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74. Bronchoalveolar lavage and phenotyping in diffuse parenchymal lung disease

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Idiopathic interstitial lung disease with anti-ssa/ssb antibodies: a forme fruste of connective tissue disease?

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There is no consensus about immunological tests to perform at the diagnosis of interstitial lung diseases. Some experts always test patients for anti-SSA and anti-SSB antibodies. Our hypothesis was that idiopathic interstitial lung disease with anti-SSA/SSB antibodies could identify a subgroup of patient with connective tissue disease. To test this hypothesis, we retrospectively compared the characteristics of patients with an interstitial lung disease newly diagnosed with an anti-SSA or anti-SSB antibody (SSA/SSB+ group) to a group of patients with an idiopathic interstitial lung disease without anti-SSA or anti-SSB antibody diagnosed the same year (SSA/SSB- group). The patients from the SSA/SSB+ group (n=15) had more often extra-respiratory signs (xerostomia and xerophthalmia), auto-immunity features and a scannographic pattern of non specific interstitial pneumonia, than the patients from the SSA/SSB- group (n=30). Only 67% of patients from SSA/SSB+ group had positive anti-nuclear antibodies. Lung function alteration was more severe at diagnosis but long term survival was not different. Most of the patients from the SSA/SSB+ group (13/15) met the criteria for the diagnosis of undifferentiated connective tissue disease (Kinder, AJRCCM 2007;176:691-7) and 2/15 met the criteria for Sjögren's syndrome. Our results suggest that the detection of anti-SSA or anti-SSB antibodies in patients with idiopathic interstitial pneumonia identifies a sub-group of patients with a forme fruste of connective tissue disease.

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Inflammatory and regulatory cytokine IL-6 in plasma and BAL fluid of patients with pulmonary sarcoidosis

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Background and aim: Recent advances have revealed that inflammatory cytokine IL-6 play an important regulatory role in several systemic chronic inflammatory diseases including sarcoidosis. However, there are only scarce and contradictory data about IL-6 in plasma and BAL fluid of patients with pulmonary sarcoidosis. Thus the aim of our study was to measure concentrations of IL-6 in BAL fluid and correlate them with its concentrations measured in plasma of patients with pulmonary sarcoidosis.

Methods: IL-6 was measured by ELISA (Endogen Pierce, USA) in plasma and BAL fluid of 50 patients with pulmonary sarcoidosis aged 44 (24 to 76) years, 29 males and 21 women.

Results: We found significantly higher concentrations of IL-6 in BAL fluid compared to plasma concentrations (4.3, 2.1-8.1 pg/ml vs. 1.6, 1.0-2.1 pg/ml, p<0.001, values: medians with 1st and 3rd quartile). We found no correlations between plasma and BAL fluid concentrations of IL-6 and no correlation with disease stages.

Conclusions: Our results showed that local production of IL-6 in the lungs of patients with pulmonary sarcoidosis is present in all stages of the disease. Stimulus for its production is rather local than systemic, since no correlations were found between systemic and local, increased concentrations of IL-6. However what triggers this increased production of IL-6 in lungs of patients with sarcoidosis is still unknown. It is also unknown, whether these increased levels are associated with worse prognosis of sarcoidosis, what have to be determined in further prospective studies.

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Bronchoalveolar lavage fluid cellular characteristics, functional parameters and cytokine and chemokine levels in interstitial lung diseases

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Introduction: Idiopathic pulmonary fibrosis (IPF), hypersensitivity pneumonitis (HP) and sarcoidosis belong to interstitial lung diseases (ILDs) where an imbalance of regulatory, profibrotic and antifibrotic cytokines is hypothesized. The relationship of bronchoalveolar lavage (BAL) fluid (BALF) cytokines, BALF cell profile and ILD course is supposed. The aim of our study was to correlate BALF cytokine and chemokine levels with BALF cell profile and lung functions in different ILDs.

Methods: 22 sarcoidosis, 7 IPF and 11 HP patients underwent lung function tests and BAL. The BALF differential cell counts and superficial cell markers were characterised and MCP-1, MIP-1alpha, MIP-1beta, RANTES, ENA-78, FGF, G-CSF, GM-CSF, IFN-gamma, IL-1alpha, IL-1RA, IL-1beta, 2,4,5,6,8,10,17, TNF-alpha, Tpo and VEGF values measured.

