213. Diffuse parenchymal lung disease pathogenesis, biomarkers, therapy and new entities

P1764
Investigation of the citrullination pathway in the pathogenesis of fibrotic lung disorders
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Rationale: Our hypothesis was to explore whether an autoimmune process implicating anticitrullinated peptide immunity is implicated in pulmonary fibrosis, both idiopathic and autoimmune.
Materials and methods: We evaluated the expression of PADI2 and PADI4, the enzymes mainly catalyzing the citrullination process, in the bronchoalveolar lavage fluid (BALF) of 53 IPF patients, 37 patients with rheumatoid lung (RA-ILD) and 10 healthy controls. Survival was evaluated against smoking status, using proportional hazards analysis, adjusting for sex, age, disease severity (composite physiologic index [CPI]), and PADI2 and PADI4 levels.
Results: Both PADI2 and PADI4 mRNA expression levels were increased in IPF and RA-ILD compared to controls ($p<10^{-4}$). Protein expression revealed a higher expression of PADI2 and PADI4 in the RA-ILD compared to controls ($p<0.005$ for both proteins) and IPF ($p<0.005$ and $p<0.05$ respectively). PADI4 protein expression was also increased in IPF compared to controls ($p<0.005$). Finally, PADI4 mRNA expression was higher in the smokers than non-smokers ($p=0.01$). Multivariate analysis with stepwise logistic regression identified three factors that independently predict mortality in the study population: CPI, age and both PADI2 and PADI4 levels (examined in separate models). When both PADI2 and PADI4 were included in the same multivariate model, both were independently predictive of late mortality after adjustment for CPI, age, gender and smoking status.
Conclusion: These results suggest that citrullination is an active process in both autoimmune and idiopathic lung fibrosis. The role of citrullinated enzymes as biomarkers predictive of late mortality merits further evaluation.

P1765
Small moleculae ACE2 activator, diminazene aceturate attenuates bleomycin-induced pulmonary fibrosis
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Introduction: Angiotensin Converting Enzyme2 (ACE2), a member of the renin angiotensin system has been shown to render protection against lung diseases, particularly pulmonary fibrosis (PF). In this study, we investigated the effects of a recently identified synthetic activator of ACE2, Diminazene aceturate (DIZE) against bleomycin-induced PF.
Methods: A single intratracheal instillation of bleomycin (Bleo, 5U/kg) in 8-week old male rats induced PF. Animals were randomized into Control, Bleo and Bleo+DIZE groups. DIZE treatment (15mg/kg, sc) was commenced soon after bleomycin administration. Following 14 days of bleomycin instillation, right ventricular systolic pressure (RVSP) was measured, followed by heart and lung excision to examine cardiopulmonary remodeling.
Results: Control rats exhibited a weight gain of 35%, while bleomycin-challenged animals lost 10% of their initial body weight by the end of the study period. Conversely, Bleo+DIZE group demonstrated 16% weight gain. Furthermore, Bleo animals displayed marked elevation in RVSP (Control: 27±1; Bleo: 40±2 mmHg; $p<0.05$, n=5-6), with subsequent development of right ventricular hypertrophy (RVH; Control: 0.26±0.007; Bleo: 0.36±0.03; $p<0.05$). However, DIZE treatment prevented bleomycin-induced increases in RVSP (32±0.5 mmHg; $p<0.05$) and RVH (0.30±0.01; $p<0.05$). Also, DIZE attenuated both the development of PF and the ensuing increase in lung weight/tibial length ratio associated with bleomycin injury.
Conclusion: Collectively, our results suggest that DIZE prevents bleomycin-induced lung fibrosis and improves cardiopulmonary hemodynamics. Thus, DIZE treatment may represent a promising therapeutic strategy for treating PF.

P1766
KL-6 is a useful serum biomarker for early detection of interstitial lung disease
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Background: We previously generated a murine IgG1 monoclonal antibody that recognizes a sialylated sugar chain designated KL-6. KL-6 is a high-molecular-weight glycoprotein classified as human MUC1; it has been reported as a sensitive biomarker for various types of interstitial lung diseases (ILDs). However, the clinical significance of KL-6 for early detection of ILDs has not been well evaluated.
Aims: We aimed to determine whether serum levels of KL-6 are of any diagnostic value in the case of patients with early stages of ILDs.
Methods: We retrospectively collected the data of 69 patients with ILD who underwent surgical lung biopsy (28 patients with chronic hypersensitivity pneumonitis, 27 with idiopathic interstitial pneumonitis, and 14 with collagen vascular disease-associated interstitial pneumonia). Serum and bronchoalveolar lavage fluid (BALF) concentrations of KL-6 were assayed using a sandwich-type electrochemiluminescence immunoassay. In addition, KL-6 expression in diseased lung specimens obtained from surgical lung biopsy was analyzed using immunohistochemistry and digital image analysis.
Results: The proportion of serum KL-6-positive cases (KL-6, ≥500 U/ml) was 94.2% (65 of the 69 patients with ILDs). Furthermore, even when the analysis was restricted to ILD patients with normal lung function (%VC, ≥80%), the proportion of serum KL-6-positive cases was 92.6% (25 of 27 patients examined). KL-6 was

prominently expressed in regenerating type II pneumocytes, and its serum and BALF levels were correlated to its expression levels in the diseased lungs.
Conclusion: KL-6 may be a useful biomarker for ILDs even in early stages of ILDs and may greatly improve the current diagnostic methods.

