74. Biomarkers of allergic inflammation

P467

Atopy is a risk factor for COPD symptoms: Results from the EUROSCOP study

Fatemeh Fattahi[1,2], Nick H.T. ten Hacken[1], Claes-Göran Löfdahl[3], Machteld N. Hylkema[2], Wim Timens[2], Dirkje S. Postma[1], Judith M. Vonk[4]. *[1]Department of Pulmonology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands; [2]Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands; [3]Department of Respiratory Medicine and Allergology, University Hospital, Lund, Sweden; [4]Department of Epidemiology and Statistics, University of Groningen, Groningen, Netherlands*

Background: Pathogenesis of COPD is complex and remains poorly understood. EUROSCOP (European Respiratory Society Study on Chronic Obstructive Pulmonary Disease) showed that 18% of their COPD participants were atopic (Watson, L. *et al.* ERJ 2006; 28:311-8).
We investigated whether atopy affects symptoms and lung function in these COPD patients.
Methods: We included 843 male and 320 female smokers with mild to moderate COPD from EUROSCOP. Risk factors associated with the presence of atopy (positive phadiatop) as well as the association between atopy and symptoms, and atopy and lung function (FEV_1 and FEV_1/FVC) were analysed using multiple regression models adjusted for confounders. Interactions between atopy and gender, age, and smoking were also investigated.
Results: Prevalence of atopy was higher in males than females (21% vs 10%, $p<0.001$). Male gender (OR: 2.20; 95% CI: 1.47-3.36), BMI (1.04; 1.00-1.08) and age (0.97; 0.95-0.99) were associated with atopy. Additionally atopy was associated with a higher prevalence of cough (1.71; 1.26-2.34) and phlegm production (1.50; 1.10-2.03) in the total population, and with waking up with chest tightness in females (female: 2.69; 1.11-6.55, male: 0.84; 0.47-1.49, female vs male: 3.21; 1.12-9.25). There were no significant interactions between atopy and age or smoking with respect to symptoms. Atopy was not associated with lung function.
Conclusions: The present study for the first time shows that atopic COPD patients more likely report respiratory symptoms than non-atopic COPD patients. However, atopy is not associated with lung function. Of interest, male gender, higher BMI and younger age are risk factors for atopy in COPD patients.
Study was supported by AstraZeneca.

P468

Granzyme B expression in lung of fatal asthmatics

Yvonne Nussbaumer-Ochsner[1], Luiz Silva[2], Annemarie van Schadewijk[1], Marisa Dolhnikoff[2], Thais Mauad[2], Klaus Rabe[1], Pieter Hiemstra[1]. *[1]Pulmonology, Leiden University Medical Center, Leiden, Netherlands; [2]Pathology, University of Sao Paulo School of Medicine, Sao Paulo, Brazil*

Introduction: Granzymes are serine proteases mainly produced by CD8 and NK cells, and are involved in the pathogenesis of many inflammatory disorders. Granzyme B (GzmB) is released towards target cells, but can also be released nonspecifically and is capable of cleaving extracellular matrix (ECM) components contributing to ECM degradation and remodeling in chronic inflammation. Recent studies have also shown participation of GzmB in allergic inflammation, but there are no descriptions of the its expression in fatal asthmatics.
Methods: We studied large and small airways and lung parenchyma of 12 patients that died of fatal asthma (FA) and 8 controls (CTR). Using image analysis we measured the number of GzmB positive cells in the inner layer (IL), smooth muscle (SM) and outer layer (OL) of both large (LA) and small airways (SA) and in peribronchiolar (PS) and distal (DS) alveolar septa. Values [median (IQR)] were expressed as GmzB+ cells/BM length (cells/mm) in OL and IL, GmzB+ cells/SM area (cells/mm^2) in SM, and as GmzB+ cells/septal length (cells/μm) in PS and DS.
Results: In LA we found a higher number of GmzB+ cells in IL [FA=12.9 (5.1), CTR=5.8 (3.1), p=0.02)] and OL ([FA=27.2 (9.3), CTR=7.5 (2.4), p=0.001)] in FA, but no differences were observed in SM. In SA, the number of GmzB+ cells was also higher in IL [FA=8.3 (4.4), CTR=2.8 (1.8), (p=0.03)] and OL [FA=15.9 (5.3), CTR=5.2 (4.1), (p=0.002)] in FA, but no differences were observed in SM. In the parenchyma, there was a higher number of GmzB+ cells in PS of FA comparing to CTR (p=0.02), but no differences were observed in DS.
Conclusion: The results show that GzmB expression is increased in FA and may contribute to the previously described process of airway remodeling in these patients.

