418. New biomarkers for lung cancer

P4204

Volatile organic compounds (VOC) in exhaled breath in patients with lung cancer, using the analytical technique thermal desorber- gase chromatography-spectrometer mases

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Aim: Oxidative stress is increased in lung cancer (LC) and generated volatile organic compounds (VOC). We can detect VOC in exhaled breath using the analytical technique TD/GC/MS. The determination of VOC, may be useful as a noninvasive screening in LC.
Objetive: To determine differences in VOC present in the exhaled breath in 3 groups: LC group, COPD group and clinically healthy volunteers.

Table 1.- Characteristics of study subjects

	Control(n=80)	COPD(n=40)	LC(n=81)
Age (year)*	49.3 (9.5)	74.3(10.0)	66.5(11.1)
Male/female	(42/47)	(37/3)	(64/17)
Smoker/ex-smoker/non smoker	20/24/36	34/3/3	50/22/9
Tabacco (packs-year)*			
Smokers	30.9(18.4)	123(29.5)	77.5(50.6)
Ex Smokers	36.5(21.4)	72.5(34.3)	63.6(34.1)
Histology (%)			
Squamous Cells			27.2%
Adenocarcinoma			39.5%
Undifferentiated			18.5%
Large Cells			2.5%
Carcinoid			1.2%
Small cells			11.1%
TNM (IA/IB/IIA/IIB/IIIA/IIIB/IV)			8.6%/6.2%/7.4%/2.5%/ 28.4%/14.8%/22.1%
GOLD (I/2/3/4) (%)		10%/32.5%/25 %/ 32.5%	13.6%/25.9%/13.6%/37.3% 29.6% without COPD

* Years (SD)

Table 2.- Frecuency. Comparation of biomarkers between different study groups. p* values

	LC vs. Control	LC vs. COPD	LC with COPD vs. COPD
Hexanal	0.276	0.698	0.668
Heptanal	0.308	0.851	**0.051**
Octanal	0.769	0.759	0.710
Nonanal	0.454	0.645	0.807
Propanoic Acid	0.534	0.533	0.802
Nonanoic Acid	**0.011**	**0.001**	**0.001**

*Wilcoxon-Mann-Whitney

Table 3: Relationship between VOC in LC vs control group and COPD group.

	LC Group vs. Control Group			LC Group vs. COPD Group		
	Control	LC	p*	COPD	LC	p*
Hexanal	1.00	1.03 (0.46-2.68)	0.941	1.00	0.43(0.18-1.04)	0.062
Heptanal	1.00	1.42(0.79-2.29)	0.274	1.00	0.88(0.41-1.91)	0.752
Octanal	1.00	0.92(0.39-2.19)	0.848	1.00	1.10(0.36-3.41)	0.869
Nonanal	1.00	1.57(0.85-2.92)	0.152	1.00	1.08(0.0-2.32)	0.839
Propanoic Acid	1.00	1.24(0.68-2.28)	0.484	1.00	1.08(0.50-2.32)	0.853
Nonanoic Acid	1.00	2.53(1.21-5.29)	**0.014**	1.00	8.99(2.01-40.1)	**0.004**

*Odds ratio and 95% confidence interval based in logistic regression.

Methods: Case-control study with 81 patients with LC, 40 patients with COPD and 89 healthy volunteers (without respiratory disease). Informed consent accepted. Collection of exhaled breath by means BioVOC™ to functional residual capacity
Analytical technique: TD/GC/MS (Markes-Agilent Tech.)
Statistical analysis: SPSS® v-15 for Windows.
Results: Description of the sample. Quantitative study and qualitative study of VOC.
Conclusions: 1. Nonanoic acid is the only VOC with statistical significance between study groups: and it is independent of age and smoke custom.
2. The probability to find nonanoic acid in LC group is higher than control and COPD groups
3. Nonanoic acid and heptanal could be useful to discriminate between LC + COPD patients versus LC without COPD patients.
4. In our sample, nonanoic acid could be useful like a LC tumorlike marker.
Supported by FIS: PI07/1116; Neumomadrid 2008 and SEPAR 2010: PI-881.

