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### 359. Novel mechanisms and targets of molecular pulmonary pathology

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**P3526****The diagnostic value of matrix metalloproteinases -8 and -9 in pleural effusions**

Smaragda Oikonomidi, Irene Tsilioni, Konstantinos Gourgoulianis, Theodoros Kiriopoulos. *Respiratory Medicine Department, University of Thessaly, Medical School, Larissa, Greece*

**Background:** The diagnosis and management of pleural effusions remains a clinical challenge with significant cost to both patients and the health care system. The aim of this study is the assessment of matrix metalloproteinases -8 and -9 (MMP-8,-9), in order to investigate their usefulness in the differentiation of pleural effusions.

**Materials and Methods:** The study included 170 pleural effusions classified as exudates (89 malignant, 24 tuberculous, 26 noncomplicated parapneumonic effusions, 31 complicated parapneumonic effusions and empyema) and 35 transudates due to congestive heart failure. MMP-8 and MMP-9 were determined by ELISA in pleural fluid.

**Results:** The levels of MMP-8 and 9 were significantly higher in patients with exudates compared to transudates ( $83.28 \pm 122.7 \text{ ng/mL}$  vs  $12.47 \pm 47.29 \text{ ng/mL}$ ;  $p < 0.0001$  and  $347.1 \pm 650.1 \text{ ng/mL}$  vs  $30.86 \pm 83.39 \text{ ng/mL}$ ;  $p < 0.0001$ , respectively). Pleural fluid MMP-8 levels were significantly higher in complicated parapneumonic effusions and empyema ( $274.9 \pm 115.1 \text{ ng/mL}$ ) compared to noncomplicated parapneumonic effusions ( $92.47 \pm 118.0 \text{ ng/mL}$ ,  $p < 0.0001$ ), tuberculous ( $49.54 \pm 55.76 \text{ ng/mL}$ ,  $p < 0.0001$ ) or malignant ( $22.94 \pm 51.58 \text{ ng/mL}$ ,  $p < 0.0001$ ) effusions. Similarly, pleural fluid MMP-9 levels were significantly higher in complicated parapneumonic effusions and empyema ( $1372.0 \pm 862.4 \text{ ng/mL}$ ) compared to noncomplicated parapneumonic effusions ( $241.7 \pm 416.6 \text{ ng/mL}$ ,  $p < 0.0001$ ), tuberculous ( $99.38 \pm 102.0 \text{ ng/mL}$ ,  $p < 0.0001$ ) or malignant ( $85.97 \pm 215.5 \text{ ng/mL}$ ,  $p < 0.0001$ ) effusions.

**Conclusions:** High levels of pleural fluid MMP-8 and -9 might be used as markers for the differential diagnosis of complicated parapneumonic effusions and empyema, indicating the pleural fibrosis that occurs in these situations.

**P3527****Z  $\alpha$ 1-antitrypsin: crystal structure and mechanism of polymerisation**

Aiwu Zhou, Robin Carrell. *Haematology and Medicine, CIMR, University of Cambridge, Cambridge CB2 0XY, United Kingdom*

The severe deficiency of plasma  $\alpha$ 1-antitrypsin associated with its Z variant predisposes to COPD and principally results from the intracellular polymerisation of the variant antitrypsin. The Z mutation, Glu342Lys is at the hinge of the peptide loop containing the reactive centre of the molecule [RCL] and proximate to the point where, in the inhibitory mechanism, the loop enters into the A  $\beta$ -sheet of the molecule. We have recently shown with activated dimers [J Mol Biol.2008;375:36-42] how the polymerisation of antitrypsin is initiated by a transitory change in conformation that results in two sites for molecular interlinkage – a partially inserted RCL and a partially opened A-sheet. Evidence from peptide-insertion studies here and with others indicate that Z antitrypsin also has a partially inserted RCL and opened A-sheet. To confirm, we determined the crystallographic structure of Z antitrypsin at 3.3Å but unexpectedly the structure clearly shows a fully exposed RCL and a closed A-sheet. This result fits into context however with other findings; the rate of polymerisation of Z antitrypsin is only marginally increased – whereas activated dimers polymerise at 37°C over a few hours, Z antitrypsin takes as many days. Our recent results with other serpins, including antithrombin, CBG, and TBG, which physiologically undergo partial RCL insertion, show that only a variable proportion of molecules are in the inserted form. We conclude

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that the mutation in Z  $\alpha$ 1-antitrypsin allows a transitory change to an activated form with a partially inserted RCL and opened A-sheet, the small population in this conformation at any one time explaining the relatively slow rate of polymer formation.

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### P3528

#### Heat shock proteins 70 and 90 expression in peripheral lung of COPD patients

G. Caramori<sup>1</sup>, P. Casolari<sup>1</sup>, P. Kirkham<sup>2</sup>, J. Marwick<sup>2</sup>, M. Contoli<sup>1</sup>, A. Zanforlin<sup>1</sup>, K. Ito<sup>3</sup>, K.F. Chung<sup>3</sup>, P.J. Barnes<sup>3</sup>, I.M. Adcock<sup>3</sup>, A. Papi<sup>1</sup>.  
<sup>1</sup>Centro di Ricerca su Asma e BPCO, University of Ferrara, Ferrara, Italy;  
<sup>2</sup>Novartis Institute for Biomedical Research, Novartis, Horsham, United Kingdom; <sup>3</sup>Airways Disease Section, NHLI, Imperial College London, London, United Kingdom

