492. Bronchoalveolar lavage and biomarkers in diffuse parenchymal lung disease

P4761
Overexpression of matrix metalloproteinase-7 (MMP-7) in bronchoalveolar lavage fluid (BALF) of IPF and lung cancer patients
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It is long recognised that lung cancer has an increased frequency in idiopathic

pulmonary fibrosis (IPF), raising questions regarding the similarity of IPF and lung cancer biology. Matrix metalloproteinases regulate remodelling of the extracellular matrix, an important function for pathological processes such as angiogenesis, tissue repair and tumor invasion. We aim to assess the expression of this pathway in BALF samples of lung cancer patients and compare with IPF patients to examine possible pathogenetic links between these two lethal pulmonary diseases.
We prospectively studied 23 newly diagnosed patients, with non small cell lung cancer (NSCLC), 10 IPF patients and 10 healthy controls (C). MMP2, MMP7, MMP9, TIMP1 and TIMP2 mRNA expression levels were measured in BALF by real time RT-PCR. MMP7 protein levels were measured in BALF supernatants using ELISA kit.
mRNA expression of MMPs 2,7,9 and TIMP1 was significantly increased in lung cancer compared to controls ($p<0.05$). The IPF population showed decreased expression of the aforementioned MMPs in comparison with lung cancer while did not show significant difference at the mRNA level in comparison to controls. MMP7 protein levels (pg/μl) were significantly higher in both NSCLC and IPF populations compared to controls (NSCLC: 24.69±4.18, IPF: 18.65±2.11, C=9.76±1.92, with p values: NSCLCvsC p=0.032, IPFvsC p=0.005).
Increased expression of MMP-7 in both m-RNA and protein level may suggest a common link between those lethal disorders. Given that our population was newly diagnosed, the over-expression of these markers could be predictive of tumor progression, migratory behaviour and metastasis potential.

P4762

Expression profiling of Th17 cell activators revealed elevation of STAT-3 in progressing sarcoidosis

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Sarcoidosis is a Th1/Th17 multisystem inflammatory disorder of unknown aetiology. Although Th17 cells have been implicated in sarcoidosis and its progression, there is limited information about the molecules involved in the Th17 immune response in sarcoidosis and its phenotypes.
We, therefore, investigated, mRNA expression of Th17 pathway activators (IL-6, IL-21, IL-23, TGFbeta, RORC, STAT-3) together with the cytokines produced by Th17 cells (IL-17A, IL-17F, IL-22) by quantitative RT-PCR in bronchoalveolar (BAL) cells from 77 sarcoidosis patients (S) and 20 control subjects (C); subanalysis was performed in sarcoid phenotypes.
Of studied Th17 activators, IL-6 (mean S/C; 0.37/0.04, p=0.0001), IL-21 (0.002/0.001, p=0.001), IL-23 (0.06/0.02, p=0.001), TGFbeta (0.86/0.51, p=0.02) and RORC (0.06/0.02, p=0.0002) were up-regulated in sarcoidosis vs. controls. Expression of Th17 cytokines did not differ between sarcoidosis and controls ($p>0.05$). The expression profiling in remitting (n=27) and progressing (n=40) sarcoidosis, as assessed by the disease outcome after 2 years, revealed elevation of STAT-3 in progressing sarcoidosis (p=0.01).
In conclusion, increased expression of Th17 activators (IL-6, IL-21, IL-23, TGF-beta, RORC) was observed in sarcoid BAL cells irrespective of clinical phenotype. Enhanced expression of STAT-3, an essential regulator of Th17 cells, was detected in patients with progressing sarcoidosis. Further studies on the role of STAT-3 and Th17 cells in the progression towards the fibrosis in sarcoidosis are needed.
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P4763

Fibrocytes are detected in bronchoalveolar lavage (BAL) fluid in idiopathic pulmonary fibrosis (IPF)