P4205

Retrospective study of treatment outcomes according to exon difference with EGFR mutations in non-small cell lung cancer patient in Korea

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Background/Aims: EGFR mutations in NSCLCs are most important biomarker for EGFR-TKI treatment. About 90% of EGFR mutations are clustered in exons 19 (deletion) and 21 (point mutation at codon 858) and patients with these mutations have great response to EGFR-TKIs. However, the response rates of NSCLC to EGFR-TKIs according to types of EGFR mutation in Korea are still not cleared. This study aimed to evaluate the genomic types of EGFR mutation and compare the influence of each genomic types on the response to EGFR-TKIs and clinical outcomes in patients with NSCLC.
Methods: We reviewed medical records from January 2007 to August 2011, and classified genotypes of EGFR mutations which were done by direct sequencing methods. Mutation status was compared with clinicopathological features. Clinical outcomes were assessed based on EGFR genotypes.
Results: EGFR gene mutations were identified in 43(20.2%) out of 211 NSCLC patients. EGFR mutations were significantly more frequent in females than in males (37.1% vs. 13.4%, $p < 0.001$), but not correlated with smoking status (22.% vs. 18.1%, $p = 0.311$). There are no significant differences between exon 19 deletion and exon 21 point mutation at cordon 858 in progression-free survival (10.9 vs. 9.1 months; $p = 0.554$), nor in overall survival (21.8 vs. 18.2 months; $p = 0.142$) and disease control rate (90% vs 85.7%; $p = 0.669$) with EGFR-TKI treatment.
Conclusion: PFS and overall survival were not significantly different between exon 19 deletions and Exon 21 L858R mutations, these results are similar to those of previous studies.

P4206

Clinical significance and functional roles of FoxM1 in non-small cell lung cancer

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Purpose: To test the role of FoxM1 in the metastasis of non-small cell lung cancer.
Experimental design: FoxM1 expression was examined in 175 NSCLC patients with or without nodal metastasis by immunohistochemistry. By lenti-virus transduction, the role of FoxM1 in metastasis was also tested by in vitro methods using different NSCLC cell lines.
Results: In this study, the FoxM1 expression was found to be significantly associated with nodal metastasis and the cumulative 5-year survival rate. Thus, an increased expression of FoxM1 is the indicator of shortened survival and high risk of metastasis of NSCLC patients. Furthermore, we also found that increased FoxM1 expression could enhance the migratory and invasive abilities of lung cancer cells, while inhibition of FoxM1 expression could reduce the migratory an invasive properties of lung cancer cells, which are the two important parameters of metastasis biology. In addition, cells with high FoxM1 expression were presented with phenotypic changes reminiscent of EMT, which was further proved by the results of immunoblotting with down-regulation of E-cadherin and ZO-1 while up-regulation of N-cadherin and Vimentin.
Conclusion: These results suggested that FoxM1 plays an important role in lung cancer metastasis and elevated FoxM1 expression could be used as an indicator of poor prognosis and high risk of metastasis of NSCLC patients.

P4207

Alterations of serum inflammatory biomarkers in the healthy and lung cancer patients before and post chemotherapy

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Introduction: Only 20% of lung cancer patients could be early diagnosed with surgical treatment. Due to the high cost, it is not practical to apply CT scan and pathological biopsy for lung cancer as regular screening tools even in high-risk population. A simple but sensitive and specific assay used for lung cancer diagnosis and prognosis is warranted.
Methods: Serum samples from 55 subjects including healthy people and patients with NSCLC (30 with adenocarcinoma and 13 with squamous cell carcinoma) were collected to measure 40 inflammatory mediators by multiplexed cytokine immunoassays. All patients have completed follow up for up to two years. A series of systematical computational analysis was applied.
Results: The set of 17 cytokines (such as IL-9, CCL16, CXCL10, etc.) prefers to identify adenocarcinoma samples from pool of the population, while the set of 2 cytokines (MSPa and IL-29) prefers to recognize squamous cell carcinoma samples. The decision trees based on these two kinds of biomarkers can both achieve about 80% accuracy in leave-one-out cross-validation. Cytokines like CXCL5, CXCL10 and CCL16 were also found to play important roles in cancer survival. The co-expressed protein interaction network (CEPIN) of cytokines related to adenocarcinoma show dynamic convergent behavior during chemotherapy.
Conclusions: This pattern of inflammatory mediators might be useful for cancer diagnosis, prognosis and evaluation of chemotherapy effects. The results of clustering of five CEPINs supported the adopted chemotherapy is effective for NSCLC patients.
This study was supported by National Basic Research Program of China (973 Program) No. 2012CB933300.

