287. Biomarkers and other new methods for lung cancer

P2783

An evaluation of the potential for inhaled xenobiotics to develop cancer in the lung using a 3D *in vitro* model of the human epithelial airway-wall

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Nano-objects (NOs) produced by combustion processes, such as the content contained within diesel exhaust particles (DEPs), can potentiate cell mutagenesis leading to lung cancer. Little is understood however, regarding the potential for engineered NOs to cause genotoxicity. The aim of this project was to use an *in vitro* human epithelial airway-model (epithelial cell layer (16HBE14o- cells) with human monocyte derived macrophages (apical layer) and dendritic cells (basolateral layer)), with different toxicological endpoints involved in NO-induced genotoxicity. Single- and multi-walled carbon nanotubes (SWCNTs/MWCNTs), as well as crocidolite asbestos fibres (CAFs) and DEPs caused no significant ($p>0.05$) cytotoxicity (lactate dehydrogenase release) up to 0.04mg/ml after 24hrs. Significant increases ($p<0.05$) in both IL-8 and TNF-a levels at 0.005-0.04mg/ml over 24hrs were observed in both the apical and basolateral layers for all NOs. The NO-cell interaction (electron tomography) was only observed with macrophages and not with epithelial or dendritic cells. Initial analysis shows no changes in cell proliferation (BrdU assay) however, increased DNA damage (comet assay), possibly via an oxidant-related mechanism, in 16HBE14o- cells compared to control levels for each NO. Investigation of oxidative stress, mutagenicity and cell death endpoints are ongoing. Initial observations suggest that SWCNTs and MWCNTs may elicit genotoxicity similar to that of CAFs and DEPs via an inflammogenic/oxidant driven mechanism *in vitro*.

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P2784

Nitrosoglutathione reductase inhibition in the airway epithelium may contribute to lung cancer risk

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Background: S-Nitrosoglutathione (GSNO) is an endogenous bronchodilator. GSNO reductase (GSNOR) depletes airway GSNO; inhibitors are in development for asthma. However, GSNO can be tumorigenic by activating wild-type (wt) Ras though S-nitrosylation. We studied whether decreased GSNO reductase promotes wt Ras S-nitrosylation and activation in the lung.
Methods: Lung non-small cell cancer lines (H157; Calu-1; H226;Calu-3; H1650; A549) were studied. Ras S-Nitrosylation was studied by biotin substitution and immunoblot. Active GTP-Ras was assayed in a Raf-1RBD-binding ELISA. Certain cells were exposed to 1) 30 ppm NO in a sealed incubator; or 2) S-nitrosocysteine (CSNO, 10 μM), GSH (2mM) and NADH (300 μM) with or without the GSNO reductase inhibitor, C3 (100 μM; 10 min; from P. Sanghani). GSNO reductase $^{-/-}$ (from J.S. Stamler) were compared with C57Black6 mice with and without intratracheal ethyl nitrite (EtONO, 10 μM).
Results: Wt Ras S-nitrosylation was increased relative to expression in cancer cell lines, though expression of was low. NO exposure for 5d, but not 2d, increased Ras S-nitrosylation relative to expression in vitro. S-Nitrosylation of Ras relative to total Ras expression was increased in the lungs of GSNO reductase $^{-/-}$ mice; and was increased in wt mice by EtONO. CSNO increased Ras activity. In the H1650 cells, this increase was partially augmented by the C3, though baseline expression and activity of GSNO reductase were low.
Conclusions: Though loss of GSNO reductase function may benefit asthma, it prevents wt Ras denitrosylation and inactivation. This effect is associated with tumorigenesis and may not be a good strategy for asthma treatment, particularly in smokers.