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Introduction: Fibrocytes are circulating precursors for fibroblasts. Their role in the pathogenesis of IPF is debated. Blood Fibrocytes are increased in patients with IPF, but there are no data concerning BAL. The aim of this study was to determine whether fibrocytes are present in the alveolar space and identify their prognostic value.
Methods: We detected and quantified fibrocytes by FACS (CD45+, Collagen1+) in BAL from 26 patients with IPF (3 exacerbations), 8 patients with Systemic Sclerosis and lung involvement, and 11 controls. BAL cells were cultured to isolate alveolar fibroblasts. MCP-1 and CXCL12 were measured in BAL fluid.
Results: Fibrocytes were not detected in controls, but were detected in 14/26 IPF (54%) and 4/8 SSc. Median% fibrocytes was 2.5% [0,4-19,7] in IPF, 3.0% [2.7-3.7] in SSc (NS). Fibroblasts were cultured from BAL in 12/26 IPF (46%), 5/8 SSc (65%) and 2 controls (P= 0,04). The detection of fibrocytes was not associated with demographics, delay from diagnosis, fibrosis HRCT score, or% survival but was associated with lower DLCO. Fibrocytes did not correlate with MCP-1 and CXCL12 BAL concentration and was not associated with the presence of fibroblasts in culture.
Conclusion: Fibrocytes are detected in BAL fluid in about half patients with IPF and SSc. Their prognostic significance is uncertain.

P4764

Characterization of myofibroblasts cultured from small volumes of diagnostic bronchoalveolar lavage fluid samples

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Myofibroblasts are supposed to have a key role in pathogenesis of fibrotic lung diseases. Our aim was to standardize process for culturing cells from small volumes of diagnostic bronchoalveolar lavage (BAL) fluid samples and to characterize the cultured cells. Small volumes of BAL samples were collected from 98 patients that underwent bronchoscopy and BAL for diagnostic purposes. Cells were visualized by electron and immunoelectron microscopy. Proliferation and invasion capacities as well as stem cell properties of the cells were evaluated. Colonies of proliferating fibroblast type cells could be seen in 62% of samples. The success rate varied significantly based on the disease being 92% in idiopathic pulmonary fibrosis (IPF), 80% in non-specific interstitial pneumonia, 89% in collagen vascular disease associated interstitial lung disease, 62% in asbestosis, 53% in sarcoidosis, 100% in allergic alveolitis, 80% in drug reaction, 40% in lung cancer and 25% in normal lung. The success was not dependent on volume or cell amount of the BAL sample. The cultured cells were either fibroblasts or myofibroblasts. Typical features of myofibroblasts were detectable in the cells by electron and immunoelectron microscopy. Some cell samples exhibited differentiation potency into osteoblasts or adipocytes. The invasion capacity varied in different disorders being the highest in IPF-patients. We concluded that myofibroblasts can be cultured from small volumes of diagnostic BAL fluid samples. This method could increase the usability of BAL fluid both in diagnostics of interstitial lung diseases and in scientific research.

P4765

Mechanisms of impaired immune surveillance in lower airways of smokers. Data from bronchoalveolar lavage (BAL) harvested in selected interstitial lung diseases (ILDs) and control group

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Background: Cigarette smoke is recognized as a cause of lung tumors, due to its carcinogenic potential. Less is known about suppressed local immune surveillance.
Methods: Alveolar lymphocytes (AL) were obtained by bronchoalveolar lavage (BAL) in pulmonary sarcoidosis (PS, n=36), idiopathic pulmonary fibrosis (IPF, n=21) and control subjects (n=13), subdivided according to smoking status. Cytokines suspected for potential impact on antitumor immunity (TNFα, IFNγ and TGFβ) were tested by ELISA in BAL supernatants. AL were phenotyped for major subsets and cytokine receptors. AL apoptosis was examined with TUNEL and cell cycle analysis.
Results: AL apoptosis rate was reduced in PS (0.7±0.2%, $p<0.05$) and increased in IPF (2.3±1.0%, $p<0.02$) nonsmokers, as compared to controls (1.1±0.3). Cigarette smoking in all groups resulted in significant increase of AL apoptosis rate (e.g. 11±7.5% in IPF smokers, $p<0.05$ as compared to nonsmokers). IFNγ levels were lower in all smoking subgroups than in respective nonsmokers. No changes were found in TGFβ and TNFα levels, but AL expression of TGF (CD105) and TNF (CD120a) receptors was higher in smoker subgroups (significant for CD105 in IPF and CD120a in PS).
Conclusions: The decreased immune surveillance in smokers lower airways include higher AL apoptosis rate and increased AL susceptibility to TGFβ and proapoptotic stimuli. Our findings suggest relative Th2 prevalence in smoker subgroups. It may be responsible for severe course of IPF, blunted PS symptoms and, in general, increased risk of local carcinogenesis.

