461. Innovative diagnostic methods for lung cancer

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Late-breaking abstract: Serum concentrations of new factors (IL-20, galectin 3, IL-29 and IL-33) involved in angiogenesis in patients with advanced non-small cell lung cancer (NSCLC)

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There are several factors involved in angiogenesis, not tested yet, whose role is not clear in patients with lung cancer. Our pilot study was carried out to analyze the serum IL-20, Galectin 3, IL-29 and IL-33 (ELISA) in patients with non-small cell lung cancer (45 NSCLC) before chemotherapy, 15 sarcoidosis (BBS), 8 hypersensitivity pneumonitis (HP) and 15 healthy controls (H). Serum concentration of IL-20 was highest in NSCLC, lower in BBS and H, and lowest in HP (45,1±2 vs 41,88±11 vs 40±5,5 vs 36,09±2,7 pg/ml; p=0,01). The levels of Galectin 3 was highest in HP, lower in BBS, NSCLC, lowest in H (2,5±0,3 vs 1,2±0,4 vs 0,9±0,5 vs 0,8±0,1 pg/ml; p=0,03). The highest level of IL-29 was in HP, lower in NSCLC and H, the lowest in BBS (85,70±12 vs 53,65±2,8 vs 45,22±2,5 vs 38,68±1,7 pg/ml; p=0,003). Serum levels of IL-33 was lowest in H, higher in NSCLC and BBS, the highest in HP (3,57±0,9 vs 4,1±0,5 vs 5,56±3,7 vs 7,24±1,5 pg/ml; p= 0,02). Serum levels of IL-29 and IL-33 were lower in patients with metastases of NSCLC (IL-29: 85,19±18 vs 48,33 pg/ml; p=0,03; IL-33: 4,54±4,2 vs 3,61±2,1 pg/ml; p=0,04). There were no significant differences in serum IL-20, Galectin 3, IL-29 and IL-33 in relation to a response to a therapy.A correlations were found between IL-33 and Galectin 3 concentrations (R= 0.60). Summing up, serum concentrations of IL-20, Galectin 3, IL-29 and IL-33 were higher in patients with NSCLC than in healthy people. Their clinical usefulness in the diagnosis of cancer seems to be weak, but this requires further investigation.

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Diagnostic accuracy of SELDI-TOF-MS (surface enhanced laser desabsorbtion ionization time of flight mass spectrometry) method in early diagnosis of lung cancer

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Objective: Early diagnosis of lung cancer is critical for successful treatment and improving the outcome of patients. The cancer cells synthesize proteins as well as all of the other cells. Cancer may be recognised with these proteins profiles analysed with any methods. With SELDI-TOF-MS method, protein profiles can be obtained from serum, pleural fluid, urine and tissue samples.
The goal of this study was to identified protein profiles and to distinguish lung cancer patients from healty individuals by serum protein profiles.
Methods: 170 patients with lung cancer, 53 individuals with no evidence lung cancer but high risk for lung cancer and 47 healthy individuals totally 270 individuals were included in this study. Venous samples were taken from all cases. All of the serum samples were analyzed by SELDI-TOF-MS method for proteomics investigation.

Results: SELDI-TOF spectra of patients with lung cancer, healthy control and high risk are shown for the most discriminatory peaks of 11480 m/z, 11547 m/z and 11679 m/z.

Discriminatory proteomic features between lung cancers and healthy controls

Proteomic feature (m/z)	p value	AUROC	Average intensity of lung cancer cases	Average intensity of healty controls
11679	3.12	0.88	1.24	–
11480	1.35	0.87	0.88	–
11547	4.87	0.87	1.03	–

These proteomic features were present in only lung cancer group, but not in healthy control or high risk groups.
Conclusion: SELDI-TOF-MS method can correctly distinguish patients with lung cancer from healthy individuals and SELDI-TOF-MS method may be a new tool in diagnosis and screening test for lung cancer.

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Bioconductance as adjunctive technique for evaluation of patients with lung masses detected by chest CT

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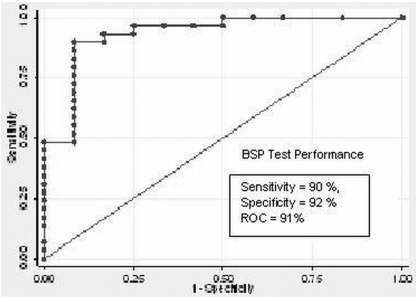
Introduction: CT screening may reduce LC mortality in high risk subjects but also find many masses with indeterminate features resulting in invasive procedures for benign lesions.
Aims: We hypothesize that trans-thoracic bioelectrical conductance measurements may discriminate benign from malignant CT-detected lesions.
Methods: 41 subjects with CT-detected masses and or lung cancer symptoms such as cough, hoarseness, dyspnea, hemoptysis, weight loss or recurrent respiratory infections enrolled. Prior to biopsy, measured 9 parameters at 62 sites were conducted with Bioconductance Scan Platform (BSP). For each subject a composite score from collected data was calculated and an optimal cut-point set to discriminate between the malignant and benign outcomes selected.
Results: 26 pathology-confirmed NSCLC, 2 SCLC, 1 carcinoid whereas 12 had a benign outcome based on pathology (9) or stable follow-up CT (3). BSP data for LC cases: 26 true positives, 3 false negatives (including the carcinoid), 90% sensitivity. For benign cases, the BSP resulted in 1 false positive,11 true negatives, 92% specificity. The overall ROC from BSP analysis was 91%.



