94. New results in molecular pathology and functional genomics of neoplastic and non-neoplastic lung disease

P760 Plasma DNA concentration and integrity measurement for NSCLC diagnostics and radical therapy effectiveness monitoring
Adam Szpechcinski1, Joanna Chorostowska-Wynimko1, Wlodzimierz Kupis2, Krystyna Maszkowska-Kopij3, Jolanta Zaleska4, Elzbieta Radzikowska5, Elzbieta Puscincka6, Pawel Slivinski7, Tadeusz Orłowski8, Kazimierz Rosekowski-Sliz9, 1Laboratory of Molecular Diagnostics and Immunology, National Institute of Tuberculosis and Lung Diseases, Warsaw, Poland; 2Department of Thoracic Surgery, National Institute of Tuberculosis and Lung Diseases, Warsaw, Poland; 3Outpatient Clinic, National Institute of Tuberculosis and Lung Diseases, Warsaw, Poland; 4Department of Lung Diseases, National Institute of Tuberculosis and Lung Diseases, Warsaw, Poland; 5Department of Diagnosis and Treatment of Respiratory Failure, National Institute of Tuberculosis and Lung Diseases, Warsaw, Poland

Plasma DNA concentration and integrity index (DII) were measured by real-time PCR in 60 NSCLC patients (stage I-IIIA), 100 patients with chronic respiratory inflammation (COPD, sarcoidosis, asthma) and control groups comprising 10 orthopedic patients and 40 healthy volunteers. NSCLC patients (8.0 ng/ml) presented significantly higher plasma DNA levels than patients with chronic respiratory inflammation (3.4 ng/ml), orthopedic patients (3.0 ng/ml) and healthy controls (2.3 ng/ml; p<0.0000). The cut-off point of >2.8 ng/ml provided 90% sensitivity and 80.5% specificity in discriminating NSCLC from healthy individuals (AU≤=0.90), while 56% specificity and 90% sensitivity in distinguishing NSCLC from any non-NSCLC subjects (AU=0.80; p<0.0001). The plasma DII was higher in resectable NSCLC (3.1) and chronic respiratory inflammation (3.7) than healthy controls (1.0; p=0.0000). Resected NSCLC (68.7 ng/ml; p<0.0000) and orthopedic patients (28.4 ng/ml; p<0.0015) presented comparable plasma DNA dynamics after the surgery. During 3-6 month follow-up plasma DNA level were significantly reduced in relapse-free NSCLC patients (2.8 ng/ml), while in relapsed subjects were higher than at baseline. The plasma DNA quantification, though insufficient for routine NSCLC detection, was still superior to diagnostic accuracy of conventional serological markers. Significant differences in DII values were associated with up-regulation of apoptosis and/or necrosis. Increased post-surgical plasma DNA concentration was due to the tissue trauma but not to the malignancy. Long-term post-operative plasma DNA follow-up might prove promising in monitoring of radical NSCLC therapy.

P761 Biomarker determinations altered by anesthesia induction
Nicolaus Kahn1, Julia Riedlinger1, Markus Ronssler1, Christina Rabe2, Thomas Mulry2, Felix Herth1, Michael Meister2, 1Pneumology and Critical Care Medicine, Thoraxklinik, University of Heidelberg, Germany; 2Translational Research Unit, Thoraxklinik, University of Heidelberg, Germany; 3Roche Diagnostics, Roche AG, Penzberg, Germany

Biomarkers are advanced tools for diagnosis, prognosis and monitoring of treatment and disease progression. The validation of biomarkers is a cumbersome process involving many steps. Serum samples from lung cancer patients were collected in the framework of a larger Lung Cancer Screening project. During the analysis of some biomarkers, differences between marker values depending on the time of blood extraction were inconsistent. Biomarker concentration differed significantly if taken before or after the induction of anesthesia. From 13 patients blood samples were drawn 1-2 days prior to surgery, on the same day and after anesthesia was applied. Markers SCC (microtiter plate), and CEA (Elecys) were analyzed. SCC showed a very strong effect in relation to the sampling time. While the first two time points were well comparable (correlation r=0.883), patients showed a highly significant (p =0.0017) increase in concentration when comparing the first two time points with the time point after anesthesia induction. The concentration of CEA had almost no variation (r=0.993 comparing time points as above).