P4766

Expression of transforming growth factor β (TGFβ) receptor, CD105, is declined in Th1 interstitial lung diseases (ILDs)

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Background: TGFβ, as the most potent activator of lung fibrosis, stimulates fibroblast proliferation and induces secretion of collagen and other extracellular matrix proteins. It reveals its profibrotic effect by receptor complex, including CD105 molecule.
Methods: TGFβ levels was examined by ELISA in bronchoalveolar lavage (BAL) supernatants. CD105 expression was tested on BAL macrophages and lymphocytes in pulmonary sarcoidosis (PS, n=16), idiopathic pulmonary fibrosis (IPF, n=9), non-specific interstitial pneumonitis (NSIP, n=6) and extrinsic allergic alveolitis (EAA, n=7). CD105 appearance was also assessed in model lung cell lines: pneumocytes type 2 (A549) and fibroblasts (HLF-1).
Results: IPF was the only disorder with significantly increased TGFβ level. CD105 expression is common on HLF-1 (98%), A549 (63%, median of 5 trials) and alveolar macrophages. In PS significantly decreased CD105 expression on BAL lymphocytes was found (all lymphocytes: 7.2±0.6%; Th cells: 4.6±0.4%, Tc cells: 1.8±0.3%, resp. control values: 13±3.4%, 6.6±2.6% and 4.7±1.1%, median±SEM, $p<0.05$). Similar results were observed in EAA. IPF was characterized by remarkably enhanced BAL CD105+ lymphocyte percentage (all lymphocytes: 23.9±5.8%, $p<0.05$).
Conclusions: TGFβ receptor, CD105, is frequently present in lower airways. Lower BAL CD105+ lymphocyte percentage in IPF, and higher one in PS and EAA, may reflect different Th1/Th2 polarization pattern. Summarizing, the key role of diverse lower airway cell reactivity to TGFβ in ILDs should be considered.

P4767

Proteological assessment of idiopathic pulmonary fibrosis and hypersensitivity pneumonitis by mean of broncho-alveolar lavage

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Introduction and aims: Idiopathic Pulmonary Fibrosis (IPF) and Hypersensitivity Pneumonitis (HP) are both interstitial lung diseases characterized by a mixture of inflammation and fibrosis. We aim to investigate the role of different pathophysiological mechanisms by analysing broncho-alveolar lavage (BAL) fluid samples.
Methods: Bronchoscopy with BAL (4 aliquots of 50 ml saline, fraction 2-4 pooled for analysis) performed at time of diagnosis were evaluated and 3 groups; HP (n=11), IPF (n=11) and control (n=10) were analyzed by multiplex ELISA (SearchLight®). Kruskal-Wallis one-way ANOVA with Dunns post-hoc test was used for analysis.
Results: Results are displayed in the table. Differences were found regarding total lymphocyte, neutrophil and eosinophil cell counts. Differences were found in pathological mechanisms of coagulation (Protein-C, PAI-1 Active), angiogenesis (VEGF), inflammation (MCP-1, MDC, IL-8, IL-12p40), fibrosis (TGF-β1, HGF), matrix remodeling pathways (MMP-8, MMP-9) and oxidative stress (MPO).