Results: The BALF VEGF values were highest in sarcoidosis (p=0.0526). IL-1RA values were higher in IPF and HP compared to sarcoidosis (p= 0.0334). IL-8/ENA-78 ratio positively correlated with BALF neutrophil counts in IPF (r=0.89, p=0.04). VC and TLCO values positively correlated with VEGF and negatively with IL-8 BALF levels in all ILDs but the correlations were most significant in sarcoidosis group.

Conclusions: We suppose that VEGF plays a role in ILDs early phases and has rather angiogenic than profibrotic effect. On the contrary IL-8 is probably upregulated in advanced ILDs with prominent fibrosis and marked lung functions decline. We state that BALF VEGF, IL-8 and ENA-78 levels and IL-8/ENA-78 ratio could become useful markers of ILDs' phase, activity and prognosis. They might be also helpful in treatment modality choice.

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Diagnostic value of total cell count in bronchoalveolar lavage fluid from patients with interstitial lung diseases

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Bronchoalveolar lavage (BAL) is a useful technique for diagnosing interstitial lung diseases. Cytological examination of the BAL fluid usually concerns analysis of the differential cell count. This study was conducted to estimate the diagnostic value of total cell count in BALF. Of 750 routine diagnostic BAL examinations we selected 222 samples which cytological pattern confirmed diagnosis of interstitial lung disease. BAL was performed according to the international standards, the total cell count (TCC) was counted using a Bürker chamber. We expressed TCC as total of recovered cells ($\times 10^6$). Control group consisted of 17 healthy nonsmoking (HNS) and 12 smoking volunteers (HS). The group of the analyzed diseases consisted of sarcoidosis-SA, idiopathic pulmonary fibrosis- IPF, cryptogenic organizing pneumonia- COP, hypersensitivity pneumonitis- HP, chronic eosinophilic pneumonia-CEP and vasculitis. For data comparison the Kruskal- Wallis test was used. TCC in the BALF of healthy and patients were presented in the Table.

BALF TCC (mean, SD)

SA	IPF	HP	COP	CEP	Vasculitis	HNS	HS
14.2±9.5	25.78±20.5	22.8±17	20.7±12.8	23.9±12.3	28.5	5.1±3.1	9.9±5.5

Statistical analysis revealed a significant difference (p<0,05) in the TCC in BALF between healthy non-smoking controls and patients with IPF, HP, COP, CEP and vasculitis. The highest TCC was observed in patients with IPF. The sensitivity and specificity in ILD diagnosis was: 60 and 96% for the TCC higher than 15×10^6 and 48 and 96% for 20×10^6 , respectively.

TCC is a valuable diagnostic marker in the cytological examination of BALF samples. High value of TCC is strongly suggestive for interstitial lung disease.

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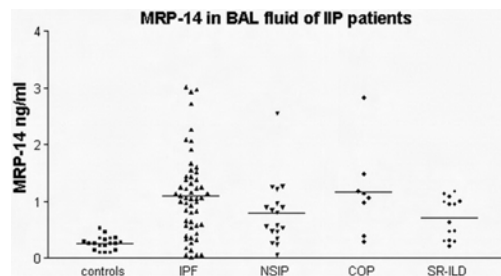
BAL levels of MRP-14 in IIPs

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Introduction: The migration inhibitory factor related proteins (MRP's) are highly expressed in neutrophils, macrophages and on epithelial cells during infections. MRP-14 (also known as S100A9 and Calgranulin B) promotes neutrophil chemotaxis and migration. MRP-14 could also stimulate fibroblast proliferation (1). MRP-14 was shown by gel electrophoresis to be elevated in IPF lungs (2). We hypothesize that MRP-14 is also elevated in the lungs of other Idiopathic interstitial pneumonia (IIP) patients, where fibrosis and increased numbers of neutrophils can be found in the lungs.

Methods: We measured MRP-14 in Bronchoalveolar lavage (BAL) fluid from 90 patients and 19 controls using an ELISA (BMA Biomedicals, Augst, Switzerland).

Results: BAL fluid levels of MRP-14 were significantly higher in IPF, NSIP, COP and SR-ILD patients compared to controls.



The average BAL level in controls was 0.26 ± 0.03 (SE) compared to 1.1 ± 0.1 in IPF patients ($P < 0.001$). BAL MRP-14 levels were significantly correlated with BAL neutrophils ($r = 0.34$, $P = 0.001$ Pearson). There was no correlation with CRP.