In this study we show the unexpectedly high influence of blood extraction timing in the concentration of the protein biomarker SCC but not in CEA. Whereas the possible causes for this alteration remains to be elucidated in further studies, these results are a caveat to make sure that biomarker sampling protocols are controlled for this type of effects.

P762 Hypoxic phenotype in pulmonary metastases of different primary tumors
Thomas Schweiger1,2, Christoph Nikolowsky1,2, Lukas Lehmann1, Robert Weribringhaus1, Gyorgy Lang2, Peter Birrer2, Walter Klepetko2, Hendrik-Jan Ankersmit1,2, Konrad Hoetzenecker1,2, 1Department of Thoracic Surgery - Division of Thoracic Surgery, Medical University of Vienna, Austria; 2Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis and Regeneration, Medical University of Vienna, Austria; 3Clinical Institute of Pathology, Medical University of Vienna, Austria

Tumor hypoxia has been shown to be a common feature in tumor growth and metastasis. It negatively affects the clinical outcome in patients with various malignancies. Although hypoxia is described in many primary tumor types, data on pulmonary metastases is lacking. We determined the expression of hypoxia-related proteins in paraffin-embedded specimens of pulmonary metastases of different types of cancer (breast cancer n= 6, colo-rectal carcinoma n=29, renal cell carcinoma n=13, sarcoma n=10). All recruited patients underwent curative metastasectomy at the Department of Thoracic Surgery, MUV, between April 2009 and December 2011. Expression of carbonic anhydrase 9 (CA9), heat-shock protein 70 and HIF prolyl hydroxylase 2 was evaluated by immunohistochemistry. Metastasis free survival and estimated tumor size and overall survival was determined for all sub-groups. Hypoxia related proteins are expressed in 66.6%, 76.9%, 7.7% and 0.0% of pulmonary metastases of breast cancer, colon-rectal carcinoma, renal cell carcinomaa and sarcoma, respectively. Furthermore, metastases with highly positive CA9-staining are associated with early tumor spreading to the lung (Disease free interval: 21.8±=7.0 months vs. 46.1±=26.6 months; p=0.008)

This study provides first evidence for hypoxia marker expression in pulmonary metastases and its clinical relevance. These findings may be important for future therapeutic targets in the therapy of generalized malignant diseases.

P763 Hypoxic phenotype in pulmonary metastases of different primary tumors
Thomas Schweiger1,2, Christoph Nikolowsky1,2, Lukas Lehmann1, Robert Weribringhaus1, Gyorgy Lang2, Peter Birrer2, Walter Klepetko2, Hendrik-Jan Ankersmit1,2, Konrad Hoetzenecker1,2, 1Department of Thoracic Surgery - Division of Thoracic Surgery, Medical University of Vienna, Austria; 2Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis and Regeneration, Medical University of Vienna, Austria; 3Clinical Institute of Pathology, Medical University of Vienna, Austria

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**P764**

**Vitronectin expression in primary lung cancer**

Lina M. Salazar-Pelaez, Magda C. Ledesma, Ana M. Herrera. School of Medicine, Universidad CES, Medellin, Antioquia, Colombia

**Rationale:** Vitronectin (Vn) plays a role in extracellular matrix (ECM) remodeling during tumorigenesis. Vn is present in human bronchial submucosal glands and it is secreted by A549 lung adenocarcinoma cell line. However, whether Vn is differentially expressed in tissues of primary lung cancer has not been explored.

**Methods:** Lung tissue from 22 primary lung carcinoma and 36 non-lung cancer subjects were obtained from fibrobronchoscopy. Sections were analyzed by hist-immunohistochemical methods. The total area occupied by ECM, surface and glandular epithelium, as well as the percentage area occupied by Vn at each of these localizations were measured. Chi-square, t-student, U-Mann Whitney and ANOVA tests were used in group comparisons. Statistical significance was tested at P<0.05.

