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

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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 (AUC=0.90), while 56% specificity and 90% sensitivity in distinguishing NSCLC from any non-NSCLC subjects (AUC=0.80; $p<0.0001$). The plasma DII was significantly 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

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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 (Elecsys) 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

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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, colo-rectal carcinoma, renal cell carcinoma 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.

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WITHDRAWN

WITHDRAWN

P764

Vitronectin expression in primary lung cancer

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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 histo-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 0.289mm^2 (± 0.032), 0.043mm^2 (±0.009), 0.084mm^2 (±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.

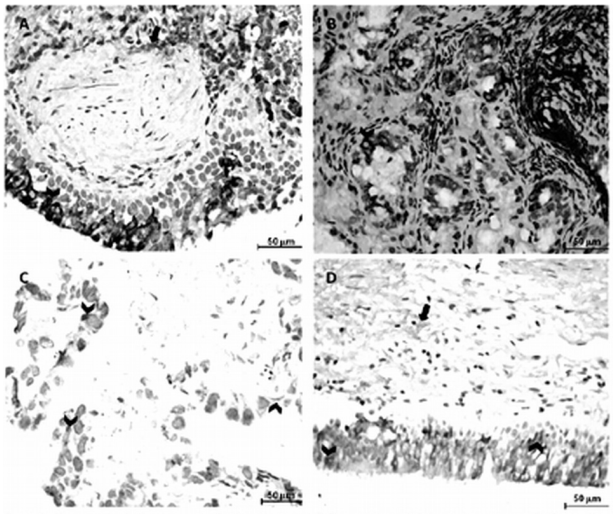

Figure 1. Vn expression in the airways. A. Chronic inflammation. B. Traumatic wound. C. Lung adenocarcinoma. D. Non-small lung carcinoma. Arrowheads: Vn expression in epithelial cells. Arrow: Vn expression in ECM.

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

P765

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

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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 clinico-pathologic characteristics.
Methods: Expression of CDK4, cyclin D1 and p16 were analysed immunhistochemically in 1082 completely resected NSCLC by means of tissue microarrays. The data were correlated with clinico-pathologic data including patients' surival 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.

P766

Ki-67 in non-small cell lung cancer

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Uncontrolled proliferative activity is one of the determinants of malignant growth and known to be a prognostic factor in a variety of human tumors. Many works have been published on this topic in pulmonary neoplasms but most studies are underpowered or have no clinical data available.
To ultimately clarify 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.
The mean proliferative fraction in NSCLC was 40.7%. Adenocarcinomas (ADC) proliferated significantly less than all other types of NSCLC but proliferation was tightly linked to specific growth patterns. Overall, proliferative activity was not associated with overall, disease free and disease specific survival. However, when patients were stratified according to adjuvant therapy, those patients with high intratumoral proliferative activity and without adjuvant chemotherapy or radiation had significantly diminished survival times when compared to patients whose tumors were proliferating slowly. These associations were reverted in the group of patients who received radio-/chemotherapy.
Our study comprehensively clarifies the impact of proliferative activity on outcome in NSCLC patients. Our data suggest that Ki-67 stains can be used as an adjunct in the selection of patients for adjuvant therapy.

P767

Feasibility of EGFR mutation testing on EBUS-TBNA and bronchial biopsy samples obtained during routine practice

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Introduction: Approximately 80% of NSCLC present with advanced disease, in whom 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.

Table 1. Tissue sample performance characteristics

	Surgical biopsy (n=67)	Bronchial biopsy (n=130)	EBUS-TBNA (n=81)
Area of Tumour			
Mean (SD)	66% (±31%)	32% (±22%)	36% (±31%)
Median (range)	80% (5-100)	28% (5-90)	30% (2-100)
Total amount of DNA			
Median (range)	2330ng (560-6240)	1700ng (250-7880)	1390ng (130-7520)
DNA control curve (Cp)			
Median Cp	29.4	29.1	29.0
Cp <32	96%	96%	88%
Cp >35	3%	1.5%	1%
EGFR mutation rate	12.3%	10.9%	12.3%

Results: The median percentage tumour cells and quantity of DNA extracted was significantly higher in surgical vs bronchoscopic samples (80% *vs* 30% and 2.3µg *vs* 1.6µg, $P<0.0001$); no statistically significant difference was observed between EBUS-TBNA and bronchial biopsies.
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%.
Conclusions: Bronchoscopic samples result in accurate EGFR 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)
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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, neratinib) 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-naïve patients with NSCLC brain metastases.
DNA was isolated from 150 paraffin-embedded tissue samples using Qiamp DNA FFPE tissue kit. We used ASP-PCR (allele-specific PCR) technique and fluorescence 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 activated EGFR gene mutations was not detected.
The primary T790M substitution of EGFR gene is detectable in chemotherapy- and TKI EGFR-naïve 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 L858 mutations in NSCLC samples with low tumor cells content
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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 characterized by different tumor cell content.
DNA samples were isolated from fresh frozen NSCLC resected tissues - (n=84), FFPE samples (n=51) or biopsy - FFPE (n=53) and cytology (n=23) materials. 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), L858P (n=1) and L858M+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 materials 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.
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
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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 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 heterogenous samples with low content of mutant allelle, 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 comparably 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 built 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 HOPE-fixed and paraffin-embedded to create cell blocks that are easy to store and convenient to handle. For routine diagnostic applications, standard marker molecules were targeted by means of immuno cytochemistry 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
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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 genetical 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 I/D, A1166C, G308A, C677T polymorphism of ACE, AT2R1, TNF-a, MTHFR 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 alelle was significantly higher among PA cases.