P2785

COPD as an independent risk factor for lung cancer in patients with bronchial squamous dysplasia

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Background: We evaluated airflow obstruction as a possible independent risk factor for lung cancer (LC) in patients with bronchial squamous dysplasia (SD).
Methods: A total of 114 patients (111 men and 3 women) with at least 1 bronchial SD, at least 1 follow-up evaluation and normal baseline chest computed tomography (CT) were evaluated; 58 (51%) were COPD and 56 (49%) were non-COPD patients. Median age was 68 years (range,44–84 yr) and median follow-up duration was 21 months (range,4-98 months). Follow-up included periodic white light and autofluorescence bronchoscopy and chest CT. Expression of iNOS (inducible nitric oxide synthase) and EGFR (epidermal growth factor receptor) in bronchial epithelium biopsy specimens was evaluated by immunohistochemistry (IHC). Diagnosis of carcinoma *in situ* (CIS) and/or LC were follow-up endpoints.
Results: Expression of iNOS and EGFR was closely related to patient COPD status (p=0.007 and p=0.018, respectively). COPD patients were more likely to have baseline high grade dysplasia (p=0.017), multiple dysplasias (p=0.045) and to develop a new dysplasia during follow-up (p=0.003). Progression to CIS or LC occurred more frequently in patients with COPD (p=0.012), positive EGFR (p=0.019), positive iNOS (p<0.001) and baseline high grade SD (p=0.035). Multivariate analysis showed that risk for progression was closely related to airflow obstruction (RR=0.959; 95% CI=0.923-0.997; p=0.044) and iNOS expression (RR=10.521; 95% CI=2.75-40.3; p=0.001).
Conclusion: In patients with bronchial SD, COPD is closely related to risk of progression to CIS or LC. This study supports the hypothesis that inflammation and oxidative stress promote lung carcinogenesis.

P2786

Role of systemic and bronchial oxidative stress and inflammation in lung cancer predisposition in patients with COPD

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Chronic airway inflammation such as that observed in COPD patients is a relevant contributor to lung cancer. Increased inflammation and oxidative stress levels have been shown in lung cancer lesions compared to non-tumor parenchyma.
Objectives: Levels of inflammation, redox balance, and angiogenesis were determined in the airways and blood of out-patients with lung cancer with and without COPD.
Methods: Oxidative stress, inflammation, angiogenesis, and growth factor levels were quantified (immunoblotting, ELISA, activity assays, and chemiluminescence) in the airways (bronchoscopy) and blood of: 1) patients with COPD and lung cancer (n=39), 2) patients with lung cancer without COPD (n=14), 3) patients with COPD without lung cancer (n=9), and 4) healthy subjects with no lung disease (n=12).
Results: In patients with COPD and lung cancer, blood superoxide anion, systemic oxidized DNA and proteins, and bronchial protein oxidation were increased compared to healthy controls. In patients with lung cancer, only protein oxidation was greater in their bronchi than in healthy controls. Antioxidant enzyme activity did not differ among groups in either blood or bronchi. TNF-alpha, interferon-gamma, and VEGF levels were higher in the blood and bronchi of patients with COPD and lung cancer and lung cancer only than in healthy controls.
Conclusions: While COPD induces an increase in systemic and bronchial oxidative stress in patients with associated lung cancer, the latter condition is related to greater content of proinflammatory cytokines and angiogenic factors in both blood and bronchi of the patients.
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P2787

Detection of patients with lung cancer out of a risk group by breath sample presentation to sniffer dogs

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Objective: Prognosis of patients with lung cancer (LC) largely depends on early diagnosis. Previous studies reported successful detection of LC in exhaled breath samples using sniffer dogs. However, questions remain concerning the discrimination potential of these approaches regarding LC and chronic inflammatory lung conditions, such as COPD.
Methods: In a prospective study we tested exhaled breath samples of patients with LC (group A), patients with COPD (group B) and healthy individuals (group C). Four sniffer dogs were trained to identify LC in human exhaled breath samples with each breath sample used only once. For analysing the diagnostic accuracy of LC detection, each dog performed the following tests in a standardized fashion: I) group A (n=10) vs group C (n=40), II) group A (n=5) vs group B+C (n=20), III) group A (n=10) vs group B (n=40).
Results: Sensitivity and specificity were in test I) LC vs healthy individuals 50% and 88% resp., in test II) LC vs mixed collective of COPD patients and healthy individuals 95% and 95% resp., and in test III) LC vs COPD 80% and 95% resp. Overall test sensitivity was 90% (CI 0,78-0,97), specificity 72% (CI 0,51-0,88), PPV 86% (CI 0,74-0,94) and NPV 78% (CI 0,56-0,93), inter-rater variability k=0,436 given the experimental conditions. Tests were analyzed for confounders (study population, smoking habits, nutrition, medication).