**Results:** Vn expression was observed in bronchial glandular and surface epithelium, as well as in ECM (Figure 1). In total, the average area of ECM, surface or glandular epithelium was 0.289mm² (±0.032), 0.034mm² (±0.009), 0.084mm² (±0.031), respectively. The percentage area occupied by Vn in ECM, surface or glandular epithelium, was 4.899 (±1.186), 7.279 (±1.623), 3.285 (±1.038), respectively; with not significant statistical differences between lung cancer and non-cancer subjects or within different types primary lung cancer.

**Conclusion:** There were no statistically significant differences in Vn expression between lung cancer and non-cancer subjects, or within primary lung carcinomas.

**Figure 1.** The expression of the vitronectin in different tissues: A. Chronic inflammation; B. Thoracic wound; C. Lung adenocarcinoma; D. Non-small lung carcinoma actinoids. White expression of vitronectin. Arrow: Expression of ECM.

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**P765**

**The expression of central cell cycle regulators in non-small cell lung cancer has therapy-dependent prognostic impact**

Judith Cortis1, Arne Warth1, Thomas Muley2, Felix J.F. Herth3, Hendrik Dienemann4, Peter Schirmacher1, Philipp A. Schnabel1, Wilko Weischer1, 1Institute of Pathology, University Hospital, Heidelberg, Germany; 2Translational Research Unit, Thoracic Hospital Heidelberg at Heidelberg University, Heidelberg, Germany; 3Pneumology and Respiratory Critical Care Medicine, Thorasklinik Heidelberg at Heidelberg University, Heidelberg, Germany; 4Department of Thoracic Surgery, Thorasklinik Heidelberg at Heidelberg University, Heidelberg, Germany

**Aims:** Cell cycle regulation in cancer is tightly linked to proliferation, metastasis, and response to chemotherapy and thus an important factor for patients' prognosis. The relevance of function and expression of central cell cycle regulators in NSCLC has been discussed with controversial and non-conclusive results. In order to shed more light on the prognostic and predictive value of cell cycle regulation in NSCLC, we evaluated expression of key regulators and correlated the data with clinicopathologic characteristics.

**Methods:** Expression of CDK4, cyclin D1 and p16 were analysed immunohistochemically in 1082 completely resected NSCLC by means of tissue microarrays. The data were correlated with clini-pathologic data including patients' survival with respect to adjuvant therapy.

**Results:** In general, high expression of all cell cycle regulators was associated with better clinical outcome in a slightly variable way, depending on protein and prognostic parameters investigated. Interestingly, after stratification for therapy, the positive association of protein overexpression with survival was only seen in adenocarcinomas without adjuvant radio- and chemotherapy. In tumors with adjuvant irradiation and/or chemotherapy, the association was switched in the opposite direction.

**Conclusions:** The expression of CDK4, cyclin D1 and p16 in NSCLC has prognostic impact depending on adjuvant therapy. As an overexpression of all three proteins was associated with improved clinical outcome in completely resected pulmonary adenocarcinomas without adjuvant therapy, these markers could be used to predict the clinical outcome of patients without adjuvant radio- and chemotherapy.

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**Table 1. Tissue sample performance characteristics**

<table>
<thead>
<tr>
<th>Area of Tumour</th>
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<th>Bronchial biopsy (n=130)</th>
<th>EBUS-TBNA (n=81)</th>
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<tbody>
<tr>
<td>Mean (SD)</td>
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<tr>
<td>Total amount of DNA</td>
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<td>1390ng (130-7520)</td>
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**DNA control curve (Cp)**

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**EGFR mutation rate**

| 12.3% | 10.9% | 12.3% |

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**P766**

**Ki-67 in non-small cell lung cancer**

Arne Warth1, Judith Cortis1, Thomas Muley2, Felix J.F. Herth3, Hendrik Dienemann4, Peter Schirmacher1, Philipp A. Schnabel1, Wilko Weischer1, 1Institute of Pathology, University Hospital, Heidelberg, Germany; 2Translational Research Unit, Thoracic Hospital Heidelberg at Heidelberg University, Heidelberg, Germany; 3Pneumology and Respiratory Critical Care Medicine, Thorasklinik Heidelberg at Heidelberg University, Heidelberg, Germany; 4Department of Thoracic Surgery, Thorasklinik Heidelberg at Heidelberg University, Heidelberg, Germany

**Aims:** Ki-67 is an important ki-67 staining and correlated the data with clinicopathoeological characteristics including therapy response and survival.