P469

Differences in responsiveness of blood neutrophils for fMLF indentify two distinct groups of COPD patients

Vera Kamp[1], Adèle Lo Tam Loi[1], Nick ten Hacken[2], Susan Hoornhorst[2], Jan Willem Lammers[1], Leo Koenderman[1]. *[1]Respiratory Medicine, University Medical Center Utrecht, Utrecht, Netherlands; [2]Respiratory Medicine, University Medical Center Groningen, Groningen, Netherlands*

In COPD severity of disease is generally diagnosed by the GOLD guidelines. The GOLD stages, however, poorly correlate with disease progression. The extend of systemic inflammation in COPD patients plays a role in the progression of the disease Therefore, the severity of the systemic inflammatory response of COPD patients was characterized by phenotyping of blood neutrophils.
51 COPD patients (GOLD I – IV), and 14 healthy smokers were included in the study. The expression of activation epitopes on neutrophils was analyzed by flow cytometry. Responsiveness for fMLF was measured by analysis of cells in the presence or absence fMLF.
Responsiveness of neutrophils for fMLF, expressed as the ratio of CD11b in the presence and absence of fMLF, identified two distinct COPD patient populations. Phenotype 1 (55%) was comparable to the healthy smokers and phenotype II (45%), showed a significantly higher mean ratio of 17.24. $\pm$ 0.78 vs. 5.6$\pm$0.53, respectively: These two groups did not differ in FEV1, but a higher expression of CD181 was found in group II compared to group I: 207.5.$\pm$ 10.24 vs. 154.5$\pm$8.9, respectively ($p<0.001$). Similar data were found for CD66b, CD33, CD64 and active FcγRII on neutrophils.
Two distinct groups of COPD patients were identified by determining the responsiveness of their neutrophils for fMLF. This was associated with differences in expression of several neutrophil activation markers. These findings demonstrate a higher systemic inflammation in patients with phenotype II. It is tempting to speculate that these patients will have a faster disease progression.

P470

A decreased integrin Mac-1 (CD11b/CD18) induced respiratory burst in neutrophils of COPD patients

Adèle Lo Tam Loi, Corneli van Aalst, Deon Kanters, Laurien Ulfman, Jan-Willem Lammers, Leo Koenderman. *Respiratory Medicine, University Medical Center Utrecht, Utrecht, Netherlands*

Introduction: Neutrophils of COPD patients are characterized by changes in activation of the respiratory burst. The aim of this study was to investigate the control of the respiratory burst in neutrophils of COPD patients upon engagement of the integrin Mac-1 (α_M/β_2; CD11b/CD18).
Methods: A cohort of 34 stable COPD patients (GOLD I-IV) was included in this study. Peripheral blood neutrophils were stimulated with formyl peptides (like fMLF) or anti-Mac-1 antibody (VIM12) and reactive oxygen species (ROS) production was measured by flow cytometry using Dihydrorhodamine 123 (DHR).
Results: The fMLF and anti-Mac-1 antibody (VIM12) induced ROS production in neutrophils from normal controls. Both fMLF and VIM12 were sensitive for priming by platelet-activating factor. The ROS production in neutrophils of COPD patients exhibited a primed phenotype in the context of fMLF. To our surprise

the ROS production upon engagement of Mac-1 was markedly reduced in COPD patients compared to normal controls (332.8±64.7 vs. 876.8±275.7 respectively; $p < 0.05$). This was not associated with a lower expression of Mac-1 on the cell surface measured by flow cytometry.
Conclusion: Our data demonstrated the complex priming phenotype of neutrophils in COPD patients. The unresponsiveness of Mac-1 induced respiratory burst of neutrophils might indicate a shift towards a phenotype more sensitive for bacterial derived molecular patterns.