Heat shock proteins (HSP) 70 and 90 are chaperones that may have roles in the pathogenesis of pulmonary fibrosis and MUC5AC secretion. Peripheral lung is the main site of airflow obstruction in COPD. The aim of our study was to investigate by immunohistochemistry the localization and expression of HSP-70 and -90 in the peripheral lung of smokers with or without COPD compared with non-smoker subjects. Lung tissue was obtained during lung resection surgery. We examined FF/PE lung sections by IHC for identification of HSP-70 and -90+ cells. The number of HSP +ve cells was determined among the alveolar macrophages and in the bronchiolar epithelium. Samples from 8 non-smokers subjects (age: 69.1 $\pm$ 3.2, 1M, FEV<sub>1</sub>/FVC ratio = 80.1 $\pm$ 2.3), 26 smokers with normal lung function (age: 67.9 $\pm$ 2.3, 22M, 48 $\pm$ 9 pack years, FEV<sub>1</sub>/FVC ratio=77.5 $\pm$ 1.3) and 19 smokers with COPD (age: 71.3 $\pm$ 1.4, 17M, 45 $\pm$ 8 pack years, post-bronchodilator FEV<sub>1</sub>/FVC ratio=60.1 $\pm$ 2.7) were analyzed. We found a significant difference in HSP-90 +ve alveolar macrophages numbers between non-smokers (35.6 $\pm$ 9.4% +ve alveolar macrophages/total number alveolar macrophages) and smokers with normal lung function (69.0 $\pm$ 5.7% +ve alveolar macrophages/total number alveolar macrophages; p<0.05) but not between non-smokers and smokers with COPD (57.3 $\pm$ 7.7% +ve alveolar macrophages/total number alveolar macrophages) nor between smokers with normal lung function and smokers with COPD. No differences were found in HSP-70 expression in the alveolar macrophages. Also, HSP-70 and -90 expression was similar in the bronchiolar epithelium of the 3 groups of subjects. These data indicates HSP-90 is up-regulated in the alveolar macrophages of smokers with normal lung function.

### P3529

#### Serum levels of proangiogenic factors before and after the correction of severe hypoxemia in COPD patients

Gordana Pavlisa<sup>1</sup>, Veljko Vrbancic<sup>1</sup>, Taida Alfircic-Ungarov<sup>1</sup>, Tajana Jalusic-Glunic<sup>1</sup>, Rada Sarac<sup>1</sup>, Slobodanka Ostojic Kolonic<sup>2</sup>. <sup>1</sup>Pulmology, Special Hospital for pulmonary diseases, Zagreb, Croatia; <sup>2</sup>Internal Medicine, University Hospital "Merkur", Zagreb, Croatia

**Introduction:** Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (b-FGF) are the two most effective proangiogenic factors. They get activated in conditions of systemic hypoxia. Resulting neoangiogenesis represents an important adaptive response of organism to hypoxia. The regulation of these physiologic mechanisms in COPD is unclear.

The aim of this investigation was to evaluate response of VEGF and b-FGF to correction of severe hypoxemia in COPD patients.

**Patients and methods:** Study group consisted of 44 typical COPD patients in acute disease exacerbation with arterial oxygen tension (PaO<sub>2</sub>) below 51 mmHg. Therapeutic goal was to reach PaO<sub>2</sub> of at least 60mmHg. Serum samples were taken before and 24 hours after hypoxemia correction. Serum levels of VEGF and b-FGF have been determined by ELISA technique (R&D Systems).

**Results:** The mean values of initial VEGF and b-FGF were 1106.1 $\pm$ 1140.7 pg/ml, and 6.1 $\pm$ 2.5 pg/ml, respectively. VEGF had a significantly positive correlation to WBC (p=0.0428), and negative to PaO<sub>2</sub> (p=0.0092) and arterial oxygen saturation (p=0.016). Following the correction of hypoxaemia, mean VEGF and b-FGF levels were 1120.9 $\pm$ 1257.6 and 6.4 $\pm$ 3.1, respectively. Both investigated proangiogenic factors have not significantly changed.

**Conclusion:** There were no significant changes of investigated factors during the study period. Our results indicate hypoxia, and possibly inflammation, as stimulators of VEGF production in this system. These findings are in line with the results demonstrated in other clinical models. In contrary to hypoxaemia, the inflammation process can not be controlled promptly which may partly explain our findings.

### P3530

#### H.Pylori DNA in paraffin embedded lung biopsy of patients with IPF

Forouzan Mohammadi, Leila Mohammadi Ziazi, S. Alireza Nadji, Shirin Karimi, Zohreh Mohammadi Taheri, Mihan Poorabdollah, Jamaati HamidReza, Moslem Bahadori. Pathology, Molecular Pathology Section, National Research Institute of Tuberculosis and Lung Disease (N.R.I.T.L.D), Tehran, Iran, Islamic Republic of

**Introduction:** IPF is a progressive fibrotic lung disease. Abnormal wound healing

in the lung results from the repeated injury and activation of alveolar epithelial cells. Chronic aspiration of acid may contribute to the lung injury. There is also evident that IPF develops in those who have H Pylori in their gastric content.

**Method:** By nested PCR we determine the DNA of H Pylori in 23 paraffin embedded lung biopsy from patient having IPF. Negative Controls include 23 block of lung parenchyma of patient who undergo surgical biopsy for pneumothorax. Positive control include block of gastric biopsy with diagnosis of H.pylori gastritis.