P4208

Anti-tumorigenic effect of age-/diabetes-related advanced glycation end-products in lung carcinoma

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Background: Clinicopathological studies indicated that lung carcinoma progression is impaired by advanced age and diabetes, which are either characterized by accumulation of advanced glycation end-products (AGEs). AGEs result from the non-enzymatic reaction of sugars with proteins in the body and in foods. Therefore, our study aimed at the effect of AGEs on the non-small cell lung carcinoma (NSCLC) progression.
Methods: AGEs were quantified by detecting the AGE fluorescence in plasma samples of NSCLC patients prior to surgery. Experimentally, the tumor effect of circulating AGEs was studied by using NSCLC spheroids and plasma samples increasingly modified with AGEs, and NSCLC-bearing mice of whom elevated AGE level were induced by AGE-enriched food.
Results: High plasma AGE levels were characterized by a later reoccurrence of the tumor after curative surgery and a higher long-term survival rate compared to patients with low levels (25% vs. 47% 5-year-survival, $P = 0.011$). In this regard, *in vitro* studies showed a lower spheroid growth of NSCLC cells in the presence of AGE-modified plasma than non-modified plasma. By *in vitro* application of plasma samples from NSCLC patients or mice with different AGE levels, we also found an inverse correlation between the NSCLC spheroid growth and the plasma AGE level. Moreover, the *in vivo* tumorigenicity assay demonstrated that mice with higher levels of circulating AGEs developed smaller tumors than mice with normal AGE levels.
Conclusion: The plasma AGE level has prognostic relevance for NSCLC patients, in which the tumor growth-inhibiting effect of circulating AGEs might play a critical role.

P4209

Increased levels of circulating interleukin 6, interleukin 8, C-reactive protein, and risk of lung cancer

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Introduction: Previous studies that were based primarily on small numbers of patients suggested that certain circulating proinflammatory cytokines may be associated with lung cancer; however, large independent studies are lacking.
Methods: Associations between serum interleukin 6 (IL-6) and interleukin 8 (IL-8) levels and lung cancer were analyzed among 123 case patients. Associations between biomarkers and lung cancer were estimated using logistic regression models adjusted for smoking, stage, histology, age, and sex. The 10-year standardized absolute risks of lung cancer were estimated using a weighted Cox regression model.
Results: Serum IL-6 and IL-8 levels in the highest quartile were associated with lung cancer (IL-6, odds ratio [OR] = 2.89, 95% confidence interval [CI] = 1.28 to 6.23; IL-8, OR = 2.46, 95% CI = 1.02 to 4.12) and with lung cancer risk (IL-6, OR = 1.93, 95% CI = 0.87 to 2.36; IL-8, OR = 1.62, 95% CI = 1.56 to 2.48), compared with the lowest quartile. Increased IL-6 levels were only associated with lung cancer diagnosed within 2 years of blood collection, whereas increased IL-8 levels were associated with lung cancer diagnosed more than 2 years after blood collection (OR = 2.03, 95% CI = 1.05 to 2.73). The 10-year standardized absolute risks of lung cancer were highest among current smokers with high IL-8 and CRP levels (absolute risk = 7.46%, 95% CI = 4.52% to 10.25%).
Conclusions: Although increased levels of both serum IL-6 and IL-8 are associated with lung cancer, only IL-8 levels are associated with lung cancer risk several years before diagnosis.