**Conclusions:** The role of proliferation in non-small cell lung cancer (NSCLC) we investigated the proliferative activity of >1000 NSCLC by Ki-67 staining and correlated the data with clinicopathological characteristics including therapy response and survival.

**Introduction:** Approximately 80% of NSCLC present with advanced disease, in which the diagnosis is often based on small samples obtained during bronchoscopy.

**Aims:** We aimed to evaluate the performance of bronchoscopic small tissue samples: (1) the percentage of tumour cells and quantity of DNA extracted; (2) the performance of EGFR mutation testing; (3) their feasibility compared to surgical samples.

**Methods:** Between Sep 2010 and Dec 2012, we screened advanced stage non-squamous histology for EGFR mutations using Therascreen. All diagnostic bronchial biopsies (n=130; 4 biopsies per patient) and EBUS-TBNA samples (n=81; 4 needle aspirations brought in cell block), as well as surgical samples (n=67) were retrieved.

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**EGFR mutation rate**

| 12.3% | 10.9% | 12.3% |
Although 25% of bronchoscopic samples had <10% tumour cells, an amount of DNA extracted <200ng and poor DNA quality (Cp >35) were observed in 1%. Control samples resulted in low T790M mutation analysis in routine practice, provided a sufficient samples are taken.

P768 Frequency of T790M substitution in exon 20 of EGFR gene in brain metastases in chemotherapy-naive non-small cell lung cancer patients (NSCLC)

Radoslaw Miklak, Paweł Krawczyk, Bozena Jarosz, Michał Skroński, Kamila Wojas-Krawczyk, Tomasz Kucharczyk, Marek Sawicki, Barbara Kuzniak, Radosław Strumowski, Joanna Chorostowska-Wynimko 1,2, Tomasz Trojanowski, Janusz Milanowski 1,2, 1Department of Pneumonology and Oncology and Allergology, Medical University of Lublin, Poland, 2Department of Neurosurgery and Pediatric Neurosurgery, Medical University of Lublin, Poland

Reversible tyrosine kinase inhibitors (TKI) of EGFR (erlotinib, gefitinib) have shown limited efficacy in patients with activating mutations of EGFR gene. The resistance to reversible TKI and the sensitivity to irreversible pan-HER inhibitors (afatinib, ceritinib) are related to secondary mutations in EGFR gene, especially T790M mutation (50% of resistance). However, data concerning the frequency of primary T790M substitution in NSCLC metastases is scarce. Our study was aimed to develop a new method for T790M EGFR gene mutation analysis and to estimate the frequency of this mutation in chemotherapy-naive patients with NSCLC brain metastases.

DNA was isolated from 150 paraffin-embedded tissue samples using QIAamp DNA FFPE tissue kit. We used ASP-PCR (allele-specific PCR) technique and fluorochrome CY5 labelled primers, which are specific for mutated or wild-type EGFR gene exon 20. The analysis was performed on ALF Fragment Analyzer. Method proved sufficient and sensitive enough to estimate the presence of T790M mutation in all examined samples. 3 patients (2% with T790M substitution were detected, one suffering from non-differentiated NSCLC (non-smoker) and two with squamous cell carcinoma (10- and 20-pack-years smokers). Coexistence of T790M and L858R gene mutations was not detected. The primary T790M substitution of EGFR gene is detectable in chemotherapy- and TKI EGFR-naive patients. It is recommended to perform complete EGFR gene mutation analysis, including exon 20, before treatment.