Median (IQR)	Control (N=10)	IPF (N=11)	HP (N=11)	ANOVA
BAL Cellular Differentiation (x10³)				
Total cells	91.5 (58.0-156.0)	173.0 (62.0-392.0)	169.0 (140.0-232.0)*	0.0376
Macrophages	79.15 (49.05-135.00)	119.70 (60.14-301.40)	111.00 (68.37-135.10)	0.28
Lymphocytes	4.95 (3.92-14.07)	8.99 (5.72-30.37)	56.76 (12.28-81.12)**	<0.01
Neutrophils	0.89 (0.08-1.18)	7.33 (7.98-18.41)**	15.31 (3.61-25.35)***	<0.01
Eosinophils	0.00 (0.00-0.11)	2.91 (0.88-11.00)***	2.32 (1.12-4.40)***	<0.01
Protein Measurements				
Protein-C	52.45(24.40-131.10)	92.10(74.40-267.50)	192.60(102.00-658.60)**	0.0087
PAI-1 Active	2.85 (2.20-4.55)	5.70 (4.00-17.00)*	7.70 (2.1.-16.70)*	0.019
VEGF	686.6 (358.2-967.0)	226.2 (202.5-288.3)**	188.8 (123.8-237.7)***	<0.001
MCP-1	51.8 (33.0-107.5)	294.1 (109.6-1307.0)***	187.7 (133.0-578.2)**	<0.001
MDC	2.90(1.05-6.50)	15.60(9.00-42.50)**	7.70(4.90-15.80)	0.0048
IL-8	29.2 (13.3-58.5)	67.2 (48.7-156.8)*	70.0 (61.1-196.3)*	0.013
IL-12p40	0.30(0.30-0.65)	0.30(0.20-0.30)	0.30(0.30-4.30)	0.0067
TGF-β1	58.2 (9.8-107.4)	54.3 (18.0-104.2)	9.8 (9.8-15.2)	0.012
HGF	219.3(131.1-376.6)	318.6(275.9-1172.0)	513.8(392.3-661.9)**	0.0089
MMP-8	4483(1288-8974)	11013(6323-22561)*	10880(4764-13810)*	0.022
MMP-9	4890(1215-9245)	17280(6545-47039)**	19204(10845-56217)**	0.002
MPO	1383 (328.5-2200)	6150 (1813-21644)*	4961 (1892-12512)	0.019

* <0.05; ** <0.01; *** <0.001

Conclusion: Differences for aforementioned mechanisms were observed in IPF and HP compared to control, as well as discriminating factors between IPF and HP. These results highlight the role of BAL in the search for new biomarkers and therapies for pulmonary fibrosis.

P4768

Diagnostic value of bronchoalveolar lavage and lymphocyte transformation test in drug-induced lung disease

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Background: The diagnostic strategy of the drug-induced lung disease (DILD) has not been established. It remains to be determined whether the lymphocyte transformation test (LTT) can be used for the diagnosis of DILD. Although bronchoalveolar lavage (BAL) is often performed, its usefulness in the diagnosis of DILD is still uncertain.
Aim: We aimed to evaluate the diagnostic values of BAL and LTT in patients with DILD.
Method: We retrospectively analyzed 47 patients who were suspected as DILD and underwent BAL and LTT between January 2004 and September 2009. The total cell number and the differential count of leukocytes in BAL fluid were determined. The levels of cytokines (eotaxin-1, -2, -3, and RANTES) in the supernatant were measured by ELISA. It was also evaluated whether these parameters were correlated with the outcome of the patients.
Results: The diagnostic sensitivity and specificity of LTT were 50% and 60%, respectively. These numbers were comparable to those in previous reports. The sensitivity of increased eosinophils (>0.5%) in BAL fluid was 90%, and the specificity was 22%. The sensitivity of increased lymphocytes (>50%) was 35%, and the specificity was 78%. Furthermore, the positive likelihood ratio of LTT and increased eosinophils and lymphocytes (>10%) in BAL fluid for the prediction of the patient recovery was 0.99, 6.22, and 5.54, respectively. On the other hand, cytokine levels in the supernatant were not correlated with the diagnosis or the prognosis.
Conclusion: It was suggested that BAL fluid findings, especially the differential count of leukocytes, may be useful for the diagnosis and the prediction of outcome of the patients with DILD.