P4210

Clinical significance of serum osteopontin levels in lung cancer

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Background: Osteopontin (OPN) is a multifunctional glycoprotein associated with lung cancer (LC) via several pathways including tumour angiogenesis.
Aims and objectives: The aim of our study was to investigate possible associations between serum levels of OPN in patients with LC and clinicopathological variables, VEGF and MMP-9 levels and overall survival.
Methods: We enrolled 51 patients (mean age 66±8.8 years) with primary LC and 30 healthy control subjects. 43 patients were ever smokers and 8 non-smokers, 12 patients had SCLC and 39 NSCLC (18 squamous, 16 adenocarcinoma and 5 NSCLC-NOS) with stage I-II/4, III/18, IV/29. Serum levels of OPN, VEGF and MMP-9 were measured by ELISA.
Results: Patients with LC had statistically significantly higher serum OPN levels than controls (45.9[10.5-266.8] vs 16[6.8-29.8] ng/ml, $p<0.0001$). ROC analysis showed that for OPN levels >23.8 ng/ml, sensitivity for detection of LC was 80.4% and specificity was 86.7%. OPN levels were also found higher in smokers ($p=0.019$) and in older patients ($p=0.026$). Moreover, patients with squamous LC had statistically significantly higher OPN levels compared to patients with adenocarcinoma. Additionally, patients with serum OPN levels lower than median value (<45.9 ng/ml) had significantly better overall survival than those with higher levels (524 days vs. 306 days, $p=0.01$) and a 1-year survival rate of 80% vs. 37%. Finally, OPN levels were positively associated with VEGF levels ($r=0.44$, $p=0.001$).
Conclusions: OPN levels were increased in patients with LC, and higher levels were correlated with worse survival, therefore suggesting a possible diagnostic and prognostic value of OPN in patients with LC.

P4211

Investigation of survivin gene polymorphism in non-small cell lung cancer patients (NSCLC)

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Introduction and aim: Survivin gene is one of the first reported inhibitors of apoptosis proteins (IAPs), which is an important family of proteins that regulate apoptosis. A common polymorphism at the survivin gene promoter (-31 G/C) has been shown to influence survivin expression and the risk for cancer development. Purpose of this study reports, relation between Turkish population who have survivin polymorphism and NSCLC also; its relevant with diseases's development and prognosis.
Methods: 146 NSCLC cases and 98 healthy control cases who were diagnosed at Yedikule Chest Diseases and Chest Surgery, Training and Research Hospital third clinic were included in this study. Pulmonary function test and routine biochemical analysis were done for all voluntaries. PCR-RFLP technique was used for genotyping.
Result: Genotype distrubition of Survivin gene's -31G/C region were detected (n=146) %77.4 GG (n=113), %.18.5 GC (n=27), %4.1 CC (n=6); at patient group and (n=98) % 6.1 GG (n=56), %47.5 GC (n=34), % 46.4 CC (n=8) (*p=0,003), at control group; -644T/C region were detected (n=146) %40.4 TT (n=59), %.48.6 TC (n=71), %11.0 CC (n=16); at patient group and (n=98) % 55.1 TT (n=54), %40.8 TC (n=40), % 4.1 CC (n=4) (*p=0,031), at control group; -625G/C region were detected (n=146) %49.3 GG (n=72), %.39.1 GC (n=57), %11.6 CC (n=17); at patient group and (n=98) % 57.1 GG (n=56), %32.7 GC (n=32), % 10.2 CC (n=10) (p=0,484) at control group.
Conclusion: These results show that Survivin gene -31 G/C polymorphism causes predisposition to lung cancer development in Turkish population.

P4212

Low levels of heme oxygenase-1 in induced sputum of lung cancer patients as a marker of defective cytoprotection

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Background: Lung cancer is a leading cause of morbidity and mortality worldwide, resulting in substantial economic and social burdens that are constantly increasing.
There is a strong correlation between inflammation and oxidative stress and malignant transformation. Heme oxygenase-1 (HO-1) is a cytoprotective enzyme that plays a central role in the defense against oxidative stress. HO-1 has anti-inflammatory, anti-proliferative and anti-apoptotic properties and is involved in the regulation of immunological balance in a wide range of lung diseases, including lung cancer.
Aims: To investigate the role of HO-1 as an anti-oxidant and anti-inflammatory enzyme in the pathogenesis of lung cancer, by comparing its activity in induced sputum (IS) of patients with lung cancer, patients with COPD and healthy nonsmokers controls.
Methods: IS was conducted according to a standard protocol. HO-1 levels were measured in IS supernatant by a biliverdin reductase-dependant reaction using bilirubin as end product.
Results: 90 subjects (31 with lung cancer, 29 with COPD and 30 healthy non-smokers) underwent IS and HO-1 level measurements. Mean HO-1 levels were significantly ($p<0.0001$) lower in lung cancer patients compares to COPD patients and healthy controls, (0.645, 1.192 and 1.628 respectively). There was a negative correlation between the lung cancer stage and HO-1 activity.
Conclusions: HO-1 activity is reduced in patients with lung cancer and correlates with disease severity, suggesting its protective effect as an antioxidant enzyme. These findings may propose a role of agents stimulating HO-1 as a novel therapeutic approach in lung cancer.