P471
IL-18 in infectious exacerbations of COPD
Nikoletta Rovina[1], Efrossini Dima[1], Georgia Papadaki[1], Theocharis Anagnostakos[1], Charis Roussos[2], Manos Alchanatis[1], Nikolaos Koulouris[1]. *[1]1st Department of Pulmonary Medicine, "Sotiria" Hospital, Athens Medical School, Athens, Greece; [2]Pulmonary and Critical Care Department, Evangelismos Hospital, University of Athens, Athens, Greece*

The nnate immune system is critical in recognizing bacterial and viral infections to evoke a proper immune response. IL-18, a pro-inflammatory and pro-apoptotic cytokine with crucial role in host defense against bacterial invasion is increased in lungs, serum and sputum of COPD patients, suggesting that IL-18 may be involved in chronic inflammation of COPD. We aimed to investigate the effect of infectious acute exacerbations of COPD (AECOPD) on IL-18 levels.
We examined 40 patients with COPD hospitalized for infectious AECOPD according to Anthonisen's criteria and 20 patients with stable COPD. We examined sputum for inflammation and for bacterial infection using PCR. IL-18 was measured in induced sputum and serum at baseline and after treatment of the exacerbation. Immunocytochemistry of IL-18 expression in sputum cells was performed using a mouse monoclonal IL-18 antibody.
All patients had no previous hospitalization the preceding 3 months and none was diagnosed with pneumonia.
IL-18 levels in sputum were found significantly lower in AECOPD compared to stable state ($p=0.05$), while right after treatment IL-18 levels were increased compared to the stable state levels ($p>0.05$). Positive staining of IL-18 was observed in macrophages in immunocytochemistry. An inverse correlation was found between IL-18 levels and sputum macrophages in AECOPD ($r=-630$, $p=0.028$). Serum IL-18 levels were elevated in exacerbations ($p>0.05$) compared to stable state, and decreased after treatment to stable disease levels ($p>0.05$).
Our data show that although IL-18 is involved in host defence against bacterial pathogens in infectious AECOPD there may be a dysregulated activation of airways' macrophages and perhaps inflammasome mediated pathways.

P472
Serum and nasal lavage fluid Clara cell protein in children with allergic rhinitis
Tharwat E. Draz[1], Terez B. Kamel[1], Mahira I. El-Mogy[2], Essam H. Moustafa[1]. *[1]Pediatrics, Faculty of Medicine, [2]Clinical Pathology, Faculty of Medicine, Ain Shams University, Cairo, Abbassia, Egypt*

Background: Allergic rhinitis is among the most common chronic disorders of childhood with prevalence of up to 40% in children. Clara cell secretory protein (CCSP) is secreted by Clara cells in the lining fluid of airways. It has an immune-modulatory and anti-inflammatory activity.
Aim of work: Study aimed at evaluating Clara cell secretory protein as a pneumoprotein biomarker in serum and nasal lavage fluid of children with allergic rhinitis.
Methods: A cross sectional case-control study was conducted on sera and nasal lavage fluid samples from 15 children with allergic rhinitis, recruited from Children's hospital, Ain Shams University, and 15 healthy children as a control group.
Results: Children with allergic rhinitis had a male to female ratio 2 to 1, with a mean age of 9.47±2.75 years, while among the healthy group, six were males and nine were females, with a mean age of 8.63±2.28 years. Rhinorrhea and nasal obstruction were the most frequent symptoms (100%) followed by itching (93.3%) then sneezing (73.3%). Serum CCSP mean±SD was 2.03±0.59μgł; it was reliable to predict allergic rhinitis ($P<0.0001$); while nasal lavage CCSP mean±SD was 12.73±8.25μgłand it was not reliable to predict allergic rhinitis. The best cut-off value was 3.75 μgłwith a sensitivity of 100%, specificity 80%, with a diagnostic accuracy of 90%.
Conclusion: In conclusion, Clara cell secretory protein is a new peripheral sensitive marker of airway injury allowing researchers to evaluate the integrity of the air blood barrier. Furthermore, serum CCSP level is a non-invasive predictor of allergic rhinitis but not nasal lavage fluid CCSP.

P473
Elevated CEA levels in sera in patients with allergic bronchopulmonary aspergillosis (ABPA)
Hirotaka Matsuzaki[1], Wakae Tanaka[1], Yasuhiro Kojima[1], Masaki Kawakami[1], Masaru Suzuki[1], Yoshio Sakamoto[1], Tadashi Horiuchi[2]. *[1]Respiratory Medicine, Kanto Central Hospital, Tokyo, Japan; [2]Internal Medicine, Okutama Hospital, Tokyo, Japan*