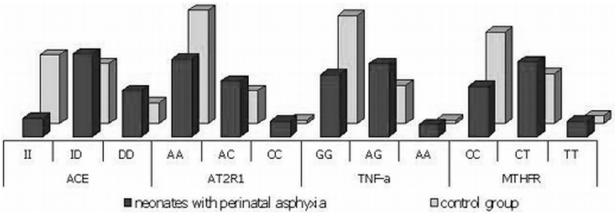

Figure 1. Distribution of investigated gene polymorphic variants.

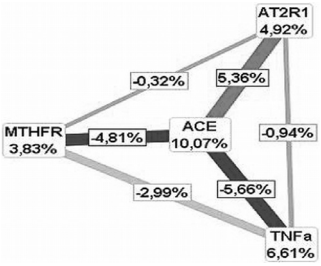

Figure 2. Interaction dendrogram for the PA dataset.

The statistical model including all investigated genes had the higher predictive value (Percentage Correct=76,5). We have found positive entropy which determined synergy interaction between ACE and AT2R1 genes.
Conclusion: We suggest that using predictive model baised on I/D, A1166C, G308A, C677T polymorphism of ACE, AT2R1, TNF-a, MTHFR genes and their combination may increase early diagnostic of PA.

P773
Asbestos and silica exposures reveal similar and divergent gene expression patterns and pathways related to fibrosis in human bronchial epithelial cells
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Background: Asbestosis and silicosis, 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 physicochemical 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/GeneSifter® analysis utilizing pairwise-comparison, hierarchical cluster analysis, 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.

P774
The effect of cigarette smoking on BAL protein profile
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Background: BAL proteomic analysis gives a panorama of the complex network of proteins of different origin and function and their modifications at alveolar level, simultaneously providing new information about events in the alveolar microenvironment and insights into lung physiology and pathology. In this study we applied the proteomic approach to the study of BAL in order to evaluate the effect of smoking exposure to BAL protein composition.
Aim: Aim of this study was to investigate qualitative and quantitative differences in BAL protein profiles from cigarette smoker and no-smoker healthy subjects.
Methods: BAL samples were obtained by 10 healthy never-smoker and 8 asymptomatic smoker subjects. After centrifugation, dialysis and denaturation of samples, BAL samples were analyzed by two-dimensional electrophoresis and proteins differentially expressed were identified by mass spectrometry.
Results: 20 BAL proteins were differently expressed: 15 were up-regulated in smokers and 5 proteins were up-regulated in never-smokers. Among these proteins some were involved in immune-regulation, host defense (i.e.Pulmonary Surfactant-associated protein A2), apoptosis, inflammatory responses (i.e. α-1-antichymotrypsin) and oxidant/antioxidant balance (i.e. Glutathione S transferase P and Annexin A5).
Conclusion: In healthy subjects smoking exposure modifies the expression of several BAL proteins implicated in the regulation of crucial biological activities (such as oxidant/antioxidant balance, inflammation and tissue matrix turnover) potentially involved in the pathogenesis of several smoke-induced lung diseases.

P775
The localization and activity of the leptins and adiponectins receptors in the thigh tissue in COPD-patients
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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 leptins and adiponectins receptors are studied using the immunofluorescence method and the quantification of the signals in the three fields of view, according to the protocol DakoCytomation. 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. Receptor cell activity in leptin and adiponectin in COPD-patients varies and depends on the stage of the disease. The most significant decrease in the number of signals to leptin occurs in stage III disease as compared with the control group. In contrast, in the same group the quantity of signals for adiponectin increases and reaches a maximum level as compared with the control group.
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 EPHX1 genes in smokers and patients with I and II stages of chronic obstructive pulmonary disease
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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 glutathiontransferase GSTP1 and microsomal epoxidhydrolase 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 105I/V 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 in smokers (OR=1.91, 95% CI: 0.83-4.4, P<0.05).
It was revealed that relative risk of COPD increased in the presence of heterozygous variant 113T/H of EPHX1 (OR 1.6, 95% CI: 0.63-4.4, P<0.05).
Molecular genetic markers, such as GSTP1 and EPHX1 can be used in predictive measures and diagnosis of increased risk of developing chronic obstructive pulmonary disease.

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Is a functional variant of *ANXA11* R230C associated with impaired apoptosis? Pilot data

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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.Grant support: IGA MZ CR NS/11117, IGA_PU_LF_2012_07, CZ.1.05/2.1.00/01.0030.

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Proinflamatory 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 LTα+252 G allele (29,58% vs. 19,30%, p<0,05), as well as homozygous GG (9,85% vs. 8,77%) and heterozygous GA genotype (39,44% vs. 21,05%) in fatigue group (p<0,05). Rare, mutated AA genotype of TNFα-308 gene was present only in fatigue group, and was absent in non fatigue group,and we found week 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 (constant parts of IgE heavy chains) mRNA semi-quantitive 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 levels (>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 sputum leucocytes and eosinophiles amount in patients with AA (ρ=-0,390; p=0,040; n=28 and ρ=-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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