**Result:** Cases include 13 male and 10 female. The average age of patient was 51.78. All had restrictive pattern on pulmonary function test and the imaging were consistent with IPF. DNA extract of all cases were positive for human  $\beta$ -actin. All patients and control specimens were negative for H pylori DNA, although positive control was positive in each run.

**Conclusion:** Our study revealed no association of IPF with helicobacter pylori. We may suggest that chronic aspiration may induce lung injury possibly through direct effect of acid.

### P3531

#### ADAMTS-1 metalloproteinase promotes tumor development through the induction of a stromal reaction in vivo

Natacha Rocks, Genevieve Paulissen, Florence Quesada-Calvo, Maria-Luz Alvarez-Gonzalez, Maud Gueders, Jonathan Hacha, Christine Gilles, Jean-Michel Foidart, Agnes Noel, Didier Cataldo. Laboratory of Biology of Tumours and Development, GIGA-Research (Groupe Interdisciplinaire de Génomprotéomique Appliquée)-GIGA-Cancer and GIGA-F. University of Liege and CHU of Liege, Liege, Belgium

ADAMTS-1 (a disintegrin and metalloproteinase with thrombospondin motifs), the first described member of the ADAMTS family, is differentially expressed in various tumors. However, its exact role in tumor development and progression is still unclear. The aim of this study was to investigate the effects of ADAMTS-1 transfection in a bronchial epithelial tumor cell line (BZR) and its potential to modulate tumor development. ADAMTS-1 overexpression did not affect in vitro cell properties such as (1) proliferation in 2D-culture, (2) proliferation in 3D-culture, (3) anchorage-independent growth in soft agar, (4) cell migration and invasion in modified Boyden chamber assay, (5) angiogenesis in the aortic ring assay, and (6) cell apoptosis. In vivo, in sharp contrast, ADAMTS-1 stable transfection in BZR cells strongly accelerated the tumor take and tumor growth after subcutaneous injection into SCID immunodeficient mice. It also promoted a stromal reaction characterized by myofibroblast infiltration and excessive matrix deposition. Conditioned media from ADAMTS-1 overexpressing cells display a potent chemotactic activity towards fibroblasts. ADAMTS-1 overexpression in tumors was associated with increased production of MMP-13, fibronectin, TGF- $\beta$  and IL-1 $\beta$ . Neutralizing antibodies against TGF- $\beta$  and IL-1 $\beta$  blocked the chemotactic effect of medium conditioned by ADAMTS-1 expressing cells on fibroblasts demonstrating the contribution of these factors in ADAMTS-1-induced stromal reaction. In conclusion, we propose a new paradigm for ADAMTS-1 contribution to tumor development which consists in the recruitment of fibroblasts involved in tumor growth and tumor-associated stroma remodeling.

### P3532

#### An immunohistochemistry (IHC) assesment of NSCLC cases

Florinel Pop<sup>1</sup>, Gabriela Barbulescu<sup>2</sup>, Laurentia Badulescu<sup>2</sup>. <sup>1</sup>Histology and Molecular Biology Laboratory, "D. Gerota" Hospital, Bucharest, Romania; <sup>2</sup>Pneumology, "D. Gerota" Hospital of Pneumology, Bucharest, Romania

The authors had decide to investigate by IHC all resected NSCLC cases, in order to evaluate the degree of aggressive potential of the tumor and to select a class of patients candidates to the genetic targeted therapy.

We decided to assess the overexpression of EGFR as a growth tumor factor and the expression of Ki-67 as a marker of proliferation on a casuistic observed during 2006-2007.

We had 50 patients with NSCLC diagnosed, after surgical excision, by classical histology method.

The cases were 43 males and 7 females, aged between 45-62 ys.

According with TNM status, the distribution of the cases was: St 1A-2 cases; St1 B-5 cases; St2 A- 10 cases; St 2B (T2N1M0)- 7cases; St2 B (T3N0M0) -10 cases; St 3B (T3 N1M0)- 5 cases; St3B (T3N2M0)- 8 cases; St 3B (T4N0M0)- 3 cases. The histological classification was as follows: - 35 squamos carcinoma; 10 adenocarcinoma; 5 large cell carcinoma.

All the fixed paraffin- embedded sections were submitted to the IHC, using an avidin-biotin HRP method. Sections were immunostained for EGFR with AC MO-Biogenex and for Ki-67 protein we used Ki-67-Biogenex. The results of staining were classified by current criteria as: 0, 1+; 2+; and 3+. We detected an overexpression of EGFR (3+) in all St 3B cases (21) and in 5 cases St 2B (with T3), and a relative expression (2+) in the rest of St 2 B cases (12). No expression was detected in st1A and 1B and st2A cases.

In all our cases Ki 67 expression was between 30 and 70%.

**Conclusion:** for 34 cases with stades 2-3 TNM, IHC provided information about tumor growth and prognosis by EGFR positive expression. These patients were referred to oncology for anti- EGFR targeted therapy also. We didn't find any correlation between Ki 67 and EGFR expression.