Conclusions: Exhaled breath analysis is a promising approach towards future non-invasive LC screening methods. By using sniffer dogs as a "detection device", our results set a benchmark for the identification of LC in exhaled breath samples and the discrimination of LC and COPD.

P2788

A new possibility of process monitoring in lung cancer: Volatile organic compounds detected with ion mobility spectrometry to follow the success of the therapeutic process

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Introduction: Lung cancer is mostly detected when it is already too late for surgery. But even when tumours can still be operated, there has not been a simple, convenient way of observing any follow-up parameters such as PSA in prostate cancer. With the non-invasive analysis of Volatile Organic Compounds (VOCs) in exhaled breath using ion mobility spectrometry (IMS) we get the chance to step up in this development.
Objectives: To develop a non-invasive technique of analysing new tumour markers in order to find a follow up biomarker for an improved monitoring of the treatment of lung cancer patients.
Methods: Exhaled breath from 33 lung cancer patients was collected and analysed with an IMS device before and after lung surgery. The patients were split into groups according to the tumour histology. Additional conditions such as COPD, medication and former radiation or chemotherapy were also taken into consideration. Furthermore, the results were statistically analysed.
Results: There were several peaks which showed differences between the pre- and post-surgery groups (such as "EV" normU: 0,062 in squamous cell carcinoma). Further results will be presented in box plots.
Conclusion: As the results show differences between the peaks before and after surgery, the analysis of VOCs in exhaled air might be a new non-invasive possibility of monitoring the process of lung cancer therapy.
In future research it would be interesting to carry out further investigations on long-term patient observations after defined time intervals.

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P2790

Volatile organic compounds in lung cancer patients before and after tumour resection

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Introduction: Ion mobility spectrometry (IMS) is a promising tool in the detection of volatile organic compounds (VOC) even in small amounts. Whether it can contribute to the diagnosis of non-small-cell cancers (NSCLC) has not been adequately evaluated. Breath analysis with IMS is based on the assessment of multiple volatile organic compound (VOC) peaks considered specific for the disease.
Objectives: We studied bronchoscopically obtained VOCs in exhaled breath with an ion mobility spectrometer coupled to a multi capillary column (MCC/IMS) in patients with NSCLC before and after tumour resection in order to find tumour specific VOCs.
Methods: In 8 patients with histologically proven NSCLC, gas samples were aspirated out of the lungs during the diagnostic bronchoscopy and after tumour resection. Gas samples were aspirated via a Teflon tube introduced in the working channel of the bronchoscope and assessed using IMS.
Results: We found 228 common peaks in the measured data. 17 of them were significantly different before and after surgery. 11 peaks could be found with a lower value after tumour resection whereas 6 had a higher value.
Conclusion: While in a former study (Poli D et al. Acta Biomed 2008; 79; Suppl 1: 64-72) using solid-phase micro-extraction no changes after lung cancer resection could have been found, IMS revealed a change in the composition of exhaled breath after surgery in our work. Therefore some VOC levels may have been influenced by the tumour and these VOCs can be detected by MCC/IMS.

P2791

New contributions in the determination of volatile organic compounds (VOC) in lung cancer (LC)

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Introduction: Determination of VOC present in exhaled breath (EB) may be useful as a noninvasive diagnostic technique in LC.
Objective: To analyze the presence of VOC in the EB in two groups of subject: LC Group and Control Group.
Methods: Descriptive, observational study. LC Group: 29 patients with LC. Control Group: 40 healthy volunteers. (All accepted Informed consent). Breath samples were collected at lung residual functional capacity, with simultaneous sampling of ambient air using BioVOC devices. Analytical technique: Thermal desorption (Markes Int.)-gas chromatography (7890A)-mass spectrometry (5975C-Agilent Tech). VOC analyzed, see table 2. Compounds identified by means of retention time + mass spectrum. Chromatographic column: DB1: 30mx0.25mmx1um (Agilent Tech).
Results:

Table 1. Characteristics of study subjects.