P1767
ProSP-B as a possible biomarker in idiopathic interstitial pneumonias (ILD)
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Introduction: Surfactant proteins (SP) can be elevated in plasma of pulmonary diseases patients. However, for SP-B only limited data are available. Aim of this feasibility study was to assess whether proSP-B and C-fragment pro SP-B (C-proSP-B) might serve as a biomarker in pulmonary diseases.
Methods: In 280 patients serum samples were obtained. In a 1st study at days 1, 7, 21 (n=120) and at 1 timepoint (n=160) in a 2nd. Measurement was based on electrochemiluminescence immunoassay with mouse monoclonal anti-proSP-B antibodies. Levels were correlated to lung function, clinical and laboratory parameters.
Results: ProSP-B and C-proSP-B levels yielded similar results with higher values for C-proSP-B. Highest C-proSP-B levels (mean, ng/ml) were found in ILD (1542, n=24) compared to infection (878 and 583, n=22 and n=31), thoracic tumors (447, n=22) and pulmonary hypertension (533 and 369, n= 21 and n=30). Low values were found in Asthma (210, n=19) and COPD (369, n=26). Levels did not differ between patients with non-invasive versus invasive ventilation (204, n=49 versus 243, n=51) and were higher for smokers, higher BMI and females. A correlation between treatment and values was not found during monitoring. No significant correlations were found between SP-B levels and lung function, right ventricular-function (PH), disease stage or to most subtypes of pulmonary disease entities. However, in ILD highest levels were found for IPF patients.
Conclusions: Plasma pro and C-proSP-B might serve as a biomarker in pulmonary diseases with alveolar or interstitial damage like ILDs, especially in IPF and pneumonias. Its role in long term monitoring of such diseases and in obstructive disease has to be clarified further.

P1768
Serum SP-A as predictor of disease progression in patients with pulmonary alveolar proteinosis
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Background: Surfactant protein A (SP-A) serum levels is known to be increased in pulmonary alveolar proteinosis (PAP),a disorder characterized by alveolar accumulation of surfactant lipoprpoteins. The prognostic value of SP-A in PAP is still unknown and has been investigated in our study.
Patients and methods: 28 consecutive patients with PAP were studied prospectively. Serum SP-A was measured by ELISA.We evaluated the correlation between initial SP-A levels, clinical variables and other serum biomarkers. Disease progression was defined as deterioration of symptoms, lung function or chest imaging.
Results: The median follow-up time was 510 (90-1890) days. Initial serum SP-A levels correlated inversely with baseline PaO2, FVC und TLCO (r=-0.405, p=0.004; r=-0.46, p=0.001 und r=- 0.462, p=0.003) and directly with AaO2 (r=0.315, p=0.026). Serum SP-A also correlated with LDH (r=0.451, p=0.001) and KL-6 (r=0.363, p=0.008). A correlation between changes in TLCO, PaO2 and AaO2 and changes in serum SP-A during the follow-up was seen (r=-0.7, p=0.002; r=-0.538, p=0.007; r=0.436, p=0.033 respectively). Serum SP-A was higher in patients with disease progression (n=14) (p=0.001). At a cut-off level of 490 ng/mL, serum SP-A predicted disease progression with a sensitivity of 88% and specificity of 75% and the necessity of whole lung lavage (WLL) with a sensitivity of 86% and specificity of 70%. In the multivariate analysis, the initial serum SP-A level was an independent predictor of disease progression (HR 3.9, p=0.046) and of the necessity of WLL (HR 7.2, p=0.003).
Conclusions: Serum SP-A appears to have a predictive value for disease progression in PAP.

P1769
Thrombin induces epithelial-mesenchymal transition via PAR1, PKC and ERK1/2 pathways in A549 cells
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Introduction: Thrombin activates the protease-activated receptor (PAR)-1 and induces a myofibroblast phenotype in normal lung fibroblasts. The origin of myofibroblasts are from the resident fibroblasts, fibrocytes and epithelial-mesenchymal transition (EMT). We investigated the effects of thrombin, which is an important mediator of the interstitial lung fibrosis, on EMT in A549 human alveolar epithelial cells.
Methods: A549 cells were stimulated with thrombin (2 units/ml) and PAR1 agonist, TFLLRN (300 μM). The development of EMT was confirmed by real-time RT-PCR, western blot and immunofluorescence staining. A549 cells were transfected with small interfering RNA (siRNA) directed against PAR1 mRNA and thrombin inhibitor, argatrovan were added before thrombin for the inhibition experiment. To determine the possible PKC and ERK1/2 signaling pathways in the development of thrombin-induced EMT, 10 nM of GÖ6976 (PKC-α inhibitor), 4 μM of rottlerin (PKC-δ inhibitor), 10 μM of PKC-ε antagonist peptide and PD98059 (ERK1/2 inhibitor) were used. The amount of collagen 1 and TGF-β in the cell culture supernatants were also measured by ELISA.
Results: Thrombin induced the α-SMA expression and decreased the E-cadherin expression from the A549 pulmonary epithelial cells. This EMT phenomenon was accompanied by increased PAR1 expression. Transfection of PAR1 siRNA or argatrovan inhibited the EMT and PAR1 expression simultaneously. Thrombin and TFLLRN also increased the production of TGF-β and collagen 1 from the A549 cells. In addition, we confirmed that this thrombin-induced EMT was mediated through the PAR1 activation and PKC-ERK1/2 phosphorylation.