P4769

Clinical and functional features in idiopathic pulmonary fibrosis (IPF) with and without haemosiderin-laden alveolar macrophages on BALF

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We have previously reported increased frequency of haemosiderin-laden alveolar macrophages in patients whit IPF in comparison to other infiltrative lung diseases. We performed a retrospective analysis of 49 patients who received diagnosis of IPF. The diagnosis of IPF were in accord to the ATS/ERS guidelines. BAL was performed in all subjects.We evaluated the occurrence of haemosiderin-laden alveolar macrophages in patients whit IPF.Perls Prussian blue stain was used to detect haemosiderin laden alveolar macrophages. ≥400 MΦ were examined for the number of cells that stained with Perls' Prussian Blue stain, and a percentage score was estabilished by dividing the number of Prussian-blue-positive cells by the total number of cells counted. Perls positivity were defined as percentage of BALF MΦ > 50%. Patients were divided in 2 groups based on BALF Perls positivity: IPF BALF MΦ P+ 15/49 (30%) and IPF BALF MΦ P – 24/49 (70%).Variables compared between the 2 groups included cellular BALF data, pulmonary function tests, arterial blood gases, and PAPs estimated by echocardiography.
DLCO% th and% oxygen desaturation during 6MWT were significantly reduced in IPF BALF MΦ P+ in comparison to IPF BALF MΦ P- (respectively 29±9 vs 41±14, p=0,03; and 18±5 vs 12±1, p= 0,02) while FVC%th (68±18 vs 71±19) and resting paO2 mmHg 64±17 vs 69±9) were not significantly different.
Conclusions: Increased haemosiderin laden alveolar macrophages is associated to more severe impairment of lung diffusion and higher oxygen desaturation during exercise.

P4770

Differential cell count in BAL by flow cytometry using CD15 FITC/CD16PE/CD45 PERCP/HLA-DR APC monoclonal antibodies

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Introduction: Usually inflammatory cell counts in bronchoalveolar lavage (BAL) are done manually on optical microscopy (OM) despite the high intra-and interobserver variability. The objective was to compare inflammatory cell counts performed by OM with these counts by flow cytometry (FC) with a new combination of monoclonal antibodies.

Methods: 34 BAL samples were analysed in a 2-laser cytometer (FACSCalibur). The results were compared with those obtained by optical microscopy. The proposed combination of monoclonal antibodies identify leukocytes as CD45 + cells and lymphocytes as $CD15^-$, $CD16^-$ and $CD16^{dim+}$(NK lymphocytes), $HLA\text{-}DR^-$ and $HLA\text{-}DR^+$ (B cells and activated lymphocytes) cells; neutrophils as $CD15^{bright+}$, $CD16^{bright+}$, $HLA\text{-}DR^-$ cells; eosinophils as $CD15^{bright+}$, $CD16^-$, $HLA\text{-}DR^-$ cells and alveolar macrophages as $CD15^{dim+}$, $CD16^{bright+}$, $HLA\text{-}DR^{bright+}$ cells. Macrophage's autofluorescence (AF) was overcome using the monoclonal antibody anti-HLA-DR conjugated with the dye APC as the main identification marker.
Results: Agreement analysis for both methods shown high correlations (r=0.70 to 0.93; $p<0.001$) but FCM overestimates lymphocyte population +13 (15.8)%, and conversely underestimates alveolar macrophage population –15.6 (19.6)%.
Conclusions: The monoclonal antibodies combination proposed is effective and reliable to identify leukocyte populations in BAL. The process is simpler and faster than manual optical microscopy but some differences in macrophages and lymphocytes counts should be considered.

P4771
LSC 2011 Abstract: Bronchoalveolar lavage in radiation pneumonitis after radiotherapy for breast cancer
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Radiation pneumonitis is a complication of radiotherapy which limits its application in cancer therapy.
Aim: To compare the bronchoalveolar lavage (BAL) findings in patients with symptomatic radiation pneumonitis (RP) versus asymptomatic RP.
Material and method: We evaluated 65 female patients with RP after radiotherapy for breast cancer.
Results: Forty-nine patients were symptomatic (fever, cough and/or dyspnea) and 16 were asymptomatic. All patients had a newly discovered infiltrate or consolidation on chest radiography, corresponding to the radiation field. BAL in symptomatic patients had an increased number of cells.