Discussion: MRP-14 levels are significantly elevated in the BAL of IIP patients. MRP-14 might be involved in neutrophil migration to the lung. Neutrophils are thought to be involved in fibrosis formation, and MRP-14 could therefore be an important factor in disease evolution in idiopathic interstitial pneumonias.

References: 1. Shibata, F. et al. *Biol Pharm Bull* 2005;28:2312-4.
2. Bargagli, E. et al. *Inflammation* 2008;31:351-4.

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BAL F proteomic analysis in polymyositis/dermatomyositis patients with interstitial lung disease

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Idiopathic inflammatory myopathies (IIMs) are a heterogeneous group of disease in which interstitial lung disease (ILD) is not a rare finding; the prevalence of lung involvement in this setting is different according to disease subsets evaluated. In fact in classic polymyositis (PM) and dermatomyositis (DM) ILD occurs in 20-30% of cases, whereas in anti-synthetase syndrome (AS) the prevalence ranges from 50 to 85%. Recently it has been emphasized the occurrence of overlapping features between myositis and systemic sclerosis (OM). Beside the prevalence, ILD impact in IIMs is different also for prognosis and treatment. On this basis, we assessed by 2DE the BALf protein profile of 3 DM, 4 AS and 4 OM patients, all with active untreated ILD, focusing on proteins exclusively expressed by DM patients.

Among the 258 protein spots characterizing DM patients, only 9 were not found in AS and OM patients, being so exclusive of DM. These 9 spots corresponded to 9 proteins with known function: 4 proteins were involved in oxidative stress and inflammation (Peroxiredoxin I, Enolase, Coenzyme Q10, 3-Hydroxybutyrate), 4 are classified as cytoskeletal proteins (gelsolin, cofilin, myotonic dystrophy kinase, vimentin) and the last Fatty acid-binding protein 5 which might be involved in surfactant lipid metabolism. To the best of our knowledge this is the first study providing evidences of a unique BALf protein profile in DM patients with ILD. Our preliminary results support that the proteomic analysis of BALf could be helpful in the explanation of the differences observed between DM and other IIMs subsets.

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Bronchoalveolar lavage (BAL) lymphocytes from patients with interstitial lung diseases (ILD) produce hepatocyte growth factor (HGF)

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Background: HGF in lower airways is secreted by macrophages, fibroblasts and pneumocytes. It exerts biological activities by its tyrosine kinase receptor, proto-oncogene MET. Since HGF strongly inhibits pulmonary fibroblasts, it is used in experimental therapies of lung fibrosis, including gene therapy of idiopathic pulmonary fibrosis (IPF).

Methods: HGF expression in lower airways was tested by ELISA in BAL supernatants from pulmonary sarcoidosis (PS), IPF/UIP, extrinsic allergic alveolitis (EAA) and nonspecific interstitial pneumonia (NSIP) patients ($n = 17, 12, 7, 6$, resp.). BAL cells were stained for superficial MET and IFN γ receptor (CD119) as well as for intracellular HGF expression.

Results: HGF level was increased in all patient groups (EAA: 160 ± 22 , IPF: 129 ± 14 , $p < 0.05$; PS: 123 ± 45 pg/mL) as compared to controls (74 ± 5 pg/mL, median \pm SEM). Systemic therapy with steroids resulted in decline of HGF expression in respective IPF and PS subgroups. Increased MET expression on BAL lymphocytes and macrophages as well as CD119 on BAL Th cells was shown in PS, EAA and especially in IPF (e.g. MET+ macrophages: $27 \pm 7\%$ in IPF vs $1 \pm 1\%$ in controls; $p < 0.05$). Surprisingly, relatively high rate (12-100%) of BAL lymphocytes was positive with intracellular HGF, as compared to BAL macrophages; this event was independent on the tested disease.

Conclusions: Enhanced HGF is a characteristic finding in all ILD examined in the study. However, increased percentage of both MET+ and IFN γ R+ BAL immune cells may suggest relative local HGF deficiency in IPF. Lower airway T cells should be considered as an important source of HGF.

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Clinical characteristics and follow-up of pulmonary MALT lymphoma

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MALT lymphoma is a low grade B-cell lymphoma and the most frequent subset of primary pulmonary lymphoma (PPL). MALT PPL is a rare disease and prior reports are based on limited patient numbers.