		Control Group (n=40)	LC Group (n=29)
Age		M: 51 (8,7) F: 50 (9,6)	M: 72 (9,2) F:57 (8,5)
Gendre (M/F)		M: 20 (50%) F: 20 (50%)	M 22 (75,8%) F: 7 (24,2%)
Tabacoo	Smokers	F: 1 (2,5%) M 3 (7,5%)	F: 1 (3,5%) M: 9 (31,0%)
	Non-smokers	F: 13 (32,5%) M: 7 (17,5%)	F: 3 (10,3%) M: 0 (0,0%)
	Exsmokers	F: 7 (17,5%) M: 9 (22,5%)	F: 3 (10,3%) M: 13(44,9%)
Hystologic	SCLC		1 (3,5%)
	NSCLC		28 (96,5%)
TNM	IA		1 (3,5%)
	IB		1 (3,5%)
	IIA		-
	IIB		-
	IIIA		1 (3,5%)
	IIIB		5 (17,2%)
	IV		21 (72,4%)
COPD			26 (89,7%)

Table 2. Volatile Organic Compound (VOC) found in exhaled breatl

VOC	Control Group n=40	Lung Cancer Group n=29	p
CARBONYL COMPOUNDS			
2butanone	6(15%)	11(37,9%)	0,029
Hexanal	1(2,5%)	6(20,7%)	0,036
Heptanal	1(2,5%)	7(24,1%)	0,008
Octanal	9(22,5%)	8(27,6%)	0,628
Nonanal	7(17,5%)	27(93,1%)	<0,001
ORGANIC ACIDS			
Propanoic Acid	17(42,5%)	23(79,3%)	0,002
Octanoic Acid	5(12,5%)	5(17,2%)	0,732
Nonanoic Acid	12(30%)	19(65,5%)	0,003
HYDROCARBONS			
Isoprene	37(92,5%)	29(100%)	0,258
Pentane, 2methyl	1(2,5%)	2(6,9%)	0,568
Pentane, 3methyl	4(10%)	0(0%)	0,133
Hexane	12(30%)	16(55,2%)	0,036
Hexane, 2methyl	6(15%)	2(6,9%)	0,453
Hexane, 3methyl	10(25%)	6(20,7%)	0,675
Heptane	4(10%)	3(10,3%)	1
Octane	4(10%)	3(10,3%)	1
Nonane	6(15%)	1(3,4%)	0,225
Decane	26(65%)	19(65,5%)	0,964
Tridecane	5(12,5%)	5(17,2%)	0,732
Tetradecane	14(35%)	19(65,5%)	0,012
Pentadecane	2(5%)	2(6,9%)	1
AROMATIC COMPOUNDS			
Benzene	40(100%)	29(100%)	-
Toluene	39(97,5%)	29(100%)	1
Styrene	18(45%)	12(41,4%)	0,765
Phenol	38(95%)	29(100%)	0,506
Benzaldehyde	38(95%)	27(93,1%)	1

Conclusions: 1.The presence of VOC (2butanone, Hexanal, Heptanal, Nonanal, Propanoic Acid, nonanoic Acid, Hexane and Tetradecane) exhibits significative differences between groups. 2. Quantification of VOC is extremely difficult because of the great amount of interfering compounds and the low concentrations found. 3.Clinical application of VOC as a noninvasive diagnostic technique in LC is not possible yet due to lack of standardization of methodology.
Acknowledge to Javier Martín from Agilent Tech. for his collaboration.
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P2792

Discriminating NSCLC from COPD using patterns derived from an electronic nose

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Introduction: Lung carcinoma is a leading cause of death. Early detection of the disease will improve the survival rate. An easy, inexpensive method for early detection or even screening at the practitioners office is lacking. Electronic noses use arrays of chemosensors for patterns describing individual breath. This abstract

describes a proof of principle study for discriminating NSCLC from COPD using an electronic nose.
Methods: 66 newly diagnosed (nd) NSCLC patients in the lung cancer group were compared to 73 COPD patients using a Cyranose 320 (Smith Detection) electronic nose. Gas samples were taken immediately after exhalation and raw files were transferred to a self developed pattern recognition software (DiagNose) to learn a common pattern for each ndNSCLC and COPD and then group each file.DiagNose software empoys neuronal network principles for pattern recognition.
Results: Pattern analysis of all files correctly identified 62 of the 73 samples as COPD and 59 of the 66 samples as Carcinoma patients (sensitivity 84%; specificity 89%).
Diskussion Patterns derived from breath analysis of NSCLC and COPD patients analyzed with a special software based on neuronal network techniques were able to correctly discriminate between cancer and COPD patients in the great majority of cases. Sensitivity and Specificity were both high. Further improvements with even more sophisticated algorithms may further improve these results. Our study demonstrates the potential of pattern recognition for early detection of lung carcinoma in a populationat risk.