	Symptomatic patients		Asymptomatic patients	
	Mean	SD	Mean	SD
No of cells (x10^6/dL)	17.71	12.15	11.9	6.15
Macrophages (%)	51.92	22.1	69.1	15.37
Lymphocytes (%)	34.9	18.81	26.14	14.3
Neutrophils (%)	8.88	12.63	3.34	2.28
Eosinophils (%)	2.56	5.99	1.22	2.35
Mastocytes (%)	0.43	0.88	0.21	0.25
CD3 (%)	87.18	9.54	88.67	1.53
CD4 (%)	58.94	15.89	46.33	15.04
CD8 (%)	26.41	9.96	38.67	16.04
CD4/CD8	2.72	1.55	1.5	1.15

Lymphocytosis was present in all patients with RP, but it was higher in symptomatic (34.9±18.81% vs. 26.14±14.3%). Macrophages were decreased in all patients. Neutrophils were slightly increased (8.88% in symptomatic and 3.34% in asymptomatic) and eosinophils were normal in both groups (2.56% and 1.22, respectively). Almost all lymphocytes were T type (CD3+). CD4+ lymphocytes were increased in both groups with normal CD4/CD8 ratio (2.72 in symptomatic and 1.5 in asymptomatic group).
Conclusion: Lymphocytic alveolitis with T lymphocytes was present in all patients with RP with a higher proportion in symptomatic patients.

P4772
Detection of differences in volatile organic compounds (VOCs) by ion mobility spectrometry (IMS) of exhaled breath in patients with interstitial lung diseases (ILDs) compared to healthy controls (HC)
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Introduction: ILDs comprise a heterogenous group of disorders involving the lung parenchyma. Rapid and accurate diagnosis is often complicated by the need of assessing the pathological changes in the lung parenchyma by bronchoalveolar lavage, transbronchial biopsies or open lung biopsy.
Objectives: The aim of our study was to compare the pattern of VOCs in exhaled breath of patients with extrinsic allergic alveolitis (EAA), non-specific interstitial pneumonia (NSIP), idiopathic pulmonary fibrosis (IPF) or sarcoidosis (SA) to healthy controls (HC).
Methods: Detection of VOCs in exhaled breath was performed by ion mobility spectrometry (IMS) coupled to a multi-capillary column (MCC) for pre-separation (MCC-IMS, B&S Analytik). ILD patients with EAA (n = 16), NSIP (n = 28), IPF (n = 26), SA (n = 21) were compared to HC (n = 39).
Results: Overall 215 peaks could be detected in exhaled breath of patients and controls. The EAA and the HC groups differed in 115 peaks. The IPF and the HC groups differed in 26 peaks, the NSIP and HC groups in 63, the SA and HC groups in 3 peaks.
Conclusions: IMS seems to be a promising technique to discriminate different ILDs from healthy controls. However, confirmation of our findings in a larger study population is needed. Also further investigations have to be undertaken, to address the question, whether different ILDs can be distinguished from one another by distinct peak patterns.

P4773
Phospho-ERM localization in UIP specimens: An immunohistochemystry approach
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Background: Idiopathic Pulmonary Fibrosis (IPF) is a progressive, fatal lung disease of unknown etiology still lacking of effective therapy. IPF has a poor prognosis with a median survival of 2.5-3.5 years, and it is associated with lung cancer with a prevalence ranging from 4.8% to 48%. Molecular mechanisms of carcinogenesis occurring in IPF remain to be clarified.
Aim and objective: The family of ezrin/radixin/moesin (ERM) proteins is essential for maintenance of cell shape, cell adhesion, migration and division, serving as an important cross-linker between the plasma membrane and cytoskeleton. Recent studies showed that ERM is upregulated in multiple types of metastatic cancers. In the current investigation, we tested the hypothesis that ERM interacts and play a key role in epithelial-mesenchimal transition (EMT) in alveolar epithelial cells.
Methods: In order to identify the relationship between lung cancer and IPF we assessed an immunohistochemistry analysis for phospho-ERM in the following pulmonary biopsy specimens: 20 IPF/UIP, 4 adenocarcinoma, 6 cryptogenic organizing pneumonia (COP), and 4 normal controls.
Results: Our preliminary data showed in normal lung samples a totally negative phospho-ERM immunostaining. We found a weak positivity in COP samples, whereas in UIP samples we found a higher global expression, in particular in activated type II pneumocytes and basal bronchiolar cells.
Conclusion: We hypothesize that activation of ERM proteins could be involved in UIP pathogenesis, leading to possible contribute to the EMT process of lung epithelial cells.