Our objective was to analyze the characteristics at diagnosis and outcome of patients with MALT PPL.

All chest and pathological departments of adult teaching hospitals in Paris were contacted to identify patients with a histological diagnosis of MALT PPL. Sixty-three cases meeting the study criteria were identified. Sex ratio was 1 and median age was 60 years. Thirty-six percent of cases had no symptoms at diagnosis. On CT-scan, 48% have a bilateral lung involvement. Forty-six percent had at least one extra-pulmonary location and 24% had a gastric involvement. The presence of monoclonal immunoglobulin was associated with an extra-pulmonary location. Fifteen patients had a PET-scan, among which 12 showed a mild FDG uptake in the lung. Nineteen BAL (43 performed) were available for both B-cell phenotyping and clonality analysis, and 15 had more than 15% of B-cell alveolitis corresponding to a strong B-cell clonal population. Bronchial biopsies were conclusive in 19/61 cases, transbronchial biopsies in 23/26 cases, percutaneous lung biopsies under CT scan in 8/10 cases and surgical biopsies in 18/18 cases. The estimated 5- and 10-year overall survivals were 90% and 72%, respectively. Age and performance status were the only 2 adverse prognostic factors for survival. The estimated 5- and 10-year progression free survivals (PFS) were 51% and 36%, respectively. Presence of mediastinal adenopathy on CT scan and treatment

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with cyclophosphamide or anthracyclin, when compared to chlorambucil, were associated with shorter PFS.

P713**Collagen and elastic system evaluation in infectious and non-infectious granulomatous lung diseases**

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Introduction: In this study, we evaluated lung extracellular matrix alterations (collagen system and elastic) in granulomatous lung diseases, specially sarcoidosis, hypersensitivity pneumonitis, tuberculosis and histoplasmosis.

Methods: We examined extracellular matrix (collagen and elastic fibers system) in sarcoidosis (N=21), hypersensitivity pneumonia (N=11), tuberculosis (N=11) and histoplasmosis (N=3) cases. We used the Picrosirius-polarization method, Weigert's resorcin-fuchsin histochemistry and morphometric analysis to evaluate the amount of normal parenchymal and granulomas collagen/elastic fibers.

Results: The granuloma and normal parenchymal areas measurements of collagen fibers were higher in hypersensitivity pneumonitis ($21,57\% \pm 8,37\%$ and $22,07\% \pm 9,40\%$, respectively) compared to sarcoidosis ($15,65\% \pm 2,11\%$ and $11,33\% \pm 1,75\%$), tuberculosis ($15,48\% \pm 6,80\%$ and $10,97\% \pm 5,15\%$) and histoplasmosis ($15,85\% \pm 8,93\%$ and $9,76\% \pm 1,77\%$). Equally higher was the granuloma measurement of elastic fibers in hypersensitivity pneumonitis ($2,99\% \pm 1,27\%$) compared to sarcoidosis ($1,18\% \pm 0,37\%$), tuberculosis ($0,15\% \pm 0,00\%$) and histoplasmosis ($0,42\% \pm 0,18\%$). The comparison between infectious and non-infectious diseases showed a significant ($p=0,005$) higher quantity of elastic fibers in granuloma areas of non-infectious diseases ($1,53 \pm 2,02$) compared to infectious diseases ($0,29\% \pm 0,21\%$).

Conclusion: We concluded that granulomas of HP are more fibroesclerotic than sarcoidosis and infectious, indicating a more intense reparative process due immune response activation after chronic and repeated inhalatory injury.

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P714**Human napsin a is a useful serum marker of interstitial lung disease, compared to KL-6, and surfactant protein-D**