P769 Diagnostic utility of PNA-LNA PCR clamp method for detection of EGFR exon 19 deletions and exon 21 codon L858R mutations in NSCLC samples with low tumor cells content

Michal Skroński, Zuzanna Langfort, Krystyna Maszkowska-Korpij, Paulina Jagus 1, Adam Szpechcinski 1, Tomoaki Tanaka 1, Tadeusz Orłowski 1, Koichi Hagiwara 3, Joanna Chorostowska-Wynimko 1,2, 1Department of Molecular Diagnostics and Immunology, National Institute of Tuberculosis and Lung Diseases, Warsaw, Poland; 2Department of Pathology, National Institute of Tuberculosis and Lung Diseases, Warsaw, Poland; 3Department of Respiratory Medicine, Saitama Medical University, Moroyama-Machi, Saitama, Japan, 1Department of Thoracic Surgery, National Institute of Tuberculosis and Lung Diseases, Warsaw, Poland

PNA-LNA PCR clamp method demonstrates potent accuracy and ability to detect mutant alleles even if present in low fraction of cells, while direct sequencing has limited sensitivity. Our aim was to compare PNA-LNA PCR clamp diagnostic utility for detection of most common EGFR mutations in clinical specimen with low tumor cells content.

DNA samples were isolated from fresh frozen NSCLC resected tissues (n=84), FFPE samples (n=51) or biopsy - FFPE (n=35) samples. Samples were analyzed by PNA-LNA PCR clamp method and/or direct sequencing. In all analyzed samples exon 19 deletions (n=8), exon 21 L858R (n=14), L858R (n=1) and L858R/L861Q double mutation (n=1) were detected. Distribution of total n=24 detected mutations among groups characterized by different tumor cells content was: In samples with >50% tumor cells - 14/24 mutations were detected by both PNA-LNA PCR clamp and direct sequencing, in samples containing 10-50% tumor cells - 5/24 mutations by PNA-LNA PCR clamp while only 2/24 by direct sequencing, in samples with less than 10% - 2/24 mutations were detected by PNA-LNA PCR clamp only. Further analysis proved that sensitivity of mutation detection depended only on tumor cells content, regardless sample type or tissue formalin fixation. Therefore proved usefulness of PNA-LNA PCR clamp method as compared to direct sequencing enables considerably more sensitive and reliable detection of EGFR exon 19 and 21 mutant alleles in resected, FFPE and cytology specimen with low cancer cell content.

P770 Evaluation of PNA-LNA PCR clamp method sensitivity for detection of key EGFR gene exon 19 and exon 21 mutations

Michał Skroński, Adam Szpechcinski 1, Paulina Jagus 1, Tomoaki Tanaka 2, Radosław Strumowski 3, Koichi Hagiwara 3, Joanna Chorostowska-Wynimko 1,2, 1Laboratory of Molecular Diagnostics and Immunology, National Institute of Tuberculosis and Lung Diseases, Warsaw, Poland; 2Department of Respiratory Medicine, Saitama Medical University, Moroyama-machi, Saitama, Japan

Highly-sensitive, robust and reliable diagnostic method is crucial for clinically useful detection of EGFR mutations in NSCLC. Due to low sensitivity, direct sequencing is restricted for samples containing at least 50% of tumor cells. PNA-LNA PCR clamp method represents allele-specific approach to gene analysis and demonstrates high accuracy and ability to detect mutant alleles present in low fraction of tumor cells.

Aim: To compare the in vitro sensitivity of PNA-LNA PCR clamp method versus direct Sanger sequencing in detecting EGFR exon 19 deletions and exon 21 L858R mutation.

Methods: Mutated DNA was isolated from cell lines harboring endogenous exon 19 deletion or L858R mutation. Sensitivity of direct Sanger sequencing and PNA-LNA PCR clamp method was analyzed in serial dilutions of mutant allele intermixed with wild-type DNA.

Results: PNA-LNA PCR clamp method reliably detected both exon 19 deletion and L858R down to 1% DNA admixture level. Direct sequencing presented considerably lower sensitivity detecting only down to 50% of mutated exon 19 allele in DNA mixture and down to 5% in samples with L858R mutant allele.