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Serum napsin A is a novel diagnostic and monitoring marker for interstitial lung disease
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KL-6, and Surfactant Protein (SP)-A and SP-D have been reported as sensitive markers for interstitial lung disease (ILD). However, a more useful serum marker for diagnosis and activity of ILD may be found in napsin A, an asparatic protease, mainly expressed in alveolar type-II cells and the kidney. This study sought to evaluate the diagnostic and monitoring value of napsin A in patients with ILD in comparison with KL-6, SP-A, and SP-D, and to determine whether serum napsin A levels reflect renal dysfunction.
Subjects consisted of 34 patients with ILD, 20 patients with lung adenocarcinoma, 12 patients with kidney disease, and 20 healthy subjects. Serum samples were analyzed for each marker by ELISA. The area under the receiver operating characteristic curves (AUC) were used to determine appropriate cut-off levels resulting in optimal diagnostic accuracy using napsin A as a marker; differences in serum levels of these markers were investigated. Further, 10 patients with ILD were treated and evaluated for treatment efficacy and usefulness of napsin A as a monitoring marker.
The AUC values for ILD patients in comparison with control subjects were 0.949 for napsin A, 0.978 for KL-6, 0.896 for SP-A, and 0.791 for SP-D. As specific markers to distinguish ILD from lung cancer, the AUC values were 0.907 for napsin A, 0.939 for KL-6, 0.762 for SP-A, and 0.672 for SP-D. The accuracy of changes in these serum markers reflecting response to therapy were 80% for napsin A, 50% for KL-6, 70% for SP-A, and 70% for SP-D. Moreover, serum napsin A level reflected no renal dysfunction.
Serum napsin A is a novel diagnostic and monitoring marker for ILD that is superior to SP-A and SP-D, and equivalent to KL-6.

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Extracellular matrix profile of lung in idiopathic pulmonary fibrosis
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Rationale: Idiopathic pulmonary fibrosis (IPF) is a progressive parenchymal lung disease of unknown aetiology and poor prognosis, characterized by progressive fibrosis of the alveolar interstitium. The ECM is a critical component in regulating cellular homeostasis and appropriate wound healing. The aim of our study was to determine the extracellular matrix protein profile of fibrotic lungs.
Methods: ECM gene expression and protein profile of lung of 12 patients with IPF, 5 chronic obstructive pulmonary disease (COPD) patients with emphysema and 7 subjects with pneumothorax (control group) were analysed by cDNA microarrays, rt-PCR and immunohistochemistry.
Results: Microarray analyze showed a total of 20 ECM proteins upregulated and 6 proteins downregulated in fibrotic lungs compared with normal lungs. Interstitial structural proteins like collagen I and III had a significant increase in IPF compared with normal lungs and emphysema. Migratory proteins (tenascin C and versican) were also significant augmented in fibrosis. These proteins were located in fibroblastic foci by immunohistochemistry. The main basal membrane protein, collagen IV, were downregulated in fibrotic lungs. There was a statistically significant increase of some metalloproteins in lungs of IPF patients compared with normal and emphysema lungs (MMP-1, MMP-7, MMP-9, MMP-12 and MMP-13).
Conclusion: ECM protein profile of fibrotic lungs show profibrogenic properties that could help disease progression and perpetuation of lung fibrosis.These findings should be considered in the IPF physiopathology for future antifibrotic therapeutic approaches.
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Elevation of serum tumor markers in patients with interstitial lung disease
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The risk of lung cancer is high in patients with interstitial lung disease (ILD). It was reported that tumor markers was increased in ILD patients. The serum levels of CEA, CA19-9, CA125 and NSE were measured in 58 ILD combines lung cancer (ILD-CA) patients, 632 ILD patients and 632 control subjects. The analysis of covariance, multiple linear regression, Logistic regression analysis and ROC curve were used for statistical analyses. The serum levels of CEA, CA19-9, CA125 and NSE showed decrement tendency in ILD-CA group, ILD group and the control group ($P < 0.01$). They were higher in patients with ILD-CA than those in patients with ILD and control ($P < 0.05$). The levels of CEA and CA125 in ILD group were higher than the control group ($P < 0.01$). The ILD group included idiopathic pulmonary fibrosis (IPF) subgroup of 214 cases, non-IPF idiopathic interstitial pneumonia (nonIPF-IIP) subgroup of 97 patients, collagen vascular disease associated ILD (CVD-ILD) subgroup of 163 cases, and other ILD subgroup of 158 cases. The serum levels of CEA, CA19-9 and CA125 were the highest in patients with IPF than the other three subgroups of ILD ($P < 0.01$). Adjusting the confounding factors such as gender, age and smoking condition, the risk of ILD-CA increased with the elevation of CEA and CA125. This study provides that the elevation of serum tumor markers in patients with ILD-CA is a common sign, and is also an important sign in patients with ILD, especially in patients with IPF. Incidence of lung cancer is increased in ILD patients with elevation of the serum levels of CEA and CA125.