P4213

Increased serum placenta growth factor level is significantly associated with progression, recurrence and poor prognosis of lung cancer

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We recently found that the expression of placenta growth factor (PlGF) in lung cancer specimens is correlated with the progression and prognosis. In this study, serum samples were obtained from 72 patients with lung cancer and from 30 normal controls. Serum PlGF levels were determined by enzyme-linked immunosorbent assay (ELISA). The mean serum PlGF levels were significantly higher in lung cancer patients than in normal controls (19.1±10.7 vs. 10.1±4.5, $P<0.001$). Serum PlGF levels dropped to near the normal control levels after surgical cancer removal. Higher pre-surgery serum PlGF levels were significantly associated with larger tumor size ($P=0.015$), positive lymph node metastasis ($P=0.001$), more advanced clinical stages ($P=0.002$), and loco-regional recurrence ($P=0.037$). The serum PlGF level was identified as an independent unfavorable prognosis factor by multivariate Cox regression analyses ($P=0.014$). Kaplan-Meier curve showed that lung cancer patients with a higher serum PlGF level had a significantly poorer cumulative recurrence-free survival than those with a lower serum PlGF level (log-rank test, $P=0.009$). When we used the serum PlGF level of 19.1 pg/ml as a cutoff point, the sensitivity, specificity, and positive predictive value for tumor recurrence was 80%, 56% and 78%, respectively. We conclude that the serum PlGF level may be a valuable biomarker for prediction of therapeutic effect, progression, recurrence and prognosis of lung cancer.

P4214

Epidermal growth factor receptor mutation status in advanced non-small cell lung cancer: A single institution experience

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Background: Epidermal Growth Factor Receptor (EGFR) mutations are found in 10-20% of non-small cell lung cancer (NSCLC) and are associated with response to EGFR tyrosine kinase inhibitors (TKIs). The aim of this study is to examine the prevalence of EGFR mutations among patients with advanced non-squamous NSCLC treated in our institution and evaluate the associations of EGFR mutations with clinicopathological characteristics.
Methods: We examined the EGFR mutations in exons 19 and 21 using sequence analysis of 133 patients with NSCLC from April 2010 till February 2012. Patients harboring EGFR mutations were treated with EGFR-TKIs, patients EGFR negative - with standart chemotherapy. 54 patients with advanced NSCLC who were not tested for EGFR mutations treated with standart chemotherapy were considered as the control group.
Results: EGFR mutations were found in 18 patients (13.5%): female 30.3% (10/33), male 8.0% (8/100) ($P<0.01$); never smokers 37.9% (11/29), former smokers 10.0% (2/20), current smokers 5.9% (5/84) ($P<0.01$); adenocarcinomas 17.4% (15/86), large cell carcinomas 7.5% (3/40) ($P>0.05$). Overall response rate was 85.7% in EGFR mutation positive, 32.9% in EGFR mutation negative and 31.5% in control groups ($P<0.05$). The median progression-free survival in EGFR mutation negative group and in control group was 5.6 months (95% CI of 4.3 to 7.0) and 5.3 months (95% CI of 4.9 to 5.7), respectively but had not been reached yet in EGFR mutation positive group ($P<0.05$).
Conclusions: The frequency of EGFR mutations is similar that presented in Europe. Screening of patients with NSCLC for EGFR mutations have a role in treatment decisions.

P4215

Role of progestrin releasing peptide (ProGRP), a serum based biomarker in early diagnosis of SCLC in cohort of high-risk patient presenting with symptoms related to lung cancer

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Lung cancer has become the top killer among malignant tumors in China during past three decades. Mortality rate of lung cancer are about 23 times and 13 times higher in current male and female smokers compared to lifelong nonsmokers. An estimated 350 million people smoke in China. Early diagnosis of lung cancer and more significantly Small Cell Lung Cancer (SCLC) is a major challenge.
In the present study we analyzed serum samples of 144 high-risk patients using a serum based biomarker panel comprising of CEA, CYFRA 21-1, SCC, and ProGRP. These patients visited our service, during June 2011 to January 2012 with symptoms related to lung cancer. The average age of patients was 64.14±8.59 years. Total 92 patients were diagnosed with lung cancer (73 NSCLC, 16 SCLC, 3 unknown), 44 patients were diagnosed of non-malignant tumors while 8 patients had unknown clinical diagnosis of the lesion. The final diagnosis was based on pathology results.
We found increased levels of ProGRP in patients diagnosed with SCLC. The mean ProGRP conc. in patients with SCLC was 3731 pg/mL compared to the mean value of 33 pg/mL for patients with NSCLC and 54 pg/mL for patients with non-malignant tumors. The results showed that ProGRP can be useful to identify patients that may have SCLC with a simple blood test on the same day which can lead to early diagnosis of SCLC by histological method and hopefully better prognosis. This test may also improve the differential diagnosis and selection of treatment for the patient.
This study was supported by National Basic Research Program of China (973 Program) No. 2012CB933300.