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KL-6, and Surfactant Protein D (SP-D) are reported to be sensitive markers for Interstitial Lung Disease (ILD). However, Human Napsin A (HNA) is thought to possess greater more sensitivity and specificity. The aim of this study was a comparative analysis of the diagnostic value of HNA and these existing markers. Subjects consisted of 34 ILD patients (25 cases of idiopathic fibrosis and 9 associated with collagen vascular disease), 20 lung cancer patients (adenocarcinoma), and 12 healthy volunteers. HNA serum levels were analyzed using a sandwich assay kit (Human Napsin A Assay Kit-IBL). Serum HNA levels were 126.5 ± 88.6 ng/ml for the ILD patients, 28.2 ± 17.7 ng/ml for the healthy volunteers, and 41.4 ± 12.7 ng/ml for the lung cancer patients. Serum levels of KL-6 and SP-D were determined using commercially available enzyme-linked immunosorbent assay kits. The cut-off levels of the serum marker's were 62 ng/ml for HNA, 500 U/ml for KL-6, and 110 ng/ml for SP-D. For the ILD patients, the serum KL-6 level was 899.4 ± 513.2 U/ml, and for SP-D 152.0 ± 160.5 ng/ml. Abnormally high levels of HNA were observed in 76.6% of the ILD patients, compared with 70.5% for KL-6, and 50% for SP-D. These observations suggest that serum level of HNA is useful in diagnosis for ILD patients.

P715**Profiling of gene expressions induced by TGF-beta1 and cyclosporine a in alveolar cells in vitro**

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Rationale: TGF-β1 has pivotal roles in pulmonary fibrosis but its precise effects on alveolar epithelial cells and alveolar capillary endothelial cells, which are supposed to be crucial as well as fibroblasts, have not been elucidated yet. In addition, the plausible effects of cyclosporine A (CsA) against pulmonary fibrosis have not been understood through molecular biology.

Aim: To clarify the effects of TGF-β1 and CsA on alveolar cells using microarray-based gene-expression profiling.

Methods: Three distinctive cell lines of alveolar cells: human fetal lung fibroblasts (MRC-5), human microvascular endothelial cells (HMVEC-L) and human alveolar epithelial cells (A549), were treated with TGF-β1 with or without CsA, and the alterations of gene expression were analyzed by microarray technique.

Results: The differentiation of MRC-5 to myofibroblasts induced by TGF-β1 were blocked by CsA, which decreased expressions of 197 genes out of 608 genes

upregulated by TGF-β1 and increased 72 out of 841 genes downregulated by TGF-β1. In A549, 8 out of 628 genes downregulated by TGF-β1 and 32 out of 3554 genes upregulated by TGF-β1 were recovered by CsA. In HMVEC-L, 4 out of 299 genes downregulated and 13 out of 31 genes upregulated by TGF-β1 were recovered by CsA.

Conclusions: These results revealed simultaneous gene-alterations by TGF-β1, which are recovered by CsA in the alveolar cells independent of immune-modulation.

P716**Differences of angiogenic factors in BALF in ILD**

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The same factors play role in angiogenic activity in idiopathic pulmonary fibrosis (IPF) and chronic extrinsic allergic alveolitis (EAA): epithelial neutrophil activating peptide (ENA)-78, interleukin (IL)-8 and vascular endothelial growth factor (VEGF).

The aim of our study was to find a possible difference in angiogenic activity between both diseases. We examined the influence of concentration of angiogenic chemokines in bronchoalveolar lavage fluid (BALF) on high resolution computed tomography (HRCT) pattern of IPF and EAA.

Seven IPF and 11 chronic EAA patients were enrolled to the study. Concentrations of IL-8, ENA-78 and VEGF in BALF supernatants were quantified using multiplex bead array assay.

IL-8, ENA-78 and VEGF in BALF

	IL-8	ENA-78	VEGF
IPF	44,6±41,1*	74,6±76,5*	16,9±15,5*
EAA	83,5±105,5*	78,7±79,5*	30,2±41,1*

Values are means ± SD. Chemokine levels are in pg/mL, *p>0.05.

The VEGF level in BALF of IPF group negatively correlated with the HRCT interstitial score ($p < 0,05$). IL-8 BALF level positively correlated with the alveolar score ($p < 0,05$) in the same group.

To our knowledge this is the first study that investigates the relationship between BALF angiogenic chemokine concentrations measured with the multiplex bead array assay and HRCT scores in IPF and chronic EAA.

Our data indicate that angiogenic chemokine profiles in BALF of chronic EAA and IPF patients may be similar. Positive correlation between IL-8 BALF concentration and HRCT alveolar score in IPF group ($p < 0,05$) may reflect IL-8 production by fibroblasts and other cells in the initial phase of fibrotic response. Changes of cytokine production during the disease course may cause the magnitude of variability of results.