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**P3533****A novel vitamin K epoxide reductase complex subunit-1 (VKORC1) mutation and the risk of bleeding**

Fanak Fahimi<sup>1,2</sup>, Shadi Baniyasi<sup>1</sup>, Bahram Kazemi<sup>3</sup>, Neda Behzadnia<sup>4</sup>, Bijan Shafaghi<sup>5</sup>, Samira Beizae<sup>2</sup>, Mojgan Banehpour<sup>3</sup>. <sup>1</sup>Pharmaceutical Care, TB and Lung Disease Research Center, NRITLD, Masih Daneshvari Hospital, Shahid Beheshti University, M.C., Tehran, Iran, Islamic Republic of; <sup>2</sup>Clinical Pharmacy, School of Pharmacy, Shahid Beheshti University, M.C., Tehran, Iran, Islamic Republic of; <sup>3</sup>Cellular and Molecular Biology Research Center, School of Medicine, Shahid Beheshti University, M.C., Tehran, Iran, Islamic Republic of; <sup>4</sup>Cardiology, TB and Lung Disease Research Center, NRITLD, Masih Daneshvari Hospital, Shahid Beheshti University, M.C., Tehran, Iran, Islamic Republic of; <sup>5</sup>Toxicology, School of Pharmacy, Shahid Beheshti University, M.C., Tehran, Iran, Islamic Republic of

**Background:** Warfarin is widely used for venous thromboembolism (VTE) treatment and prophylaxis and exerts its anticoagulant effect by inhibiting the vitamin K epoxide reductase enzyme complex subunit-1 (VKORC1).

**Objectives:** Gene variant of the VKORC1 have been associated with large interindividual differences on the anticoagulant response to warfarin.

VKORC1 polymorphism among patients with VTE who received warfarin and referred to warfarin clinic was evaluated.

**Methods:** Sixty patients (33 males and 27 females) with stable INR (2-3) were selected from warfarin clinic. A complete clinical data was obtained from all subjects. Blood samples were drawn for VKORC1 genotype analysis. Amplifications of exon1,3 and 3' untranslated region (3'UTR) were achieved using sense and antisense oligonucleotide. To identify VKORC1 mutations, Confirmation Sensitive Gel Electrophoresis (CSGE) was performed followed by direct sequencing.

**Results:** Three VKORC1 frameshift mutations were detected. The first variation was addition of two nucleotides at position 51 and 52 in the exon3 (2 patients). The second frameshift mutation was deletion of two nucleotides at position 92 and 93 in the exon3 (1 patient).

All the 3 patients reported bleeding during warfarin use, while no other bleeding was reported during the study period.

**Conclusions:** The use of a fixed dose warfarin for all patients and in-range INR may not be sufficient for warfarin monitoring. Many factors including unknown ones may also play an important role in highly variable response among patients. Our data for the first time, suggested a new possible call for screening to reduce the risk of bleeding.

**P3534****PTGER4, a promising novel DNA methylation marker for lung cancer measured in a clinical case control study using blood plasma**

Bernd Schmidt<sup>1</sup>, Volker Liebenberg<sup>2</sup>, Theo DeVos<sup>2</sup>, Juergen Distler<sup>2</sup>, Reimo Tetzner<sup>2</sup>, Anke Segebarth<sup>2</sup>, Sabine Weickmann<sup>1</sup>, Christian Witt<sup>1</sup>, Michael Fleischacker<sup>3</sup>. <sup>1</sup>Pneumologie, CCM, Charité – Universitätsmedizin Berlin, Berlin, Germany; <sup>2</sup>Epigenomics AG, Berlin/Seattle, Germany; <sup>3</sup>Onkologie/Haematologie, CCM, Charité – Universitätsmedizin Berlin, Germany

**Introduction:** Molecular markers are an emerging field in early lung cancer detection. During the last years several methylation markers, e.g. RASFF1A, p16, p53 have been extensively investigated. They appear to be meaningful as well as stable epigenetic modifications, which are highly informative and robust, but their analysis is often limited to tissue and their clinical utility hampered by poor performance.

**Patients/Methods:** In this study we determined the performance of PTGER4, a novel biomarker for non small cell lung cancer discovered earlier using differential methylation hybridisation (DMH) by Epigenomics. We included plasma samples (~4 mL) from 50 patients with confirmed lung cancer and from 50 pts. with non-malignant lung disease (e.g. bronchitis, pneumonia, benign airway stenosis etc.). DNA was isolated and after bisulfite conversion the presence of marker was determined by real time PCR.

**Results:** Amplifiable DNA could be extracted from all but 8 samples. At a specificity level of 91% PTGER4 showed a sensitivity of 69%.

**Conclusions:** Together with subsequent studies showing no significant differences in sensitivity between early and later stage NSCLC. These results suggest, that the measurement of the DNA methylation marker PTGER4 in plasma might become a promising tool in lung cancer detection.

**P3535****New aspects of COPD ethiopathogenesis**

Viacheslav Kobylansky<sup>1</sup>, Igor Ivanov<sup>2</sup>, Evgeniy Gamal<sup>3</sup>, Gulnara Babadzhanova<sup>1</sup>, Tatyana Petrova<sup>4</sup>. <sup>1</sup>Laboratory of Genetics of Multifactorial Diseases, Research Institute for Pulmonology of the Russian Public Health Service, Moscow; <sup>2</sup>Laboratory of Genetics, Pediatric Medical Academy, Saint-Petersburg; <sup>3</sup>Pulmonology, Saint Elisabeth Hospital, Saint-Petersburg; <sup>4</sup>Pulmonology, Central Medical and Sanitary Unit 122 of the Ministry of Public Health of Russia, Saint-Petersburg, Russian Federation

**Introduction:** One of the most important COPD pathogenic mechanism is mucociliary system distress and, in particular, its main, ciliary, chain, which activity is determined by dinein. However, the role of these disorders in COPD haven't been studied at cellular-molecular level.