P2793
Neutrophil elastase levels is higher in patients with lung cancer than chronic obstructive pulmonary disease
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Neutrophil elastase (NE) is a specific serine proteinase and it is a modulator of inflammation. Systemic and pulmonary inflammation has been associated with both non-small cell lung cancer (NSCLC) and chronic obstructive pulmonary disease (COPD). Still, an exact role of NE in pathogenesis of NSCLC, COPD and in coexistence of both diseases is unknown. The aim of the study was to evaluate NE levels in NSCLC with and without COPD.
Methods: Serum and BAL fluid (BALF) levels of NE were measured by enzyme-linked immunosorbent assay (ELISA) in 28 patients with NSCLC, 20 patients with moderate COPD, 25 patients with NSCLC and moderate COPD (NSCLC/COPD) and 10 healthy non-smoking individuals (HI). Periferial blood and BALF were collected from each patient before any treatment.
Results: Serum NE levels were significantly higher in groups with NSCLC: NSCLC, NSCLC/COPD, compared to COPD or HI (620.6±77.5 ng/mL and 609.8±52.7 ng/mL vs 409.2±61.6 ng/mL and 243±23.9 ng/mL, $p<0.05$). BALF NE levels were lover than serum NE levels but significantly highter NE levels was found in groups with NSCLC: NSCLC (1.47-fold), NSCLC/COPD (1.53-fold) compared to COPD (1.21-fold, $p<0.05$) or HI (basal level). Serum NE levels were correlated with the clinicalopathological status of patients with NSCLC.
Conclusions: Our data suggest a higher levels of NE both in NSCLC and NSCLC/COPD than in COPD or HI.

P2794
The eosinophilic infiltration of lung cancer tissue and its relationship with the expression of IL-5
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Objective: Some studies showed that high eosinophilic infiltration in lung cancer tissues was associated an improved prognosis. And the other studies showed the high expressed of IL-5 in lung cancer cells. IL-5 has emerged as a main controlling cytokine for eosinophil production, activation, and recruitment. However, there was little data associating IL-5 production with eosinophilia in lung cancer. We studied the eosinophilic infiltration and the expression of IL-5 in tissue specimens from lung cancer patients and analyzed the correlation between the eosinophilic infiltration and IL-5 expression of lung cancer tissue.
Methods: Eosinophilic infiltration was detected by Chromotrope-2R staining and IL-5 was detected by immunohistochemistry in 45 lung cancer tissues and 36 corresponding normal lung tissues.
Results: There were high tissue eosinophilic infiltrations in 17 of 45 (37.78%) cases. In the high eosinophilic infiltration cases, the counting of eosinophil in tumor tissue was significantly higher than that in corresponding normal tissues ($P<0.05$). In NSCLC, the rate of high tissue eosinophilic infiltration cases was significantly higher than that of SCLC ($P<0.05$). IL-5 was high expressed in all 45 cases and low expressed in corresponding normal tissues. Eosinophilic infiltration was not correlated with the expression of IL-5 in tumor cells ($r=0.026$, $P>0.05$).
Conclusion: There were high eosinophilic infiltrations of tumor tissues in some cases of lung cancer. Compared with SCLC, the rate of high tissue eosinophilic infiltration cases of NSCLC was significantly increased. There was no relationship between the eosinophilic infiltration and the expression of IL-5 in lung cancer tissue.