Conclusion: BSP bioconductance measurements is associated strongly with a thoracic cancer or benignity. A technology that non-invasively provides adjunctive information to CT scanning to decide whether biopsy or further follow-up is appropriate will be an important clinical tool.

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Amorfous components of the extracellular matrix have impact as new biochemical markers on malignancy characterization and prognosis of non-small cell lung cancer

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Introduction: Many have reported that glycosaminoglycans (GAGs) have different behaviors in the presence of malignant tissues.In this study, we examined different GAGs concentrations and their impact on diagnosis and/or prognosis of patients with non small cell lung cancer.
Methods: Sulfated GAGs and Hyaluronic Acid (HA) were examined in tumoral and non-tumoral tissues from 45 lung cancer patients.Tissue samples were dehydrated and incubated with a proteolytic enzyme.The levels of HA were measured by a noncompetitive ELISA-like fluorometric assay. The sulfated GAG chains (heparan, dermatan and chondroitin sulfate - HS, DS and CS), were precipitated, dissolved in DNAse and their concentrations were identified after gel electrophoresis.
Results: HS and HA showed significantly higher concentration in tumoral than in normal areas (p=0.02 and p=0.0001, respectively; Fig. 1A/B). The Kaplan-Meier survival curves shows that tissues with lower concentrations of HA have better long-term survival than those with higher concentrations (Log Rank=3,59; p=0.05; Fig. 1C). One hundred% of tumoral areas presented CS while the normal almost never (p=0.0001; Fig. 1D).

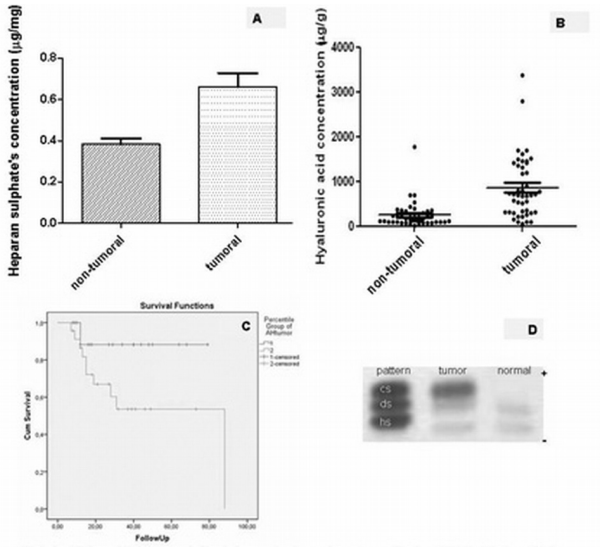

Fig 1: A – HS showed higher concentrations in tumoral than in non-tumoral areas (Fig A; p=0.02) **B** – HA showed higher concentrations in tumoral than in non-tumoral areas (p=0.0001) **C**- The Kaplan-Meier survival curves shows that tissues with lower concentrations of HA have better long-term survival than those with higher concentrations (Log Rank=3,59; p=0.05) **D**- One hundred % of tumoral areas presented CS while the normal almost never (p<0.0001). PDA gel electrophoresis assay showing the different GAG types and concentration in a pattern, normal and tumoral tissues.

Conclusions: The results presented suggest a possible role of these molecules on lung cancer development, but more importantly provide potential biochemical markers for differentiating normal from lung cancer patients.

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Interferon (-alpha, -beta, and -omega) receptor 2 (IFNAR2) is a prognostic biomarker for lung cancer

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Background: The type I interferon receptor subunit, interferon (-alpha, -beta, and –omega) receptor 2 (IFNAR2), has been reported to be overexpressed in several malignancies, primarily adenocarcinomas (ADCs). However, the biological significance of IFNAR2 in human lung cancer has not yet been studied.
Methods: Immunohistochemical analysis of 113 surgically resected non-small cell lung cancer specimens was performed. Serum concentrations of IFNAR2 were also determined by an enzyme-linked immunosorbent assay in 157 lung cancer patients and 164 healthy volunteers.
Results: IFNAR2 overexpression was observed in all histological types of lung cancers examined. Furthermore, strong IFNAR2 expression was associated with shorter progression-free survival (PFS) and overall survival (OS) ($p < 0.0001$ and $p = 0.0110$, respectively) in non-small cell lung cancer patients. Multivariate analyses confirmed its independent prognostic value for PFS and OS ($p < 0.0001$ and $p = 0.0222$, respectively). IFNAR2 serum levels were also significantly higher in lung cancer patients than in healthy volunteers ($p < 0.0001$).
Conclusions: IFNAR2 overexpression was observed in various histological types of lung cancers, and appears to be associated with lung cancers that behave aggressively. The results of this study strongly support the potential of IFNAR2 as a diagnostic and prognostic biomarker for lung cancer.