**Aim:** To study a number of polymorphisms (PM) of some genes as a causative factor of COPD ethiopathogenesis.

**Materials and Methods:** On the basis of the suggested original concept of COPD pathogenesis and using PCR-reaction with the help of allele specific primers identification of PM G/C G3519R (1) and T/C V1023A (2), as well as T/C V4134A (3) and A/G 12682V (4), corresponding to genes DNAH5 and DNAH11 was performed in two groups of patients. The first group consisted of 60 COPD patients and the second included 75 patients with chronic bronchitis and healthy persons (control group). COPD was diagnosed by standard diagnostic criteria due to ERS recommendations. The obtained data theoretically were extrapolated for 100 persons of each group.

**Results:** Of PM studies only PM<sub>3</sub> and PM<sub>4</sub> were identified whereas PM<sub>1</sub> and PM<sub>2</sub> were not found in any group. PM<sub>3</sub> frequency was higher in COPD comparing with control group, but only in case of homozygous mutant genotype (GG) was significant (p < 0.05). PM<sub>4</sub> was identified less frequently in COPD patients (p > 0.05) and was not found in control group.

**Conclusion:** Studies of PM of genes, coding dinein, as a causative factor of COPD ethiopathogenesis opens new perspectives in COPD predictive diagnostics.

**P3536****High serum levels of YKL-40 in IPF**

Nicoline M. Korthagen<sup>1</sup>, Coline H.M. van Moorsel<sup>1</sup>, Nicole P. Barlo<sup>1</sup>, Henk J.T. Ruven<sup>2</sup>, Jules M.M. van den Bosch<sup>1</sup>, Jan C. Grutters<sup>1</sup>. <sup>1</sup>Pulmonology, St. Antonius Hospital, Nieuwegein, Netherlands; <sup>2</sup>Clinical Chemistry, St. Antonius Hospital, Nieuwegein, Netherlands

**Introduction:** YKL-40 is a cartilage glycoprotein (human cartilage glycoprotein 39) encoded by the chitinase 3-like 1 (CHI3L1) gene. High serum YKL-40 protein levels have been shown in several diseases such as rheumatoid arthritis, Crohn's disease and sarcoidosis. A genetic polymorphism in the CHI3L1 gene (-329 G/A) was found to influence YKL-40 serum concentrations in healthy controls. Healthy controls with the GG genotype have serum levels more than two times higher than AA carriers. The exact function of YKL-40 is unclear but it has been found to promote fibroblast growth.

**Aim:** This study aimed to investigate whether serum YKL-40 levels are altered in idiopathic pulmonary fibrosis (IPF).

**Method:** YKL-40 levels were determined in serum from 356 healthy controls and 73 patients with IPF. In addition, their genotype for the CHI3L1 -329 G/A polymorphism was determined.

**Results:** IPF patients had significantly higher serum YKL-40 levels (181 ng/ml, 137.2-224.8) compared to controls (49.9 ng/ml, 43.0-55.2) (mean, 95% CI, p<0.001). The -329 allele frequency in IPF patients was significantly different from healthy controls (P=0.0033, odds ratio=1.8). -329A in IPF was 33% versus 21% in controls. The -329A allele carrier frequency had an odds ratio of 2.8 (CI= 0.18-3.6, p=0.0005). The polymorphism did not correlate with YKL-40 serum concentrations in IPF patients. Since the -329A allele correlates with low serum levels in healthy individuals we hypothesize that during lung insults with unknown cause low serum levels predispose to IPF.

**Conclusion:** YKL-40 serum concentrations are significantly higher in IPF patients than in controls. IPF patients have a significantly different -329 G/A allele frequency.

**P3537****Diagnostic value of IL-8 in differentiating between exudative and transudative pleural effusion**

Mostafa Elshazly, Khaled Hasanein, Badawi Elkholy, Mohamed Ghanem. *Chest Diseases, Kasr Elaini Faculty of Medicine, Cairo, Egypt; Chest Diseases, Kasr Elaini Faculty of Medicine, Cairo, Egypt; Chemical Pathology, Kasr Elaini Faculty of Medicine, Cairo, Egypt; Chest Diseases, Alexandria Chest Hospital, Alexandria, Egypt*

Interleukin-8 (IL-8) was proved as a diagnostic procedure in differentiation between exudative and transudative pleural effusions.

**Aim OF The Work:** Is to evaluate IL-8 as a diagnostic biomarker for differentiation between pleural effusions of different etiologies both in serum and pleural fluid samples.

**Subjects & Methods:** included 30 patients with pleural effusion subdivided into: Group I: Included 15 cases with transudative pleural effusion (6 cases with liver cell failure, 5 cases with chronic renal failure, 4 cases with congestive heart failure). Group II: Included 15 cases with exudative pleural effusion (9 cases with malignant effusion & 6 cases with tuberculous effusion).

The pleural fluid and serum samples of all the patients were completely analyzed, chemically, bacteriologically and cytologically including estimation of IL-8 (pg/ml) both in serum and pleural fluid.

**Results and Conclusion:** Serum level of IL-8 (pg/mL) was higher in exudative than transudative pleural effusion group and this difference was statistically highly significant (P < 0.01).