P2795
Association of genetic polymorphisms of matrix metalloproteinase (MMP)14 to the susceptibility of non-small cell lung cancer
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Background: Matrix metalloproteinase (MMP)14 is a cell surface proteinase that displays a broad spectrum of activity against extracellular matrix components and promotes the invasion/metastasis of cells. MMP14 is overexpressed in NSCLC, and the level is correlated with poor survival. We investigate the MMP14-165, +7096 and +8153 genetic polymorphisms for their role in the susceptibility to NSCLC.
Methods: Through a case-control study design, genomic DNA samples isolated from peripheral lymphocytes of 267 NSCLC patients and 328 controls, who were age and sex-matched and recruited from the health check-up unit, were subjected to polymorphism analysis with PCR-RFLP technique.
Results: The distribution of the genotype frequencies of MMP14 -165 (G/T), MMP14 +7096 (T/C) and MMP14 +8153 (G/A) were significantly different between the lung cancer patients and the healthy controls. Logistic regression analysis revealed that higher odds ratios (ORs) for lung cancer were seen for individuals with MMP14 -165 T/T genotype against GG/GT genotypes (an OR of 1.84, 95% CI 1.22-2.77, p=0.005), MMP14 +7096 CC genotype against TT/TC genotypes (an OR of 1.79, 95% CI 1.19-2.70, p=0.005), and MMP14 +8153 GA genotype against GG genotypes (an OR of 2.07, 95% CI 1.36-3.14, p=0.001).

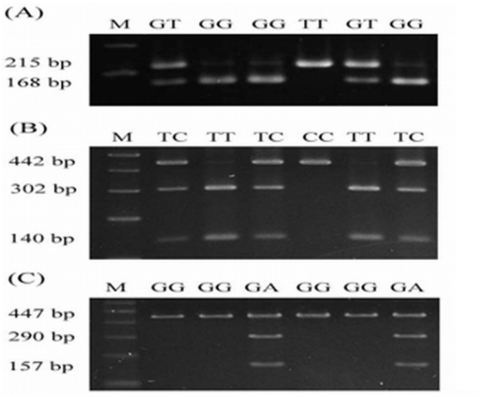

Figure 1. PCR-RFLP of MMP-14 gene. (A) PCR products of MMP-14 -165 gene polymorphism. (B) PCR products of MMP-14 +7096 gene. (C) PCR products of MMP-14 +8153 gene polymorphism.

Conclusions: A significant association between the polymorphisms of MMP14 gene and the susceptibility to NSCLC was demonstrated.

P2796
The expression and clinical significance of PD-L1$^+$CD68$^+$ macrophages in peripheral blood mononuclear cells of non-small cell lung cancer
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Purpose: To explore the roles of PD-L1$^+$ (programmed death-1-ligand 1) CD68$^+$ macrophages in peripheral blood mononuclear cells (PBMCs) of non-small cell lung cancer (NSCLC) by flow cytometry (FCM).
Materials and methods: 60 squamous cell carcinoma patients, 60 adenocarcinoma patients and 60 healthy controls were recruited in the study. PD-L1$^+$CD68$^+$ macrophages were isolated from PBMCs of the 180 persons with FCM.
Results: Squamous cell carcinoma and adenocarcinoma group had higher PD-L1$^+$CD68$^+$ macrophages expression compared to healthy controls ($P<0.001$ and $P=0.013$ respectively), and PD-L1$^+$CD68$^+$ macrophages from squamous cell carcinoma were higher than that from adenocarcinoma group [(19.03±12.28)% vs (14.21±11.88)%, $P = 0.031$)].
Conclusions: PD-L1$^+$CD68$^+$ macrophages in PBMCs have marked relationship with squamous cell carcinoma and adenocarcinoma, especially can produce profound effect on squamous cell carcinoma.

P2797
Circulating CD133(+) stem cells in lung cancer patients
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The presence of circulating tumor cells in lung cancer patients are potentially

responsible for the spread of cancer. Lung cancer stem cells (LCSCs) found in the tumor samples express both: CD133 and Epithelial Cell Adhesion Molecule (EpCAM). Previously we observed that CD133(+) stem cells are present in the lung cancer tissue. The aim of this study was to identify LCSCs in circulation and to correlate their proportion with the histological type of lung cancer and the stage of the disease.
Flow cytomery was applied to identify LCSCs in the peripheral blood of 20 patients with lung cancer in IIIB, IV stage of the disease. Blood samples were stained with anti-CD133 and anti-EpCAM antibodies. LCSCs were also studied in the resected lung cancer tissue samples from 7 patients.
Putative LCSCs of CD133(+)EpCAM(+) phenotype were found in the tumor samples from 6/7 patients and constituted 0,01-1,32% of the tumor cells. Analysis of the peripheral blood samples revealed the presence of CD133(+) cells in 16/20 cases (mean 0.395%), EpCAM(+) cells in 16/20 cases (mean 1.25%) and the CD133(+)EpCAM(+) cells in 5/20 cases (mean 0.078%). We did not find EpCAM cells in patients with small cell lung cancer (4 patients). We observed higher proportion of CD133+ cells in patients with M1b stage of disease (with presence of distant metastases) when compared with those with intrapulmonary spread (p=0.08).
We confirmed the presence of the putative lung cancer stem cells in the tumor samples and the presence of the CD133(+)EpCAM(+) cells in the circulation of lung cancer patients. Our study may have important implications in understanding of the mechanisms of the metastases spreading.