P4216

Investigation of a relationship between NF-KB1A gene polymorphism and non small cell lung cancer (NSCLC)

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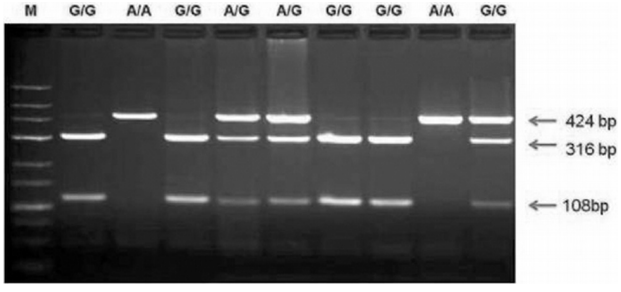
Nuclear factor kappa b (NF-κB) is defined as a protein family. NF-κBIA (IκBα) is inhibitory of NF-κB transcription factor. It binds NF-κB transcription factor and blocks carrying NF-κB factor to nucleus and binding to DNA. NF-κBIA (IκBα) is a gene that contains six exons and locates in 4q13 chorosomal region. Purpose of study reports relation between Turkish people who have NF-κBIA gene polymorphism and NSCLC.
Method: 99 lung cancer cases and 99 healthy control cases who were diagnosed in our hospital included in the study. PCR-RFLP technique was used for genotyping.
Results: Genotype distrubition of NF-κBIA gene's relevant region were detected (n=99) 17.2% AA (n=17), 48.5% AG (n=48), 34.3% GG (n=34) at patient group and (n=99) 21.2% AA (n=21), 45.5% AG (n=45), 33.3% GG (n=33) at control group. (p=0.766).



Genotypes and allele distribution	Control N:99		Patient N:99		P value	χ2
NF-κB1α	N	%	N	%		
GG	33	33.3	34	34.3		
AG	45	45.5	48	48.5	0,766	0,532
AA	21	21.2	17	17.2		
Allele						
G	111	56,06	116	58.59	0,611	0,125
A	87	43.94	82	41.41		

Conclusion: According to this study's results, a significant difference was not detected between Turkish people who have NF-κBIA polymorphism and NSCLC as statistical. We have opinion that significant results can be gained by increasing cases's numbers.

P4217

Lung cancer metabolomics in plasma, urine and broncheoalveolar lavage. A pilot study

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Lung cancer (LC) has high morbimortality rates. Today, research in biomarkers is a hot topic, and among these, the least frequently studied, are those of the metabolomics field.
Our aims are to present preliminary data using metabolomics technics for detection of differentiated patterns between patients with LC and non-lung cancer (NLC) patients, to obtain an effective screening method.
We obtained blood, urine, and broncheoalveolar lavage (BLA) samples from a group of patients who underwent bronchoscopy, for further analysis through mass spectrometry methods. These techniques provide us with metabolic fingerprinting, allowing the study of the metabolites involved in the process.
Identification of the resultant metabolites was performed trough mass-mass fragmentation procedures. Guided by mass spectrum, the results where processed by *Partial Least Squares Discriminant Analysis*. We compared the results from both groups.
Initially, 7 LC patients and 7 NLC subjects' samples were included. We found differences in metabolite profiles among these groups, allowing us to differentiate between LC and NLC cases. Including the blood and urine samples, we where able to identify potentially overexpressed markers, such as choline, phosphocholine and propionylcarnitine, leaving analysis of BLA samples results pending.
In conclusion, both groups shown different metabolomics profiles in the analyzed samples, this allows for its statistic discrimination.
Metabolites that are responsible for this discrimination have been identified and correlated with previously described neoplastic processes.
The preliminary data raises the possibility of further studies that will allow the development of early screening technics.