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SHOX2 DNA methylation is a promising biomarker for the diagnosis of lung cancer in plasma

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Background & objectives: *SHOX2* DNA methylation (^{m}SHOX2) has been shown

previously to identify lung cancer in bronchial aspirates and a test for ^{m}SHOX2 is available in Europe as an *IVD* test to aid pathologists in the diagnosis of lung cancer. DNA methylation biomarkers can also be used to detect tumor-derived circulating DNA in blood. The objective of the present study was to develop a modified assay for detection of ^{m}SHOX2 in plasma and to evaluate the clinical performance in patients.
Methods: A real time PCR duplex assay originally developed for quantification of ^{m}SHOX2 in a high background of unmethylated DNA in bronchial aspirates was modified for the unique requirements of plasma. Following assay optimization, quantitative real-time PCR was used to analyze ^{m}SHOX2 in plasma samples (n = 411). A training study was performed to determine a cut-off for patient classification (n = 20 lung cancer patients, n = 20 controls) and the resulting cut-off was verified in a testing study (n = 202 stages I – IV lung cancer patients, n = 169 controls, including patients with other cancers like e.g. of prostate).
Results: The assay reliably detected 15 pg of methylated DNA in a background of 50,000 pg unmethylated DNA. The ^{m}SHOX2 assay differentiated lung cancer patients from controls with a sensitivity of 60% and a specificity of 90%. Patients with stages II (72%), III (55%) and IV (83%) lung cancer were detected at a higher sensitivity as compared with stage I patients (27%). Small-cell lung cancer (80%) and squamous cell carcinoma (63%) were detected at higher sensitivity than adenocarcinoma (39%).
Conclusions: ^{m}SHOX2 is a promising biomarker for detection of malignant lung disease in plasma.

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Circulating tumor cells in chronic obstructive pulmonary disease patients
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Introduction and hypothesis: Migration of circulating tumor cells (CTC) in the blood stream is certainly an early event occurring during lung carcinogenesis. Chronic obstructive pulmonary disease (COPD), even in early stages, is associated with a higher incidence of lung cancer in smokers and ex-smokers. The purpose of this study was to look for the presence of CTC in a cohort of smokers having a COPD.
Methods: The presence of CTC was searched in 50 COPD patients, using both the CellSearch (CS) and the isolation by size of epithelial tumor (ISET) cells methods. Results were compared to the detection of CTC in a group of 30 patients with stage IIIB or IV non-small cell lung cancer (NSCLC) and in a group of 30 healthy individuals.
Results: 4/50 (8%) COPD patients had CTC (mean=4; range, 2-8 CTC) detected by CS and/or ISET methods. 18/30 (60%) of advanced stages NSCLC patients showed CTC (mean=16; range, 2-86). No CTC were detected in healthy individuals.
Conclusion: The detection of CTC using the CS and/or the ISET methods is a rare biological event in COPD smoker patients. In this regard, the potentiality of the CS and the ISET methods as tools for detecting early blood biomarkers of lung carcinogenesis is challenging.

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A step towards easier diagnosis of lung cancer: Detection of volatile organic compounds in air releasing tumour samples with ion- and differential mobility spectrometry
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Introduction: Lung cancer is the tumour with the highest mortality rate in the western world.
Unfortunately, the diagnostic process is still extensive.
The detection of Volatile Organic Compounds (VOCs) in air releasing tumour samples might be a method to accelerate the process of gaining first results.
Objectives: The goal in this study was to develop a method using VOCs in the detection of characteristic peaks to determine tumour containing tissue and to specify tumour entities with air samples.
Methods: Employing an Ion Mobility Spectrometer (IMS) and a Differential Mobility Spectrometer (DMS), 30 tumour and lung samples were placed into an aluminium lung simulator and analysed by the DMS and IMS via side-stream Teflon tube.
Tumour containing samples were compared to non-tumour samples from the same indivudual in order to distinguish between characteristic peaks. Furthermore the peaks were statistically analysed.
Results: The results showed various characteristic peaks and clusters in both measuring devices. These were able to differentiate between tumour and non-tumour samples (e.g. peak“P38”, p=0,0003 in adeno carcinoma samples), as well as between the tumour entities. In addition certain peaks (e.g.“P38”in the squamous cell carcinoma samples) were lower in the tumour group.
Conclusion: VOCs are able to discriminate between tumour entities and also to detect tumour containing tissue. In future research projects IMS and DMS should be compared with a closer view on standardizing peaks in order to and gain further opportunities for more precise tumour detection.