P2798
Evaluation of IL-6 level in serum and bronchoalveolar lavage fluid of patients with non-small cell lung cancer (NSCLC)
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Aim: To study the relationship between the interleukin-6 (IL-6) level in serum and broncheoalveolar lavage fluid (BALF) and non-small cell lung cancer (NSCLC).
Methods: The concentration of IL-6 in serum or BALF of 82 NSCLC patients, 56 patients of pulmonary inflammatory diseases (PID) and 26 normal controls were measured by ELISA.
Results: The concentration of IL-6 in BALF of NSCLC patients was significantly higher than that of PID patients and normal controls ($P<0.01$). The concentration of IL-6 in sera of NSCLC patients was higher than that of normal controls ($P<0.01$) but not statistically different from that of PID patients. In patients with NSCLC, the concentration of IL-6 in BALF was slightly higher than that of serum IL-6, but this difference was not statistically significant ($P>0.05$). Serum IL-6 level was higher in NSCLC of advanced stages (stage IIIa, IIIb and IV), as compared with that in NSCLC patients of early stages (stage Ia-IIb). BALF IL-6 level of NSCLC patients at different stages was not different. IL-6 (level in serum but not in BALF) of PID patients correlated with the serum concentration of C reactive protein (r=0.69).
Conclusion: IL-6 in BALF might be used as a marker for NSCLC. Serum IL-6 level might be an indicator for the stage of NSCLC. The serum IL-6 can also reflect the severity of acute lung inflammatory response.

P2799
Magnolol can induce apoptosis in non-small cell lung cancer via caspase-independent pathway
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Magnolol is a traditional Chinese herbs drug purified from Magnolia officinalis which has multiple pharmacological properties such as antioxidant and anti-inflammatory effects. Magnolol exhibited remarkable inhibitory effects on tumor growth. However, the effect of Magnolol on non-small cell lung cancer (NSCLC) has not been clarified. In this study, the NSCLC cell lines (A549, H520, H441 and HPAEC) were treated with different concentrations (0～100μM)of Magnolol for 24 hrs and the cytotoxic effect of Magnolol positively correlated with its concentration. Moreover, the chemotherapy drug Cisplatin at the dose of 25μM had the same cytotoxic effect as Magnolol at 80μM on NSCLC cell lines. Flow cytometry assay revealed that pretreatment with Magnolol at the dose of 80μM for 24 hours could induce apotosis in A549 cells. We also illustrated that membrane potential of mitochondria in A549, H520 and H441 cells started to decrease within 15 min after treatment with Magnolol by staining cancer cells with JC-1 dye. To clarify the mechanism of Magnolol-induced cell apoptosis, the mitochondria and nuclear proteins of NSCLC cell lines were measured by western blotting after treatment with Magnolol at various concentrations for 6hr, and the results revealed that apoptosis-inducing factor (AIF) and endonuclease G (Endo G) induced by Magnolol translocated from mitochondria to nucleus, suggesting that the Magnolol-induced apoptosis is mediated through caspase-independent pathway. In conclusion, Magnolol can induce apoptosis of NSCLC cells. In addition, the apoptosis in NSCLC cells involves, most likely, caspase-independent pathway in NSCLC cells.

