect cat allergen on their clothing, most likely by both airborne and direct contact. Repeated low-level allergen exposure may induce chronic airway changes, specifically increases in airway hyperresponsiveness.

**Test with formoterol among school-age children with bronchial asthma and lung structural changes**

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Asthma is the most common chronic lower respiratory disease in childhood throughout the world. It is widely known that this disease exists with difficult pathological process of bronchi remodeling. Unfortunately, there is no data about bronchodilators therapy efficacy. We have performed examination of 38 school-age children with asthma moderate to severe. We have formed 2 groups. The first one included 25 patients during exacerbation period: 56% (14/25) boys, 44% (11/25) girls, average 11,1[6:16]. The second group consisted of 69,2% (9/13) boys, 44% (4/13) girls, average 11,9 [6:16], who have been examined during remission period. There were 12% (3/25) cases severe asthma and 88% (22/25) moderate asthma in the first group, and 23,1% (3/13) patients with severe asthma and 76,9% (10/13) with moderate asthma in the second one. Chest computer tomography have revealed lung structural changes (pneumofibrosis, bula, eosinofil infiltrates, emphysema, subpleural nodules) among 68% (17/25) patients of the first group and 69,2% (9/13) children of the second one. The results of computer spirometry in the first group 88% (22/25) children had obstruction limitation of airflow, 12% (2/25) mixed disorders. The positive bronchodilations test with formoterol in the first group in 15 minutes after inhalation 50% (4/8) patients without lung structural changes and 41,2% (7/17) patients with lung structural pathology, in 30 minutes ≤37,5% (3/8) and 23,5% (4/17), in 120 minutes ≤62,5% (5/8) and 58,8% (10/17) accordingly. The second group showed following data ≤37,5% (4/9) patients with lung structural pathology, in 30 minutes ≤50% (2/4) patients without lung structural changes and 44,4% (4/9) patients with lung structural pathology, in 30 minutes ≤50% (2/4) and 53,8% (6/9), in 120 minutes ≤50% (2/4) and 66,6% (6/9) accordingly.(p>0,05). Thereafter, there is evidence of
decreasing efficacy of broncholitic among children with lung structural changes, there is no significant data about it efficacy difference in exacerbation and remission.

### 41 Occupational Asthma due to Polyvinyl chloride and Methylmethacrylate, “hidden in an adhesive”

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Polyvinyl chloride (PVC) is a thermoplastic polymer widely used in industry, reflected in the number of tons consumed annually in the world. We present a 48 year old male patient with no history of atopy, plumber by profession for 30 years, developed progressive dyspnea and dry cough in the last 3 years, triggered in the work environment and persist outside it, related it to manipulate an adhesive called Tangit, whose components are PVC powder and methylmethacrylate. Never presented skin lesions, or used protection. Were performed blood tests, chest radiography, skin tests, spirometry, bronchodilator test and fraction of exhaled nitric oxide, methacholine challenge test was positive (PC 20, 8mg/dl). After signing informed consent, we performed Specific bronchial provocation (SBP) in a 7m3 dynamic chamber for 30 minutes of cumulative exposure, simulating working conditions with placebo, the Tangit adhesive provoked a late asthmatic response with a maximal fall in FEV1 of 33% at 7 hours, PVC powder and methylmethacrylate generated dual asthmatic responses, with a maximum drop of 17% at 30 minutes and 17.3% at 7 hours, 22% at 2 minutes and 20% at 9 hours, respectively. The patient during the study was on sick leave from to 24 months. We report a case of occupational asthma by PVC and Methylmethacrylate, shown by specific bronchial challenge in a plumber exposed to these agents. There are cases of occupational asthma published by the degradation products of PVC or manipulated workers manufacturing bottle caps1 and packaging. It is the first case of occupational asthma by PVC content in an adhesive. So far been known that adhesives containing acrylates, capable of inducing asthma.