P4218

Increased levels of plasmatic dopamine in human small cell lung cancer

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Dopamine (DA) is a monoamine neurohormone with pleiotropic effects that exerts an immunomodulatory action inhibiting proliferation and cytotoxicity of CD4+ and CD8+ T cell. In human malignancies increased plasmatic DA levels are documented. In addiction recent reports also indicate an active production of DA by some human tumor cell lines. Small cell lung cancer (SCLC) is a very aggressive neuroendocrine human tumor able to produce several molecules with neurohormonal effect. Actually no data are available about DA plasmatic levels in patient affected by SCLC.
Aim of this study is to assess plasmatic DA levels in SCLC patients in comparison with non-small cell lung cancer (NSCLC) and healthy subjects and to correlate this data with the plasmatic levels of neuron specific enolasis (NSE).
Before treatment whole blood was collected from patients affected by lung cancer (n=50; SCLC n=15; NSCLC n=35), healthy subjects (n=10), and plasma was separated to assess its DA content by Hight Performance Liquid Chromatography (HPLC).
DA levels are significantly increased in patients affected by SCLC comparing with NSCLC [102,5 pg/ml ±18,3(SEM) *vs* 52,3 pg/ml ±5,8(SEM); p< 0,05] and with healthy subjects [102,5 pg/ml ±18,3(SEM) *vs* 38,9 pg/ml ±13,5 (SEM); p< 0,05].
In SCLC patients these increased levels are inversely correlated with the NSE plasmatic values (r= - 0,5; p < 0,05).
The data here presented show increased plasmatic DA levels in SCLC patients comparing with NSCLC and healthy subjects. Further studies are needed to assess if this increased plasmatic levels represent an active ectopical secrection of DA by SCLC and if this may exerts a possible role in the tumor growth.

P4219

MAdL: A new diagnostic marker for adenocarcinomas

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With regard to the growing number of targeted therapies in lung cancer, a specific and reliable sub-differentiation is of crucial importance. Without discriminating adenocarcinomas from squamous cell carcinomas, molecular based diagnostics that e.g. target certain mutations in the EGFR gene are not applicable and hence, the patients cannot profit from the new therapies. To generate an antibody that, reliably detects adenocarcinomas of the lung in addition to the established markers, we immunized mice with primary human alveolar cells type II. Hybridomas were produced to obtain cell culture supernatants that were screened on tissue micro arrays. Among others, we identified one clone that strongly binds human adenocarcinomas of the lung. Since most of patient material are Formalin-fixed and paraffin-embedded, we established an antigen retrieval protocol that works on FFPE tissues.
Here we present a monoclonal antibody, designated MAdL as a new specific marker for adenocarcinomas of the lung.

P4220

Potential angiogenic biomarkers in patients with non small cell lung cancer: Possible implications

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To date, no single agent has gained a sufficient prognostic significance for NSCLC patients. So, there is an urgent need for new innovative biomarkers in NSCLC.
The present study was designed to: 1) Evaluate the role of Nitric Oxide (NO), Sialic Acid (SA) and Glutathione S-Transferase (GST) as prognostic indicators in NSCLC.2) Correlate the above parameters levels with the clinicopathological status of the patients.
The study included 30 patients with newly diagnosed histopathologically confirmed NSCLC, as well as 10 healthy volunteers with matched age and sex as controls. Blood samples and lung tissue biopsies were taken from all subjects on admission and after chemotherapy with and without Nimesolide (Cox-2 inhibitor).
Results: Serum and tissue levels of NO, SA and GST activities were significantly higher in NSCLC patients compared to controls. These levels decreased significantly after chemotherapy (specially if Cox-2 inhibitors were added). The serum and tissue levels of the studied parameters decreased significantly in the responders compared to resistant cases.
In conclusion, NO, SA, besides GST correlated significantly with the clinicopathological status of NSCLC patients and are considered cheap sensitive prognostic biochemical indices.