P717**Increased mRNA expression of collagen V gene in pulmonary fibrosis of systemic sclerosis**

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Background: Our previous studies done with animal model of systemic sclerosis (SSc) had proven that fibrosis was due to increased amount of abnormal collagen type V (Col V). We propose to analysis Col V in lung tissue of SSc patients.

Methods: We examined the amount of collagen V and mRNA chains expression using immunofluorescence, Real-time PCR and computer morphometric analysis in 15 open lung biopsies of patients with SSc. The pulmonary function tests were analyzed and correlated with collagen amount and PCR chains expression. Normal lung tissue was obtained from 8 individuals who had died from traumatic injuries.

Results: Immunofluorescence showed abnormal dense thick Col V bundles in the interstitium and histomorphometry revealed higher amount of distorted Col V fibers, when compared with control group. In SSc patients there were increased [$\alpha 1(V)$] and [$\alpha 2(V)$] mRNA chains expression when compared with control, but [$\alpha 2(V)$] was proportionally raised compared to control group. High levels of collagen V were inversely associated to VC ($r = -0,72$; $p=0,002$), FVC ($r = -0,76$; $p<0,001$) and FEV1 ($r = -0,89$; $p<0,001$) pulmonary function tests.

Conclusions: We conclude that abnormal Col V fibers are overproduced in SSc patients and it could plays an important role in the pathogenesis of SSc, since this molecule regulates tissue collagen assembly. This aberrant histoarchitecture observed in SSc can be related to overexpression of [$\alpha 2(V)$] gene of unknown origin.

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P718**A clinical study of chronic eosinophilic pneumonia (CEP)**

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Background: CEP is a relatively rare disease, which remains an enigma in many respects and presents diagnostic challenges as antibiotic-unresponsive pneumonia. Studies on a large series of CEP cases are few, so that we investigated from various angles CEP cases that we diagnosed at our hospital.

Methods: Study subjects were 74 cases that were diagnosed as CEP during a period of 20 years from April 1988 to August 2008.

Results: Mean age was 57 years, with 34 males and 40 females. Current smokers were 11 cases, while non-smokers 63 cases. A history of allergic disease such as bronchial asthma was found in 23 cases (31.1%). Clinical symptoms included cough most frequently in 85.1%, followed by fever in 62.2%, sputum in 47.3%, dyspnea in 41.9%. A result of blood examination was: 10137/ml for WBC; 21.6% for eosinophil; and 1169 IU/ml for IgE. BALF examination revealed increases in the total cell count, eosinophil percentage, and lymphocyte percentage. In pulmonary function tests, any of VC, FEV1.0, and DLco was found to be decreased. Images revealed predominantly non-segmental, bilateral, peripheral, and wandering shadows. In half of the cases, subpleural nodular shadows and/or air bronchograms were observed. All cases were treated with corticosteroid. The dramatic response to corticosteroid therapy was observed in all patients, but about 50% of patients relapsed when corticosteroid was tapered. However responsibility to steroids remained even after relapse.

Conclusions: CEP was mostly found in non-smokers or was most frequently associated with a history of allergic disease. Although patients presented with a variety of clinical symptoms or images, they responded to steroids very well and their prognoses were good.

P719**Immunohistochemical study of Langerhans'cell histiocytosis**

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Clinical and morphological analysis of 62 cases of Langerhans'cell histiocytosis (LCH) in lungs was performed. The isolated lung affections were in 43 cases, combined ones – in 19 cases. Middle age of patients was 26.5 years. Morphological picture didn't correlate with clinical manifestation of disease in all cases. The eosinophile and histiocyte infiltration and granulomas are markers of the process activity. For verification the LCH it is necessary to carry out immunohistochemical research with antibodies for CD1a and S-100 – specific markers of Langerhans'cells. Without immunophenotyping diagnosis was difficult due to impaired tissue architectonics (sclerosis, bullous transformation) and artifacts that were inevitably present during lung biopsy. The antigen expression (p53, bcl-2, bax, cyp 32) was studied for clearing up the peculiarities of apoptosis regulation. The received regularities of antigen expression (p53, bax, bcl-2) illustrate conditions for the programmed Langerhans'cell death. These results can be used in search of new methods to therapy of Langerhans'cell histiocytosis.

P720**Clinical picture of amiodarone pneumonitis 2001-2008**

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Amiodarone is an effective antiarrhythmic agent used to treat serious cardiac disorders. Its pulmonary toxicity represents serious risk for the patients treated with this drug. The aim of our study was to prospectively evaluate the clinical picture of amiodarone pneumonitis in our patients.