### 42 Severe asthma database in Hungary, initial steps

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**Background:** Patients with severe asthma represent a significant unmet clinical need, however the evidence base for the management of these patients is small. Published literature on prevalence of severe asthma is quite heterogeneous and we do not have accurate data in Hungary. In 2010 we started to build up a severe asthma database involving patients who were treated in the pulmonary care network in Hungary and met the ATS criteria for severe asthma. The aims were to determine severe asthma prevalence and to define and characterise clinical phenotypes further.

**Methods:** The survey was carried out by using a special severe asthma questionnaire regarding information of sex, age, disease onset and duration, lung function, atopy, smoking habits, systemic steroid claim, exacerbations. To date 454 patients were recruited. 354 of them were registered by the pulmonary clinics over the country (group1) while the remainders (n=100) were registered by the asthma outpatient clinic of our institute (group2). The latter group was operated as a reference group to verify the reliability of questionnaire data received from other centres. Identification of severe asthma phenotypes was started in patients of group 2, and it is based on more detailed clinical features and determination of pattern of airway inflammation using induced sputum, exhaled breath condensate sample analysis and measurement of exhaled NO.

**Results:** There were no differences between the two groups in gender distribution, prevalence of atopy, systemic steroid dependency and the mean value of personal best FEV1. On the other hand the mean age of the group 1 was significantly higher, with no difference in asthma duration between the two groups.
Considering that the significant indicators of the two groups proved to be quite homogeneous, the combined clinical data of 454 patients were compared to the data published in the literature (ENFUMOSA, SARP, TENOR studies) and no relevant differences were found. Phenotypes of severe asthma with early onset atopic disease, severe asthma with salicylate intolerance and systemic steroid dependency and severe asthma induced by airway infection and characterized by mixed eosinophilic-neutrophilic airway inflammation and systemic steroid dependency were found. **Conclusion:** Data of patients registered in our severe asthma database are similar to the international data, but a phenotype of severe asthma induced by airway infection was detected.

### 43 Assessment of patient-reported outcomes in interventional randomized clinical trials in asthmatic children registered in a public-access trial registry

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**Background:** Asthma is the most common chronic illness among children that requires approved treatment based on specific trial-based data. Asthma and its management still pose a challenge to children and parents that may affect their functional health and well-being. Despite the importance of patient-reported outcomes (PROs), concerns about which outcomes should be measured in randomised clinical trials in children with asthma persist. The aim of this study is to assess whether the choice of using PROs can be ascertained by trial characteristics in clinical trials in children with asthma.

**Methods:** On October 12, 2010, ClinicalTrial.gov was searched for phase 2 through 4 randomized clinical trials (RCTs) enrolling asthmatic children younger than 17 years of age. Of 313 RCTs, 99 met eligibility criteria. The study characteristics were collected and outcomes were determined and classified as PROs, non-PROs, and objective outcomes.

**Results:** Of the 99 registered RCTs included in this analysis, 2 trials did not report any outcome and secondary outcomes were not reported in 8 more trials. 83 trials were phase 3 or 4 and 75 were sponsored by industry. Primary outcomes were PROs in only 7 of 99 trials (7%; 95% CI, 2% - 12%), non-PROs in 34 of 99 (34%; 95% CI, 25% - 43%), and objective outcomes in 56 of 99 (57%; 95% CI, 47% - 67%). PROs were reported as primary or secondary endpoints in 28 of 99 (28%; 95% CI, 19% - 37%) trials. Of the 28 RCTs reporting PROs as primary or secondary end point, only 11 trials employed validated PRO measures; 6 generic and 5 disease-specific measures. The results obtained from univariate and multivariate analyses did not show significant association between the reporting of PROs and independent predictors of trials.

**Conclusion:** Although the sample size was too small to answer the research question, the findings document that only 7% of ongoing registered trials in children with asthma will measure PROs as primary end point while PROs are more likely to measure as secondary end points especially in combination with other outcome domains.