Background: ABPA is an allergic disease associated with peripheral blood eosinophilia and elevated total IgE in serum. It sometimes accompanies with bronchiectasis filled with mucus plug detected by chest X-ray- or CT films or fiberotic bronchoscopy. Mucus plug is not infrequently misdiagnosed as lung tumor. Carcinoembryonic antigen (CEA) is one of tumor markers and measured as a diagnostic as well as surrogate marker of bronchogenic carcinoma. It has been reported that some patients with ABPA demonstrated elevated CEA levels in their sera.
Aim: To evaluate clinical significance of serum CEA level in patients with ABPA.
Methods: Ten patients (6 women), aged from 39 to 78 (median; 67) with ABPA were evaluated. All patients were never smokers or ex-smokers. Serum CEA level, eosinophil number, total IgE, *Aspergillus fumigatus* (*Af*)-specific IgE- and precipitating antibodies were measured. Chest roentgenogram and or CT were examined when blood test was performed. For comparison, serum CEA levels were also evaluated in patients with bronchiectasis (n = 20).
Results: Among 10 patients with ABPA, five demonstrated elevated serum CEA levels (6.2 – 140.0 ng/ml; normal range ≤ 5 ng/ml). Massive mucus plugs were demonstrated in four out of five patients on chest CT or X-ray films. Two out of five patients were treated with systemic glycocorticosteroids resulting in the decrease in CEA levels. Another patient showed the decrease in CEA spontaneously in parallel with the decrease in eosinophil number.
Conclusion: Massive mucus plug may induce CEA synthesis from airway epithelial cells lining on the ectasic bronchi.

P474
Clinical implication of interleukin-33 in acute eosinophilic pneumonia compared with chronic eosinophilic pneumonia
Naoko Mato, Hideaki Yamasawa, Masayuki Nakayama, Masashi Bando, Yukihiko Sugiyama. *Respiratory Medicine, Jichi Medical University, Shimotsuke, Tochigi, Japan*

Background: Both acute eosinophilic pneumonia (AEP) and chronic eosinophilic pneumonia (CEP) show prominent eosinophilia in lung tissue; however, they differ in clinical course and pathological findings. Thus far, there have been some reports discussing the clinicopathological differences of AEP and CEP, however, biological approaches defining their differences were few.
Objective: Interleukin-33 (IL-33) is a new cytokine known as inducer of Th2 cytokine and it potently enhances increase of eosinophils. We focused on IL-33 and compared its levels as well as Th2 cytokines in AEP and CEP.
Methods: IL-33, Th2 cytokines (IL-4, IL-5) and IgE were measured in serum from healthy controls (n=8), and patients with AEP (n=3) and CEP (n=12) at admission. IL-33, IL-4, and IL-5 in bronchoalveolar lavage fluid (BALF) were also measured from patients with AEP (n=3), and CEP (n=8).
Results: Serum IL-33 was dramatically elevated in patients with AEP (1684.1±1025.8 pg/ml), but not in controls (64.7±112.8 pg/ml) and CEP (143.9±200.4 pg/ml). In BALF analysis, IL-33 was under detectable levels in controls and CEP; by contrast, it was prominently elevated in AEP (394.7±318.8 pg/ml). Further, IL-4 and IL-5 were also significantly elevated in AEP, and levels of serum IL-33 was positively correlated with serum IL-4 and IL-5, and BALF IL-33 was also positively correlated with BALF IL-5.
Conclusion: Our results suggested that IL-33 is a key molecule triggering the eosinophilia in AEP. This is the first report to establish the impact of IL-33 in eosinophilic pneumonia and to demonstrate the biological differences between AEP and CEP through IL-33 level.

P475
Possible role of IL-33 in the pathophysiology of asthma: A cross-sectional study
Hideki Inoue, Isao Ito, Akio Niimi, Hisako Matsumoto, Tsuyoshi Oguma, Hitoshi Nakaji, Tomoko Tajiri, Toshiyuki Iwata, Tadao Nagasaki, Michiaki Mishima. *Respiratory Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan*