Also malignant effusion group (MPE) showed the highest mean value of serum IL-8 69.7 ±35.3pg/ml While in T.B effusion group a mean value of serum IL-8 25.4 ±18.9 pg/mL. The level of pleural fluid IL-8 was higher among exudative than transudative pleural effusion group and this difference was statistically highly significant (P < 0.01). Thus IL-8 (pg/mL) can be used as a diagnostic procedure in differentiation between transudative and exudative pleural effusion patients. In

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patients with adenocarcinoma & mesothelioma effusion there was highly strong positive correlation between serum IL-8 and pleural fluid IL-8.

**P3538****Different expression of miRNA in non-small cell lung cancer according to its clinical stage**

Arturo Huerta<sup>1</sup>, Alfons Navarro<sup>2</sup>, Ramon Marrades<sup>1</sup>, Angels Quera<sup>3</sup>, Nuria Viñolas<sup>4</sup>, Josep Ramirez<sup>3</sup>, Carles Agusti<sup>1</sup>, Elena Gallardo<sup>4</sup>, Antoni Torres<sup>1</sup>, Mariano Monzo<sup>2</sup>. <sup>1</sup>*Servei de Pneumologia (CIBER 06/06/0028), Hospital Clinic de Barcelona, Barcelona, Spain;* <sup>2</sup>*Departament d' Anatomia (Facultat de Medicina), Universitat de Barcelona, Barcelona, Spain;* <sup>3</sup>*Servei d' Anatomia Patològica, Hospital Clinic de Barcelona, Barcelona, Spain;* <sup>4</sup>*Servei d' Oncologia Mèdica, Hospital Clinic de Barcelona, Barcelona, Spain*

MicroRNA (miRNA) are small molecules of RNA that act as negative regulators in proteic synthesis. Their function, performed in the cytoplasm, is to bind specific messenger RNAs (RNAm) and downregulate its traduction. They participate in the regulation of multiple biologic processes including development of cancer.

**Aim:** To determine the expression of miRNA in normal lung tissue as well as in neoplastic tissue and to analyze the relationship between their expression at different stages according to TNM classification.

**Methods:** In 31 patients with non-small cell lung cancer (NSCLC) we analyzed 62 bronchial biopsies (31 tumoural and 31 normal) obtained during diagnostic fibrobronchoscopy. We determined the expression of miRNA by qRT-PCR.

**Results:** The hierarchical clustering of miRNA expression showed statistically significant difference between the following groups:

- Tumoural tissue vs. normal tissue: Underexpression of *let7c* and overexpression of *mir 17-5p*, *mir-221* and *mir 19a* (miRNAs involved with the expression of K-ras protein (carcinogenesis) and cell proliferation)
- NSCLC stage I vs. all other stages: Underexpression of a miRNA of the *let 7* family (*let7a*, *let7c* and *let7e*) and overexpression of the *mir 17-5p*, *mir 19a* and *mir-502* (miRNAs involved in cell proliferation)
- NSCLC with M<sub>1</sub> vs. all others: Overexpression of *mir 21*, *mir 491* and *mir 50* (miRNAs involved in apoptosis and cell polarization)

**Conclusion:** The expression of miRNA at different stages may be useful in understanding the mechanisms involved in tumour proliferation in the lung. These miRNA might be good targets for new therapeutics strategies.

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**P3539****Increased expression of Lipocalin2 suppresses allergen-induced airway disease in mice**

Martin Krokowski<sup>1</sup>, Hellmuth-Alexander Meyer<sup>1</sup>, Anna Dittrich<sup>2</sup>, Jack Cowland, Eckard Hamelmann<sup>1</sup>. <sup>1</sup>*Pediatric Allergy, Charité University Medicine, Pediatric Pneumology and Immunology, Berlin, Germany;* <sup>2</sup>*Collaborative Research Center 587, University Medicine Hannover, Hannover, Germany;* <sup>3</sup>*Department of Hematology, University of Copenhagen, Copenhagen, Denmark*

**Background:** Bronchial asthma is a chronic airway disease in which the interplay of various genes with environmental factors regulates inflammatory responses in the lungs. The aim of the project was to identify and validate new genes involved in the development of allergic airway disease in a murine asthma model.

**Methods:** RNA microarray analysis of expression profiles from lungs of two mouse strains with different allergic susceptibility (BALB/c vs. C57/B16) was used to identify common genes regulated upon allergen sensitization and airway challenges. Target regulation and localization were confirmed by western blot of BAL-fluid and immunohistochemistry. Requirements for induction of target genes were assessed in epithelial cell cultures via RT-PCR and TUNEL-assay. Functional relevance was assessed utilizing knock-out mice.

**Results:** Lipocalin2 (Lcn2) was found to be upregulated in lung tissues of sensitized and challenged mice vs. control animals. Lcn2 expression in lung tissue increased in both strains after induction of airway inflammation on RNA/protein level. Functional analysis revealed induction of Lcn2 in epithelial cells via different pro-inflammatory cytokines and LPS suggesting a pro-apoptotic effect of Lcn2. Allergen sensitization and airway challenges of Lcn2 knock-out mice resulted in vastly elevated airway inflammation and hyperresponsiveness, associated with decreased levels of apoptosis in lung tissue.

**Conclusion:** These data suggest a protective role for Lcn2 in allergic airway disease with the pro-apoptotic effect as underlying mechanism. Lcn2 thus is a newly discovered mediator between innate and adaptive immune responses with a potential protective role in allergic airway disease.