Conclusions: PNA-LNA PCR clamp method is the highly sensitive tool for detection of EGFR activating mutations. It might be particularly useful in heterogeneous samples with low content of mutant allele, like biopsy material. Direct sequencing presents lower sensitivity, limited by type of mutation.

P771 HOPE-BAL: A novel tool to expand the methodological capabilities in pulmonary research

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Bronchoalveolar lavage cells are essential biomaterials for both, basic research and clinical applications. Easy to acquire with comparatively little risks for the patients, biomaterials even from non-malignant diseases like COPD or Asthma can be used to aid diagnostics or deliver samples from the alveolar space for basic research. However, these samples are transient materials which require timely read-out if used freshly. In case of formalin-fixed, paraffin-embedded cell blocks they are left to last but with the drawback of protein cross-linking and the severe side effects like degradation of nucleic acids. Since the rapid developments in molecular based techniques, archived samples increasingly need to meet more and more requirements for modern read-outs. Here we present a novel approach to BAL cells with all the possibilities of the omics-techniques.Human BAL cells were fixed in formalin and paraffin-embedded to create cell blocks that can be stored and convenient to handle. For routine diagnostic applications, standard marker molecules were targeted by means of immuno cytchemistry without antigen retrieval. Furthermore we show that HOPE-fixed, paraffin-embedded BAL cells can be used for proteome analysis by application of 2D gel electrophoresis. In addition these BAL Blocks contain enough RNA of sufficient quality for transcriptome analysis on Agilent Whole Genome arrays. Differential regulated genes show distinct expression patterns between healthy donors and patients with Asthma or COPD. In summary, HOPE-BAL is a novel powerful tool for both diagnostics and translational research. Additionally biobanks of HOPE-BAL will ensure proper accessibility for unrestrained investigations.

P772 The predictive model for perinatal asphyxia risk evaluation in the neonates Natalia Gorovenko 1, Svetlana Kyrtyachenko 2, Zoya Rosokhla 3, 1Medical and Laboratory Genetics, National Medical for Postgraduate Education named after P.L. Shupyk, Kiev, Ukraine; 2Laboratory of Molecular Genetics, Reference Centre for Molecular Diagnostics of Ministry of Public Health, Kiev, Ukraine

Background: Perinatal asphyxia (PA) is often associated with nonreversible adverse neurological outcome. Modern tests for PA diagnostic have low predictive values.

The aim of this study was to define the genetic-based predictive model for early preventive diagnostic of PA.

Methods: We performed a case-control study of 201 neonates PA cases and 110 from control group. The ID, A1166C, G308A, C677T polymorphism of ACE, AT2R1, TNF-a, MTHR genes were detected using PCR and RFLP analysis. Statistical analysis was performed to assess the effects of all analyzed genes and their combinations (logistic regression (SPSS 17.0) and MDR model (MDR 2.0)).

Results: The frequency of all investigated genotypes with mutant allele was significantly higher among PA cases.
Asbestos and silica exposures reveal similar and divergent gene expression patterns and pathways related to fibrosis in human bronchial epithelial cells

**Background:** Asbestos and silica, two common forms of pneumoconioses are caused by excessive exposure to airborne minerals, leading to pulmonary fibrosis. However, both diseases have distinct pathological presentations, which are likely due to the physiopathological characteristics of the two minerals. The epithelium is an initial target cell to come in contact with inhaled materials. The early molecular events that therefore take place in epithelial cells have a strong influence on the molecular and cellular responses that promote inflammation, lesion formation, and fibrosis.

**Methods:** Primary human bronchial epithelial cells were exposed to non-lethal doses of crocidolite asbestos and cristobalite silica for 24 hours. Affymetrix/GenoSifter analysis utilizing pairwise-comparison, hierarchical clustering, and Ingenuity pathway analysis was used to reveal similar and unique gene expression patterns.

**Results:** Both minerals altered a number of genes related to inflammation, cell proliferation, apoptosis, cell-surface receptors, and transcription factors. Genes selectively altered by asbestos were related to evasion of apoptosis, MAPK signaling, and iron metabolism. Conversely, genes altered by silica were involved in TNF-signaling, TLR-signaling, and mitochondrial biogenesis. Similar and distinct pathways associated with inflammation and fibrosis were affected by both mineral exposures.