Background: IL-33 and its receptor ST2 have been recognized as key molecules in Th2 inflammation. Clinical roles of serum levels of IL-33/ST2 have not been well evaluated in asthmatic patients.
Aims: To investigate relationships between serum IL-33/ST2 levels and clinical measurements in patients with stable asthma.
Methods: Seventy-two patients with stable, mild-to-severe asthma aged 55.0±18.4 (mean ± SD) years were analyzed cross-sectionally. Serum levels of IL-33 and ST2 were measured by ELISA. Relationships between serum levels of IL-33/ST2 and clinical measurements were evaluated. Clinical measurements include spirometric values, exhaled nitric oxide (FeNO) levels, induced sputum cell differentials, blood eosinophil count and serum total and specific IgE results.
Results: Forty-nine patients were positive for at least one IgE CAP RAST. Serum levels of IL-33 were positively correlated with disease duration ($r = 0.34$, $p < 0.01$), and negatively correlated with FEV1/FVC ($r = -0.25$, $p = 0.04$). IL-33 levels were also positively correlated with FeNO levels ($r = 0.26$, $p = 0.04$), blood eosinophil counts ($r = 0.35$, $p < 0.01$) and serum IgE levels ($r = 0.29$, $p = 0.01$). There was no difference in median values of serum IL-33 between CAP RAST-positive and CAP RAST-negative subgroups. Serum levels of ST2 had no correlation with any of the clinical indices.
Conclusions: Serum levels of IL-33 may reflect atopic status as represented by serum total IgE levels as well as degree of airway inflammation as assessed by peripheral blood eosinophilia and FeNO levels.

P476

Is analysis of exhaled breath condensate equivalent to that of bronchoalveolar lavage fluid?

Kunihiko Nakamura[1], Megumi Mikuniya[1], Shingo Takanashi[2], Akihito Hayashi[1], Takeshi Morimoto[1], Kageaki Taima[1], Ken Okumura[1]. *[1]Department of Cardiology, Respiratory Medicine and Nephrology, Hirosaki University Graduate School of Medicine, Hirosaki, Japan; [2]Health Administration Center, Hirosaki University, Hirosaki, Japan*

Background: Although the analysis of bronchoalveolar lavage fluid (BALF) is the most useful examination for the assessment of airway inflammatory markers, it is an invasive technique with limitations and risks for side effects.
Aims: The aim of this study was to examine the molecules included in exhaled breath condensate (EBC) in comparison with BALF, and to clarify the clinical usability of EBC.
Methods: EBC was collected from sixteen subjects suspected to have sarcoidosis just before BAL. The 40 different inflammatory molecules in EBC and BALF were analyzed with a protein array method.
Results: BALF levels of 6 inflammatory molecules including soluble tumor necrosis factor receptor type II (sTNF-RII) and regulated upon activation, normal T cell expressed and secreted (RANTES) were significantly correlated with the percentage of lymphocyte in BALF (%Lym). EBC levels of 13 inflammatory molecules including sTNF-RII and RANTES were significantly correlated with%Lym. We found significant correlations between the levels of EBC and BALF in 16 out of 40 inflammatory molecules. Levels of macrophage colony-stimulating factor (M-CSF), RANTES, TNF-α and sTNF-RII in EBC were significantly correlated with those in BALF. Their levels in EBC and BALF also were correlated with%Lym.
Conclusion: Highly sensitive approach to protein array in EBC allowed us to detect inflammatory molecules. Comprehensive analysis of EBC might be equivalent to that of BALF.

P477

Expresion of galectins in asthma patients

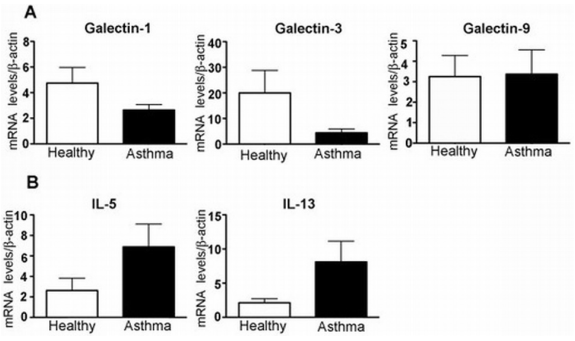
Silvia Sánchez-Cuéllar[1], Hortensia de La Fuente[2], Amalia Lamana[2], Danay Cibrián[2], Arantxa Cruz-Adalia[2], Rosa Mº Girón[1], Francisco Sánchez-Madrid[2], Julio Ancochea[1]. *[1]Pneumology Department, Hospital Universitario de la Princesa, Instituto de Investigación Sanitaria Princesa. Universidad Autónoma de Madrid, Madrid, Spain; [2]Immunology Department, Hospital Universitario de la Princesa, Instituto de Investigación Sanitaria Princesa. Universidad Autónoma de Madrid, Madrid, Spain*

Objetive: The galectins has been described in a variety of inflammation and allergic pathologies.This study have investigated the presence of galectin-1,3 and 9 in induced sputum samples.
Methods: Levels of Th2 cytokines and galectins in 24 patients with stable asthma and 18 healthy controls were determined in airway cells isolated from induced sputum samples using RT-PCR.