**P3540****Analysis of cytokine signaling pathway JAK-STAT through their activation in patients with asthma**

Valery Mineev, Lada Sorokina, Maria Radionova, Maria Hudoley, Vasily Trofimov. *Hospital Therapy, Saint-Petersburg Pavlov's State Medical University, Saint-Petersburg, Russian Federation*

Asthma is a chronic inflammatory disease of the airways caused (induced) by a multitude of cell types and inflammatory mediators and negative regulation disturbances. JAK-STAT signaling pathway mediated by Interleukin-4 is crucial

in the pathogenesis of allergic disorders. It promotes differentiation of premature lymphocytes to Th2 cells and provides with several immunological processes. An activation of STAT6 and GATA3 may lead to the predominant differentiation of T-helper 2 cells and the production of corresponding cytokines.

**Aim:** The levels of STAT6, phosphoSTAT6, GATA3 in patients with bronchial asthma (BA) was the subject of our study.

**Methods:** These proteins expressed in peripheral lymphocytes, obtained from 14 patients with BA (from moderate to severe), activated with IL-4 (Sigma) (1 ng/ml) for 15 min, were compared with control (healthy volunteers) and analyzed by Western blot after the lymphocytes were lysed. Preparation of cell lysates, and the Western blotting were performed through the standard procedure. We used indicated antibodies: against phospho-STAT6 (Cell Signaling), and STAT6 (Cell Signaling); against GATA3 (Abcam). The level of protein was analyzed according to beta-actin (Cell Signaling).

**Results:** The expression of STAT6 and GATA3 was increased in patients with BA. The level of pSTAT6 after IL-4 activation was higher compared with healthy volunteers.

**Conclusion:** In conclusion, this study suggests that asthma is associated with an active T-helper 2 cell inflammatory process involving activation of STAT6 and GATA3.

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**P3541****Extrapulmonary effects of inhaled ultrafine carbon particles in mice**

Tobias Stoeger, Dariusch Etehadieh, Shinji Takenaka, Holger Schulz. *Institute of Inhalation Biology, Helmholtz Zentrum München, München/Neuherberg, Germany*

Ambient ultrafine particles are able to translocate from the lung into the systemic circulation, but the impact of translocation on cardiovascular effects is largely unclear. Here we test the hypothesis that translocated ultrafine carbon particles (UfCP) induce significant signs of extrapulmonary inflammation and compared the effects caused by two different exposure models: whole body inhalation and intraarterial infusion.

Mice were either exposed for 4 or 24h to 440 µg/m<sup>3</sup> UfCP by inhalation, or for 4h by intraarterial infusion to the equivalent dose of estimated within 24h inhalation translocated 5×10<sup>7</sup> UfCPs. Mice were analyzed for systemic effects by haematology, peripheral leukocyte activation and plasma cytokine levels. Lung, heart, aorta and liver were investigated using a panel of inflammatory markers by RT-PCR and a protein assays.

Only UfCP inhalation caused a modest proinflammatory response of the lungs, followed by significantly reduced leukocyte activation, possibly a sign for increased retention in the pulmonary vascular bed. In this context blood leukocyte numbers increased especially after UfCP infusion. Platelet cytograms indicated mobilization and recruitment after UfCP inhalation only, accompanied by decreased blood fibrinogen levels and increased local fibrin deposition in liver samples. Finally also the aorta exhibited strongest proinflammatory gene expression after particle inhalation

Our results affirm a crucial role for the inflammatory responses in the lung and support the hypothesis, that a release of soluble mediators by the lungs or an activation of circulating blood cells in the capillary bed of the challenged lungs drives in large part particle related extrapulmonary effects.

**P3542****Levels of TNF-alpha, IL-6, sICAM-1, sVCAM-1 in NSCLC patients plazma**

Uldis Kopeika<sup>1</sup>, Peteris Tretjakovs<sup>1</sup>, Inga Bormane<sup>1</sup>, Jazeps Basko<sup>1</sup>, Natalja Jakushenko<sup>2</sup>. <sup>1</sup>*Institute of Experimental Medicine, University of Latvia, Riga, Latvia;* <sup>2</sup>*Faculty of Medicine, University of Latvia, Riga, Latvia*

**Background:** Recent studies suggest that interleukin IL-6 and TNF-alpha are directly produced by the tumor cells and involved in the development of lung cancer. The aim of this study was to investigate the concentration of TNF-alpha, sICAM-1, sVCAM-1, IL-6 and other biomarkers in the blood of NSCLC patients before and after treatment.

**Methods:** We enrolled 15 patients with histological evidence of NSCLC (11 men and 4 women, age 60±12 years) and 15 controls with non-malignant lung disease (9 men and 6 women, age 57±8 years). Adiponectin, leptin, PAI-1, sICAM-1, sVCAM-1 IL6 and TNF-alpha were measured, in patients and controls, at the beginning of the study, and in NSCLC patient group also 2 weeks after radical surgery- lobectomy or pneumonectomy.

**Results:** In control group patients TNF-alpha was 3±0.6pg/ml and IL-6 was 1.1±0.4 pg/ml. In NSCLC patients group before surgery TNF-alpha was 4.87±1.2 pg/ml and IL-6 was 1.17±0.44 pg/ml and after surgery TNF-alpha was 2.38±0.9 pg/ml and IL-6 was 1.26±0.6 pg/ml. sICAM-1, sVCAM-1 levels were different between both patients groups (p<0.05). In adiponectin, leptin, PAI-1 plasma level we did not find significant difference between both groups of patients.