Methods: Starting from January 2001 the patients with possible amiodarone pneumonitis on the basis of symptoms, chest X-ray and HRCT, pulmonary function test and BAL findings were enrolled in the study.

Results: Since 1/1/2001 up to 31/12/2008 27 patients have been enrolled, six females and 21 males with average age 68,4±14,1 years. The most common symptoms of amiodarone pneumonitis were cough and dyspnoe. The average daily dose of amiodarone was 200 mg, the duration of administration varied from 3 to 120 months (mean ± SD 30,2 + 25,5 months). The mild restrictive ventilatory impairment (TLC/mean ± SD 78,3±25,5% pred) with moderately reduced DLco (mean ± SD 50,5±7,7% pred) have been observed. Chest X-ray and HRCT scans showed ground glass, patchy infiltration, nodular and reticular patterns. BAL was performed 13 patients and confirmed lymphocytic alveolitis (Ly 26,8±8%) with predominance of CD8+T cells (IRI 0,5±0,2). The most frequent treatment option was discontinuation of the drug in 24 patients, in three patients steroids were also added.

Amiodarone is used frequently by cardiologists and therefore the pulmonary toxicity of the drug becomes more important. The information about pulmonary toxicity of the drug and the recommendation for follow-up by a pulmonologist if symptoms occur represents a very important part of patients care.

P721**Endothelial to mesenchymal transition (EnMT) as a mechanism of alveolar septal fibrosis/remodelling in emphysema**

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Defining emphysema as enlargement of alveolar spaces with loss of connectivity does not reflect the histological complexity of this disease, which includes septal thickening and matrix deposition. At histopathology we have observed a link between septal fibrosis and loss of microvasculature. We therefore investigated this relationship *ex vivo* via the response of endothelial cells to cigarette smoke injury, hypothesising that EnMT contributes to septal fibrosis.

Methods: Human lung microvascular endothelial cells were treated for 24 hrs with 3% cigarette smoke extract (CSE) and harvested after 7 days. Endothelial plasticity was investigated via cellular expression of proteins on confocal microscopy, flow cytometry and on western blotting. Immunohistochemistry was performed on paraffin embedded sections from emphysematous lungs for α -SMA, collagen I, collagen III and CD31.

Results: Cobblestone endothelial cells changed morphology to spindle shaped cells in response to CSE. CD31 expression reduced following CSE exposure on flow cytometry (MFI 42.0 vs. 23.6, $p < 0.05$) and on western blotting. On confocal microscopy, CD31 expression reduced and expression of α SMA and Vimentin increased. On immunohistochemistry, CD31 immunoreactivity appeared as granular deposits within the areas of septal thickening or as focal regions bordering the expanded septae.

Conclusions: Pulmonary microvascular endothelial cells undergo EnMT in response to CSE, a finding consistent with the histopathological association of loss of microvasculature and septal fibrosis. Understanding EnMT in emphysema may provide insights into the early pathogenesis of emphysema and novel approaches to influence remodeling.

P722**Screening tool for lung cancer based on feed-forward neural network regarding semiquantitative cytochemical analysis of alveolar macrophages**

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A feed-forward instance of an artificial neural network is the perfect way to organize gathered information into precisely defined categories regardless blurred borders between them. This network is consisted of three layers of artificial neurons with the functions similar to the biologic ones. Well-trained neural network with sufficient number of neurons in processing layer will act as a human expert concerning the specific question, with highly increased learning and data processing skills and reduced possibility of error below 0.001. In this study, two parameters were selected as input variables. Semiquantitative cytochemical analysis of alveolar macrophages (AM) obtained by bronchoalveolar lavage was performed in regard of amounts of iron and butyrate esterase activity of AM. These values have been further plotted into three-dimensional space, forming ellipsoidal segments with significant epicenter function to estimate the percentages of participation in each of the three groups: non-smokers, smokers and patients with NSCLC. Although the sample of 17 patients usually does not satisfy the minimum required for standard statistic tests, the network created and trained by Levenberg-Marquardt algorithm after several epochs provided significant results, both in sense of reliability and mean squared error reduction. Final correlation between training, validation and testing subsamples randomly generated by MatLab indicated that the close relationship between the parameters and target value is a solid ground for further estimations and predictions.