**Conclusions:** These studies exhibit initial changes in gene expression by asbestos and silica in target bronchial epithelial cells as early molecular events that may initiate the inflammatory and fibrotic responses leading to asbestosis and silicosis.

**P775**

The localization and activity of the leptins and adiponectins receptors in the thigh tissue in COPD-patients

**Objective:** To determine the localization and activity of receptors for leptin and adiponectin in the tissues of the thigh in patients with COPD.

The study included 90 patients with COPD (I stage, n = 30; II stage, n = 30; III stage, n = 30; control group n = 10). The samples of thigh tissue are obtained with fine-needle biopsy of the automatic MAGNUM system. The localization and activity of leptin and adiponectin receptors are studied using the immunofluorescence method and the quantification of the signals in the three fields of view, according to the protocol DakoCytonam. Positive expression of tags is observed in the cytoplasm and cell membrane.

The positive expression of the signals is obtained in the control group in the skin, connective tissue and muscle. The disappearance of signals to the adipokins in muscle tissue is observed with increasing of the stage of COPD.

**Conclusion:** The activity of the leptins and adiponectins receptors in muscle tissue disappears with the increasing of the COPD stage. Quantification of activity depends on the severity of the disease, the number of receptors to leptin decreases and the number of receptors to adiponectin increases.

**P776**

Polymorphism of GSTP1 and EP1X1 genes in smokers and patients with I and II stages of chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is known as disease associated with smoking. Studying the processes of genetic control of detoxication in connective tissue seems to be important.

Polymorphic variants of genes encoding glutathione transferase GSTP1 and microsomal epoxidase EPHX1 have been studied. 80 patients have been examined. They were divided into two groups: 1 group - smoking persons without COPD; 2 group - smoking patients with I or II stages of COPD (2 subgroups). The control group consisted of healthy non-smoking persons. Analysis of polymorphic loci of genes GSTP1, EPHX1 was performed by polymerase chain reaction.

The high frequency of heterozygous polymorphisms of GSTP1 and EPHX1 was established in patients with COPD. It was estimated that relative risk of COPD increased in the presence of heterozygous variant 105IV of GSTP1 (OR=2.4, 95% CI: 0.93-6.19, P<0.05).

Relative risk of COPD also increased in the presence of heterozygous variant 114A/V of GSTP1 (OR=1.9, 95% CI: 0.83-4.4, P<0.05).

Multiple genetic markers, such as GSTP1 and EP1X1, can be used in predictive measures and diagnosis of increased risk of developing chronic obstructive pulmonary disease.
P777
Is a functional variant of ANXA11 R230C associated with impaired apoptosis? Pilot data
Regina Fillérová1, František Mražek2, Monika Zirková1, Vitezslav Kolek2, Martin Petrek1, Eva Krigeová1, 1Laboratory of Immunogenomics and Immunoproteomics, Palacký University, Olomouc, Czech Republic; 2Department of Respiratory Medicine, Palacký University, Olomouc, Czech Republic

Sarcoidosis is a granulomatous disorder of an unknown etiology, where the granuloma formation has been associated with impaired apoptosis of activated inflammatory cells. There is no information concerning the influence of ANXA11 R230C, a functional annexin A11 variant associated with protection/disease modification of sarcoidosis, on the sensitivity of peripheral blood mononuclear cells (PBMC) to apoptosis yet.

We therefore compared the sensitivity to apoptosis of PBMC obtained from 30 sarcoid patients and 9 healthy controls. Tributyltin was used as apoptosis stimulus; annexin V positive cells were detected by flow cytometry. Pilot analysis was performed in subgroups according to ANXA11 R230C (rs1049550) genotype (TT, n=7; CC, n=7).