**Conclusion:** Higher concentrations of TNF-alpha and IL-6 were found in plasma of NSCLC patients compared to healthy subjects. These findings suggest that the measurement of TNF-alpha and IL-6 in plasma of NSCLC patients could be used for the diagnosis and the monitoring of evolution of this disease.

**Discussion:** We explain increased IL-6 level in NSCLC patients group after radical surgery as response to surgical trauma.

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**P3543****Oxidative stress and pleural effusions**

Irene Tsilioni, Smaragda Oikonomidi, Konstantinos Gourgoulis, Theodoros Kiroopoulos. *Respiratory Medicine Department, University of Thessaly, Medical School, Larissa, Greece*

**Background:** The imbalance between oxidants and antioxidants is referred to as oxidative stress and has been associated with various respiratory disorders. The aim of this study is the assessment of 8-isoprostane and Cu/Zn SOD in the pleural fluid in order to investigate their usefulness in the differential diagnosis and pathogenesis of pleural effusions.

**Materials and Methods:** The study included 179 pleural effusions classified as exudates (88 malignant, 24 tuberculous, 31 noncomplicated parapneumonic effusions (NCPE), 37 complicated parapneumonic effusions (CPE) and empyemas) and 34 transudates due to congestive heart failure. Both biomarkers were determined by ELISA.

**Results:** 8-Isoprostane and Cu/Zn SOD were found significantly higher in exudates compared to transudates, ( $81.7 \pm 89.7$  vs  $33.1 \pm 22.5$  pg/mL;  $p=0.032$  and  $267.8 \pm 454.2$  vs  $101.4 \pm 50.89$  ng/mL;  $p=0.002$ , respectively). 8-Isoprostane levels were significantly higher in CPE and empyemas ( $243.7 \pm 257.8$  pg/mL) compared to NCPE ( $40.57 \pm 20.72$  pg/mL,  $p<0.0001$ ), tuberculous ( $26.37 \pm 12.95$  pg/mL,  $p<0.0001$ ) and malignant ( $42.62 \pm 45.55$  pg/mL,  $p<0.0001$ ) effusions. Similarly, Cu/Zn SOD levels were significantly higher in CPE and empyemas ( $706.4 \pm 814.5$  ng/mL) compared to NCPE ( $97.51 \pm 47.25$  ng/mL,  $p<0.0001$ ), tuberculous ( $136.9 \pm 79.02$  ng/mL,  $p=0.0001$ ) and malignant ( $177.4 \pm 200.5$  ng/mL,  $p<0.0001$ ) effusions.

**Conclusions:** 8-Isoprostane and Cu/Zn SOD levels are higher in exudates compared to transudates. Both 8-Isoprostane and Cu/Zn SOD levels may be useful additional markers in the differentiation among CPE and empyemas from NCPE, tuberculous and malignant effusions.

**P3544****Evidence for a stimulating effect of hyperbaric oxygen on the transcription rate of Cu/Zn-SOD in rat lung**

Sukru Oter<sup>1</sup>, Ahmet Korkmaz<sup>1</sup>, Serdar Sadir<sup>1</sup>, Seyfettin Gumus<sup>2</sup>, Omer Deniz<sup>2</sup>, Recai Ogur<sup>3</sup>, Halil Yaman<sup>4</sup>, Hayati Bilgic<sup>2</sup>. <sup>1</sup>*Department of Physiology, Gulhane Military Medical Academy, Ankara, Turkey;* <sup>2</sup>*Department of Pulmonary Medicine, Gulhane Military Medical Academy, Ankara, Turkey;* <sup>3</sup>*Department of Public Health, Gulhane Military Medical Academy, Ankara, Turkey;* <sup>4</sup>*Department of Biochemistry, Gulhane Military Medical Academy, Ankara, Turkey*

Hyperbaric oxygen (HBO) is reported to increase both oxidative stress markers and antioxidant enzymes activities. It is thought that antioxidant enzymes were stimulated due to the overproduction of reactive oxygen species indirectly, but there are also arguments that HBO may induce these enzymes synthesis directly. Therefore, taken the lung as the main target organ affected by HBO exposure, we aimed to elucidate this matter via detecting the transcription rate of copper/zinc-superoxide dismutase (Cu/Zn-SOD).

Seventy-two male Sprague-Dawley rats were divided into 4 groups as follows: control, HBO, HBO+Glutathione (GSH) and HBO+SOD, HBO+melatonin, and melatonin. HBO was administered once for 2 hours at 3 atmospheres pressure. GSH, SOD, and melatonin were administered intraperitoneally one hour before HBO exposure. Right after exposure the lungs were taken for further biochemical assay: malondialdehyde (MDA) and protein carbonyl (PCO) levels, catalase, glutathione peroxidase and SOD activities, and reduced/oxidized glutathione (GSH/GSSG) ratio.

HBO clearly enhanced oxidative stress criteria, namely increased MDA and PCO, and decreased GSH/GSSG ratio. Antioxidant enzymes activities as well as SOD mRNA transcription rates were also increased with HBO exposure. Neither additional SOD nor GSH were able to block this effect. Interestingly, melatonin increased the SOD mRNA transcription itself, but limited (not blocked) the increasing effect of HBO.

The present findings support the suggestion that HBO have the ability to stimulate directly the mRNA transcription of SOD. Nevertheless, the limiting effect of melatonin indicates a molecular interaction and has to be further elucidated.