Clinical characteristics

Subjects (N: w/m)	Asmtha (24: 19/5)	Controls (18: 8/9)
Age (range)	50.34 (24–75)	33.88 (26–53)
ICS BDP dose <500/500–1000/>1000 mg/day (n)	8/9/7	0/0/0
Lung Function:		
FEV1% pred (ml)	94.5% (2623)	108.5% (3937)
FVC% pred (ml)	100.4% (3220)	108.2% (4789)
FEV1/FVC ratio	76	83.2
Diferencial cell count in induced sputum:		
Total cells (105)	4.7 (1–20)	4.6 (1–20)
Macrophages (%)	31.82 (27.90–35.75)	58 (52.06–63.3)
Neutrophils (%)	62 (54.23–69.13)	37.9 (34.66–41.14)
Eosinophils (%)	1.75 (0.5–2.7)	0.26 (0–0.52)
Lymphocytes (%)	0.9 (0.5–1.72)	3.85 (2–5.7)
ACQ	22 (16–25)	25
Current smokers (%) – (Y/N)	8.3% – (2/22)	44% – (8/10)

Results: Gal-1 and gal-3 mRNA levels in asthma patients (mean±SEM= 2.6±0.4, and 4.4±1.4, respectively) were lower than in healthy subjects (4.7±1.2, and 20.0±8.7). Gal-9 mRNA expression did not vary significantly between the two groups (3.2±1.3 *vs* 3.3±1.1). Asthma patients contained elevated mRNA levels of IL-5 and IL-13 ($p<0.05$).
Conclusions: The low levels of the negative regulator of galectins in human asthma may contribute to the inflammatory response present in this disease.



P478

Analysis of IgE and C-reactive protein as possible predictors of exacerbation patients with asthma

Besim Prnjavorac[1], Adlija Cauševic[2], Sabina Semiz[2], Tanja Dujic[2], Maja Malenica[2], Ekrema Mujaric[3], Tamer Bego[2]. *[1]Department of Lung Disease, General Hospital Tešanj, Tešanj, Bosnia and Herzegowina; [2]Clinical Biochemistry, Faculty of Pharmacy, Sarajevo, Bosnia and Herzegowina; [3]Internal Medicine, Cantonal Hospital, Zenica, Bosnia and Herzegowina*

Aim: To study correlation of IgE level and C-reactive protein (CRP) for exacerbation of the disease in asthmatic patients.
Methods: Asthmatic subject were examined for achieving of asthma control according to GINA recommendation. Numbers of exacerbation of asthma during one month were analyzed. The patients were followed in six month period (since first January to 30th of Jun. Average monthly days of exacerbations was calculated. IgE level in the blood was measured using Enzyme-linked Immunoassay (ELISA), and CRP was measured by immunotubidimetry. Assessment of asthma control was considered using Asthma Quality of Life Questionnaire (AQLQ).
Results: The study includes 63 patients with asthma. Average level of IgE was 674 IU/mL (SD 167), range 56-3785 IU/mL, 1 IU=3,2 ng; average level of CRP was 16,4 mg/mL (SD 6,3), range 5-48; Average number of days in exacerbation during one month was 3,6 (SD 2,4), and varied from zero, patients with no exacerbation, to 21. Using test of multiple correlation it was shown statistical significant correlation (level $p<0,05$) between IgE and CRP from one side, and number of days with exacerbation from the other. Patients with higher level of CRP were most likely to have exacerbation, than those with higher level of IgE. AQLQ was worse in those with higher level of CRP, than in those with higher level of IgE.
Conclusion: In this study CRP was shown as stronger predictor of asthma exacerbation and worse quality of life than total IgE level in asthmatic subjects.