When compared to healthy controls, lower number of annexin V positive cells was detected in tributyltin-stimulated PBMC from sarcoid patients (mean 55.9%; range 23.3–77.2%) than in cells from controls (64.8%; 54.7–74.1%; p=0.01). After subdivision according to genotypes, the number of annexin V positive cells did not differ between patients carrying TT genotype (50.8%; 23.3–58.8%) and those with CC genotype (54.5%; 41.7–71.7%; p>0.05). Genotyping and investigations on association between apoptosis and genotypes in larger patient/control groups are under progress.

In conclusion, PBMC obtained from sarcoidosis patients showed more apoptosis resistant phenotype than cells from control subjects. In our pilot cohort, wild type ANXA11 genotype and its R230C variant did not differ in terms of sensitivity to apoptosis. Further studies in a larger patient cohort are ongoing.

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P778
Proinflammatory gene polymorphism and fatigue in sarcoidosis
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Introduction: Fatigue is one of the most prominent symptoms in sarcoidosis patients. Cause of fatigue in sarcoidosis is still unknown. No relationship was found between fatigue and disease activity marker in sarcoidosis. Fatigue can persist many years after disease remission. Proinflammatory cytokines, TNF-α and LT-α, play important role in sarcoidosis pathogenesis. Elevated values of TNF-α and LT-α, are detected in patients with chronic fatigue syndrome of unknown etiology.

Aim of our study was to investigate possible association between TNF-α and LT-α gene polymorphism and fatigue in sarcoidosis patients.

Methods: One hundred and twenty eight patients with sarcoidosis were included in study. Fatigue was measured on the Fatigue Assessment Scale (FAS). Genetic tests were performed using the patients’ peripheral blood. Instances of polymorphism were examined by PCR-RFLP (polymorphism detection based on the restriction fragment length) on the DNA isolated from the blood by the commercial kit (Fermentas, Lithuania). Fatigue was present in 71 patients.

Results: Our results showed significantly higher frequency of rare LT-α+252 G allele (29.58% vs 19.30%, p<0.05), as well as homozygous GG (9.85% vs 8.77%, p<0.05) and heterozygous GA genotype (30.44% vs 21.05%) in fatigue group (p<0.05). Rare, mutated AA genotype of TNF-α-308 gene was present only in fatigue group, and was absent in non fatigue group. We found significant difference (p=0.05) in allele frequency between these two group of patients.

Conclusion: Based on these results, we concluded that proinflammatory gene polymorphism can predict the occurrence of fatigue in sarcoidosis patients.

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The role of transcription factor PAX-5 (BSAP) in asthma severity and in the activity of allergic inflammation
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PAX-5 is transcription factor of B-cell, which is crucial in asthma pathogenesis. The aim is to evaluate PAX-5 role in allergic (AA) and non-allergic asthma (NAA).

Materials and methods: Peripheral blood lymphocytes from 107 asthma patients and 22 healthy were examined. Part of lymphocytes was analyzed after 24h incubation with and without IL-4 10 ng/ml. To estimate PAX-5 and CHε mRNAs levels, semi-quantitative RT-PCR was performed.

Results: PAX-5 mRNA levels were significantly increased in patients with NAA in comparison with healthy (p<0.018) and AA patients (p<0.03) (U-crit.). We revealed important positive correlations of PAX-5 with CHε which were stronger in patients with normal and low serum IgE (<150 MU/ml) than in patients with high serum IgE (>150 MU/ml) (r=0.543; p<0.001; n=53 and r=0.474; p=0.017; n=25 resp.). We revealed significant negative correlations of PAX-5 with spumineosinophils and eosinophils amount in patients with AA (r=0.390; p=0.040; n=28 and r=0.385; p=0.043; n=28 resp.).

Significant decrease of mRNA PAX-5 was registered in lymphocytes from AA patients after 24h IL-4 action (p=0.046; n=13, W-crit.). This decrease was not significant in healthy (p=0.345; n=5, W-crit.).

Conclusion: We revealed the association of PAX-5 and NAA development. It may be explained by fact of PAX-5 prolonging B-cell life and its functioning as antigen-presenting cell. In patients with AA PAX-5 may be considered as protective factor. IL-4 may influence PAX-5 expression through the pathways which are involved in asthma pathogenesis (e.g. STAT6 signaling).

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