P4221

KI67: Analysis of its expression and prognostic significance in a resected non small cell lung cancer population

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Ki67 is a nuclear protein involved in the cellular proliferation regulation; its expression is associated with cancer and usually has a prognostic significance.
Aim: To study Ki67 expression and its prognostic significance in a resected non small cell lung cancer (NSCLC) population, in general and by types.
Population and methods: We included all the patients, with a completely resected NSCLC stage IAp to stage IIBp, seen at our hospital from 1998 to 2003. The

Table 1.	Ki67 positive expression (%)	p
Squamous cell carcinoma (n=99)	60,6%	0,046
Adenocarcinoma (n=37)	40,6%	

Table 2.	W-G	L-R	Prognostic significance
General	0,04	0,09	Negative
Squamous cell carcinoma	0,42	0,70	Not significant
Adenocarcinoma	0,05	0,04	Negative

Ki67 expression was studied by inmunohistochemistry techniques. We used the Chi square test to analyze the differences in the Ki67 expression between the squamous cell carcinoma and adenocarcinoma and a bivariate analysis to study the prognostic significance of Ki67 expression according to the 5 years survival using the Wilcoxon-Gehan (W-G) test and the log rank (L-R).
Results: We included 146 patients, 91% were men with a median age of 67 yo. 99 were squamous cell carcinoma, 37 adenocarcinoma and 10 large cell carcinoma. The Ki67 expression was positive in 56% cases, negative in 42% and no valid in 2%. The Ki67 positive expression found in the different types of NSCLC is shown in Table 1 and the prognostic significance of Ki67 expression, in NSCLC and its different types, is shown in Table 2.
Conclusion: In the population studied, the Ki67 expression was higher in the squamous cell carcinoma and was associated with a bad prognosis.

P4222
Increased activity of Lyn tyrosine kinase causes multiple chronic obstructive pulmonary disease-like changes in mouse
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Lyn is a member of the Src family of tyrosine kinases and was first discovered to be an key regulator of B cell activation. There are many studies now showing that Lyn also plays a role in the progression of myeloid leukemia and several types of epithelial cancer. The Lyn gain-of-function (Lyn up/up) mutant mouse was created to investigate putative proto-oncogenic roles of Lyn. Lyn up/up mice develop chronic lung inflammation and emphysema at young age. The extent of alveolar airspace enlargement in the Lynup/up mouse model is more severe than any other gene-targeted or smoke-induced mouse model of emphysema to date. Chronic lung inflammation, characterised by increase in macrophages, neutrophils and T cells and development of skeletal muscle wasting and osteoporosis in COPD patients were also observed in Lynup/up mice. Lung tumors have also been found is some aged mice although penetrance is low. Histological assessment of lungs in 4 week old Lyn up/up mice shows that alveolar epithelial cells are hyperplastic and there were a lack of blood vessels formation around the alveoli. There is also reduced numbers of apoptotic cells in the lung as detected by TUNEL assay. This suggests that alveolar airspace enlargement is not a result of emphysematous destruction caused by chronic inflammation but a possible attribute of perturbed signaling in endothelial and/or epithelial cells that needs further investigation. The complex process that leads to the occurrence of emphysema together with lung cancer is still not understood. The Lyn up/up mouse will be an excellent model to investigate the underlying co-determinant of the two seemingly opposite outcome of lung disease.

P4223
Genetic polymorphisms of k-ras gene in smoking related diseases
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Ras is a family of genes that have many biological functions but mainly control cell growth and development. Chemicals in cigarette smoke cause mutation in ras gene. Smoking causes lung cancer because the carcinogen bound strongly to the precise site in K-ras gene. Our aim of the study is to detect genetic polymorphism of K-ras gene in smoking related diseases.
The study included 50 patients, 20 with chronic obstructive pulmonary disease (COPD), 20 with lung cancer and 10 normal subjects. All patients and normal subjects were smokers. Serum samples were evaluated, DNA was extracted and mutational analyses performed using a PCR assay. Two (10%) out of 20 COPD patients and four (20%) out of 20 lung cancer patients had mutated k-ras gene, while there was no mutation in the control group. The mutation of k-ras gene was associated with smoking history, severity of COPD and cell type of lung cancer. Mutations were observed in heavy smokers in COPD (13.3%) and lung cancer (22.2%) patients. Moderate (14.3%) and severe (12.5%) obstruction in COPD patients were associated with mutations. All k-ras mutations were observed in non small cell lung cancer (NSCLC (95%).
In conclusion, k-ras mutation is detected in the lung cancer and COPD patients suggesting that COPD patients were in the early stages of developing cancer. For COPD patients the ras gene might be a biomarker for cancer as a screening of DNA in serum using a noninvasive technique.