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Interferon-g or azathioprine plus corticosteroids do not alter the expression of apoptotic markers in alveolar macrophages of patients with IPF
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Introduction: Pathologic apoptosis is described in IPF. Macrophage activation is implicated in the pathogenesis of fibrotic lung diseases. The apoptotic profile of alveolar macrophages (AM) in IPF is unclear.
Aim: To examine the expression of apoptotic proteins in AM of patients with IPF after therapeutic intervention.
Methods: Twenty newly diagnosed IPF patients were randomised in group A treated by a combination of interferonγ-1b and prednisolone and group B treated by azathioprine and prednisolone. Groups were compared regarding clinical deterioration, lung function, and bronchoalveolar lavage (BAL) apoptosis markers.
We analyzed by immunohistochemistry, the expression of the anti- apoptotic markers bcl-2 and the pro-apoptotic markers bax, fas, fas ligand in AM obtained from BAL before and after treatment. We measured apoptosis by TUNEL.
Immunohistochemistry results were evaluated with a digital image analysis assay.
Results: No difference was observed regarding age, gender, smoking habit between the two groups of IPF patients. The patients of both groups had similar FEV1, FVC and DLCO values at entry and after six months of treatment.
We found no difference in the expression of apoptotic markers in AMs before and after treatment between groups. We found no correlation between clinical and functional parameters and change in apoptotic markers.
Conclusions: We specifically examined the expression of apoptotic markers in AMs of patients with IPF and we report that different treatment options do not affect the expression of these markers in IPF. These results may be related to the ineffectiveness of pharmacological therapies for IPF.

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Reliability of ATS/ERS criteria for the diagnosis of idiopathic pulmonary fibrosis
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Background: The 2002 ATS/ERS Consensus Classification has been widely accepted as the standard classification for interstitial lung diseases (ILD). However, the diagnostic accuracy of the major/minor criteria in the ATS/ERS Classification for idiopathic pulmonary fibrosis (IPF) is still controversial.
Aims and objectives: To evaluate the reliability of the major/minor criteria in the ATS/ERS Classification for IPF.
Methods: Patients with ILD admitted to Ruhrlandklinik (Essen, Germany) were retrospectively studied. All patients presenting with an insidious onset and a duration of illness of >3 months were included. Because of the concept that the exclusion of known causes for ILD is not always easy, we also enrolled patients with secondary ILD (dust/drug exposure or CVD-associated). The diagnostic accuracy of the major/minor criteria for IPF was evaluated.
Results: A total of 163 patients with suspected ILD were studied. The final diagnoses of the enrolled patients were IPF (n=82), other IIP (n=42) and secondary ILD (n=39). In the univariate analysis, the p-value of the criteria (%VC<80%,%TLC<80%,%D_{LCO}<80%, A-aD_{O2}>=25Torr, PaO_2<60Torr, typical findings for IPF in HRCT, BAL lymphocytosis <30% age>50, and bibasilar crackles) for the diagnosis of IPF were 0.04, 0.94, 0.65, 0.08, 0.29, <0.0001, <0.0001, 0.97, and 0.0015, respectively. In the multivariate analysis, the typical findings for IPF on HRCT ($p<0.0001$) and BAL lymphocytosis <30% ($p=0.0001$) showed independent diagnostic significance for IPF.
Conclusions: Typical findings for IPF in HRCT and BAL lymphocytosis <30% were of diagnostic significance for IPF.