P2800
Emerging prognostic biomarkers in non small cell lung cancer patients: Impact of treatment with nimesulide (COX-2 inhibitor) combined with chemotherapy
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To date, the treatment outcome of non small cell lung cancer (NSCLC) is still not satisfactory and new treatment options are urgently needed. The present study was designed to: 1) evaluate the effects of the antiangiogenic drug; nimesulide (NSAID, a COX-2 inhibitor) combined with chemotherapy on NSCLC treatment progress, 2) Evaluate the role of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), as prognostic indicators in NSCLC, 3) Correlate the above parameters levels with the clinicopathological status of the patients during the therapy. The study included 30 NSCLC.
Methods: The patients were divided to: group I, included 17 patients received chemotherapy alone and group II included 13 patients received the same chemotherapy with Nimesulide and 10 as controls. Serum and biopsies were taken for all subjects on admission and 3 weeks after the completion of treatment.
Results: Serum and tissue levels of VEGF and bFGF, were significantly higher in NSCLC patients and decreased significantly after treatment specially in group II compared to group I. The serum and tissue levels of the studied parameters decreased significantly in the responders as compared to resistant cases. The response rate after combined therapy was 69% versus 53% after chemotherapy alone.
Conclusion: In conclusion, Nimesulide appears to boost the efficacy of the traditional chemotherapy as its co-administration showed encouraging effects on improving and normalization of the proangiogenic parameters levels and in turn the vascular supply of tumors. This may have good impact on the patient outcome, prolongation of their survival rate and prognosis.

P2801
Investigation of biomarkers for pulmonary carcinomatous lymphangitis in patients with lung cancer
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Introduction: It is a crucial issue to perform differential diagnosis on the newly emerging interstitial opacities with dyspnea in the advanced lung cancer patients during chemotherapy. Many possible causes of these image findings should be considerd, such as interstitial pneumonia including drug-induced, infections, and pulmonary carcinomatous lymphangitis (PCL). The treatments for these diseases are different from each other, but urgent matters.
Aim: To establish biomarkers for PCL of lung cancer.
Method: Serum samples are collected from the subjects, which includes patients with lung cancer with lymphangitis (Lym, n=7), with enlarged lymph nodes (LCN, n=4), without lymphangitis or enlarged lymph nodes (LC, n=7), interstitial pneumonia (IP, n=8), infectious pneumonia (Inf, n=8), and healthy volunteers (Cnt, n=13). We measured serum VEGFR3, VEGF-C, and Lyve-1, which are important receptors and a ligand for lymphangiogenesis, by ELISA. We also measured CRP, KL-6, and SP-D. Statistics were performed by ANOVA and post-hoc analysis.
Result: Serum VEGF-C and Lyve-1 were not different among these groups. Serum VEGFR3 levels were higher in LCN and in Cnt compared with Lym. CRP levels were significantly increased in Inf than that in several other groups. KL-6 and SP-D levels were significantly increased in Lym and IP than other groups. However, any marker could not be useful for PCL by itself. KL-6/SP-D is significantly higher in Lym than that in the others.
Conclusion: Although three molecules regarding to lymphangiogenesis were not useful for the diagnosis of PCL in patients with lung cancer, KL-6/SP-D may be a promising biomarker for PCL.

P2802
Microparticle-associated tissue factor activity is increased in late stage lung cancer
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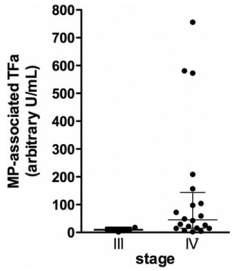
Background: Microparticles (MP) are small (.05-1 μm) vesicles shed by cells upon activation or during apoptosis. MP are rapidly gaining importance as a new family of physiologically relevant mediators. Tissue factor (TF) is the transmembrane receptor for factor VII(a) essential in the activation of blood coagulation; TF is also involved in several pathways relevant to tumor growth and metastasis including angiogenesis, and it has been postulated that it contributes to cancer progression. Recent evidences indicate that TF circulates associated with MP. Preliminary data indicate that MP-associated TF is increased in lung cancer (LC) patients.
Aim: To investigate whether MP-associated TF correlates with disease stage in LC patients.

Methods: Plasma MP-TF activity was measured in 33 patients with LC of different stages. Blood was collected at the time of inclusion; MP were collected by ultracentrifugation; TF activity was measured with a one stage clotting assay. Informed consent was obtained from all patients.
Results: The blood of patients with stage IV LC had a higher MP-associated TF activity compared to stage III (45.56 [14.88-143.70] vs. 9.45 [3.47-16.23] arbitrary U/mL) (median [interquartile range]); p<.05 by Mann-Whitney test (fig. 1).



Conclusions: MP-associated TF activity increases in late stage LC. This observation is consistent with the hypothesis that MP-associated TF is involved in LC progression.