P479

Structure-function relationship between extracellular matrix in airway smooth muscle and dynamics of lung function in asthma

C.Y. Yick[1], D.S. Ferreira[2], R. Annoni[2], P.W. Kunst[1], E.H. Bel[1], R. Lutter[1], T. Mauad[2], P.J. Sterk[1]. *[1]Respiratory Medicine, Academic Medical Centre, Amsterdam, Netherlands; [2]Pathology, São Paulo University Medical School, São Paulo, Brazil*

Rationale: Asthma is characterized by an increased deposition of extracellular matrix (ECM) within the airway smooth muscle (ASM) [Panettieri 2008, Araujo 2008]. We hypothesized that ECM composition within ASM is associated with the dynamics of lung function.
Aim: To investigate the fractional areas of collagen I and III within ASM, and their association with spirometry and respiratory resistance R_{rs} and reactance X_{rs} in asthmatics and controls.
Methods: Atopic mild asthmatics (n=10, no ICS) and controls (n=17) were included in this cross-sectional study. Spirometry, PC_{20}, R_{rs} and X_{rs} (forced oscillation technique during tidal breathing and deep inspiration) were measured. Paraffin sections from endobronchial biopsies were stained for collagen I and III by immunohistochemistry, and fractional areas were obtained by image analysis.
Results: There was no difference in fractional areas collagen I and III within ASM between asthmatics and controls ($p>0.05$). However, ASM collagen III was correlated with the change in R_{rs} after deep inspiration in asthmatics (r=0.74,

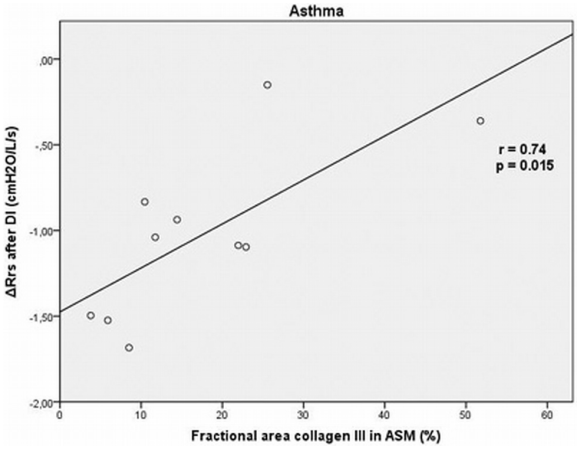


Figure 1

p=0.015, Fig.1). ASM collagen I and III were not associated with spirometry or PC_{20}.
Conclusion: These data show that collagen III composition within ASM is correlated with the dynamics of R_{rs} after deep inspiration in asthmatics *in vivo*. This suggests that ECM within ASM is positively associated with impaired airway mechanics in asthma.

P480
Peanut sensitisation is independently associated with increased airways inflammation in allergic asthma
Andrei Malinovschi[1], Christer Janson[2], Pia Kalm-Stephens[3], Katarina Nisser[2], Lennart Nordvall[3], Kjell Alving[3]. *[1]Dept. of Medical Sciences: Clinical Physiology, Uppsala University, Uppsala, Sweden; [2]Dept. of Medical Sciences: Respiratory Medicine and Allergology, Uppsala University, Uppsala, Sweden; [3]Dept. of Women's and Children's Health, Uppsala University, Uppsala, Sweden*

Background: The fraction of nitric oxide in exhaled air (FeNO) is increased in allergic asthma and the degree of IgE sensitisation to aeroallergens relates to FeNO levels. An additional IgE sensitisation to food allergens is known to increase asthma risk, but the relation between IgE sensitisation to food allergens and FeNO has been little studied previously.
Aim: To investigate in an ongoing asthma study if the presence of food allergy influences the levels of exhaled NO and which food allergens appear to be important.
Methods: Within the frame of an industry-academy collaboration on minimally-invasive diagnostics (MIDAS), measurements of FeNO and specific IgE (sIgE) against the allergens included in ImmunoCAP Phadiatop and fx5 (Phadia AB, Sweden) were done in 170 patients with physician-diagnosed asthma.
Results: Asthmatic subjects with IgE sensitisation to both aero- and food allergens (n=77) had higher FeNO levels than subjects sensitised to only aeroallergens (n=93) (21.2 ppb (18.4, 24.4) vs 15.6 ppb (13.2, 18.6), p=0.006). This difference was confirmed (p=0.03) in a multiple linear regression model after adjustments for sex, height, lung function and age. The sIgE levels to aeroallergens were higher in subjects also sensitised to food allergens ($p<0.001$). Peanut sensitisation was most common (63/77 subjects) and peanut IgE levels were independently associated with increased FeNO, even after adjustment for the sum of sIgE to aeroallergens.
Conclusion: Peanut sensitisation was independently associated with increased airways inflammation in allergic asthma. A possible mechanism might be a further enhanced Th_2 cytokine-driven inflammation related to peanut IgE sensitisation.

P481
ADRB2, ADAM33 and AAT gene polymorphisms and IgE-mediated asthma in Russian patients
Elena Dmitrieva-Zdorova[1], Marina Gabaeva[1], Elena Latysheva[2], Olga Voronko[1]. *[1]Laboratory of Chemical Genomics, Institute of Biomedical Chemistry, Moscow, Russian Federation; [2]Dep. of Allergology and Immunotherapy, Institute of Immunology, Moscow, Russian Federation*

Background: Asthma is complex disease which pathogenesis is a mix of environmental and genetic factors. A predisposition of airways to bronchoconstriction is one of main risk factors of asthma. Therefore genes which are directly or indirectly involved in bronchoconstriction are of great interest in asthma genetics.
The aim of our study was to evaluate an effect of polymorphisms *Arg16Gly* and *Glu27Gln* in beta-2-adrenergic receptor (*ADRB2*) gene, *Glu264Val* and *Glu342Lys* in alpha1-antitrypsin (*AAT*) gene and *ST+4* in a disintegrin and metalloprotease domain 33 (*ADAM33*) gene in predisposition to IgE-mediated asthma in Russian patients from Moscow.
Methods: 230 patients with IgE-mediated asthma and 214 healthy individuals were examined. The genotyping was performed by MALDI-TOF mass spectrometry.
Results: Allele *G* of *Arg16Gly* and allele *C* of *Glu27Gln* (both markers in *ADRB2* gene) showed strong association with atopic bronchial asthma (*OR* = 2.51 and 1.46, respectively, $p<0.05$). Allele *A* of *Arg16Gly* and allele *G* of *Glu27Gln* of that gene was found to have protective effect (*OR* = 0.76 and 0.69, respectively, $p<0.05$). Additionally one of haplotypes of *ADRB2* gene (A-G) also was associated with protection from asthma development (*OR* = 0.43, p=0.008). No association was detected between other studied SNPs and development of asthma.
Conclusion: Our results suggest that *Arg16Gly* and *Glu27Gln* polymorphisms in *ADRB2* gene strongly contribute to predisposition to IgE-mediated asthma. Probably *ADAM33* and *AAT* genes are not directly involved in predisposition to asthma and those genes possibly realize their effects when asthma is already developed.

P482
Gene polymorphism of epidermal growth factor receptor (EGFR) and airway hyperresponsiveness (AHR) in young allergic subjects without respiratory symptoms
Takahiro Yoshikawa[1], Hiroshi Kanazawa[2]. *[1]Sports Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan; [2]Respiratory Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan*

Background: Airway hyperresponsiveness (AHR) in young asymptomatic adults with atopy can be one of indicators of future symptomatic asthma. We aimed to examine genetic association of epidermal growth factor receptor (EGFR) with the asymptomatic AHR.
Methods: We recruited one-hundred and eighty-nine allergic volunteers (mean age 19.5±1.4 y.o.) without history of respiratory symptoms, measured bronchial responsiveness to methacholine (0.3 to 10.0 mg/ml) and classified them into subjects with AHR and those without AHR according to a cutoff level of PC_{20} (8.0mg/ml). Then, we genotyped 12 SNPs on EGFR gene, and estimate total imbalance of haplotypes frequencies within haplotype block between two groups.
Results: Seventy-two (38.1%) in 189 subjects exhibited 20% decrease in FEV_1 from the baseline values by inhalation of methacholine 8.0mg/ml or less. Significant results were observed in the following haplotype block: *rs* 4947972 - *rs* 12718945 - *rs* 2072454 (block-1), *rs* 4947972 - *rs* 12718945 - *rs* 2227983 (block-2), or *rs* 4947972 - *rs* 12718945 - *rs* 2293347 (block-3). In each haplotype block, higher frequency of a haplotype, C-T-C (block-1), C-T-A (block-2) and C-T-A (block-3), was observed in subjects with AHR than in those without AHR (odds ratio and the corresponding *P* value; 3.289 and 0.00087 (block-1), 3.436 and 0.00055 (block-2), and 4.036 and 0.00181 (block-3), respectively). No significant difference was observed in serological parameters and pulmonary function between two subject groups.
Conclusion: Our results indicate that the EGFR gene polymorphism might be associated with presence of asymptomatic AHR in young allergic adults.