P1770
Inhibitory effect of statins on fibrogenic mediator production from lung cells
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Introduction: The pathogenesis of idiopathic pulmonary fibrosis remains largely unknown. Pulmonary fibrosis also remains a devastating clinical disorder for which there are limited therapeutic options. Statins are 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors of cholesterol biosynthesis, and they have been reported to exert pleiotropic effects on cellular signaling involved in tissue inflammation and also in organ fibrosis/remodelling.
Objective: To examine the preventive effects of statins on fibrogenic mediator expression and production in lung cells.
Methods: Normal human lung fibroblasts and type II pneumocyte A549 cells, cultured with pitavastatin, pravastatin, or medium alone, were stimulated by transforming growth factor-β1 (TGF-β1). Then, mRNA expression and protein secretion of several mediators from these cells were evaluated by real-time PCR, ELISA, or multiplex assays.
Results: The TGF-β1-induced expression or production of mediators, such as CXCL8, platelet-derived growth factor, vascular endothelial growth factor, and collagen-1, were significantly suppressed in both lung cells pretreated with statins, compared to non-treated cells.
Conclusion: Statins inhibited TGF-β1-induced fibrogenic mediator production from lung fibroblasts and airway epithelial cells. Although further evaluation of the signaling pathways for these phenomena is needed, our results suggest the possibility of statins as anti-fibrotic agents for pulmonary fibrosis.

P1771
Interdependence of endothelin-1 and transforming growth factor-β_1 on Wnt3a expression in idiopathic pulmonary fibrosis
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Introduction: The Wnt signaling pathways may be involved in the development of idiopathic pulmonary fibrosis (IPF). Wnt has been demonstrated to down-regulate the expression of CCAAT enhancer-binding protein α (C/EBPα), and the pro-fibrotic cytokines transforming growth factor-β (TGF-β) and endothelin-1 (ET-1) are controlled by Wnt3a signaling.
Aim: To study the effect of TGF-β and ET-1 on ECM production and Wnt expression in primary human IPF fibroblasts.
Methods: Fibroblasts were isolated from IPF lungs (n=4) and from non-fibrotic controls (n=4). After stimulation with TGF- β_1 and/or ET-1, ECM was measured by ELISA. Total protein was harvested and immuno blot analysis was performed.
Results: TGF-β (0.5 – 10 ng/ml) dose dependently increased total ECM deposition by 180%. ET-1 alone (0.5 – 10 ng/ml) had no effect on ECM. When combined, ET-1 super-induced the TGF-β-effect in a synergistic manner. Expression of Wnt3a was up-regulated by TGF-β in IPF fibroblasts whereas no effect was seen after ET-1 treatment. When ET-1 was added together with TGF-β, Wnt3a expression was further enhanced in comparison to TGF-β alone. Expression of Wnt3a was weak in control fibroblasts, and no induction by ET-1/TGF-β was observed. Expression of total C/EBPα in IPF fibroblasts was lower than in controls.
Conclusions: In IPF fibroblasts, ET-1 exerted its pro-fibrotic effect only in the presence of TGF-β, and a similar interconnection was observed for the up-regulation of Wnt3a expression. This suggests a disease-specific and interdependent pro-fibrotic effect of ET-1 and TGF-β, which might be mediated via the up-regulation of Wnt3a and the down-regulation of the C/EBPα.

P1772

Occupational and environmental impact on the clinical course of autoimmune pulmonary alveolar proteinosis

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Background: Existence of GM-CSF autoantibodies in sera and lung are a diagnostic for autoimmune pulmonary alveolar proteinosis (aPAP). In our large cohort study in Japan (n=223), 57% of patients were current or ex-smokers, and 26% had history of dust exposure (Inoue et al. AJRCCM. 177: 752, 2008). In order to confirm the impact of occupational and environmental factors on the clinical course/prognosis of aPAP, we conducted the longitudinal study of aPAP in Japan.
Subjects and methods: We analyzed the registered cohort data from our database, and collected new follow-up data. Clinical data from 98 patients with aPAP were analyzed. All patients had positive serum GM-CSF autoantibody. Occupational, and environmental exposure histories were analyzed.
Results and discussion: 36 patients (37%) experienced spontaneous improvement, and 15 patients (15%) died during the study period. Median survival was 17.4 years. The survival time of the patients with dust exposure (n=38) had significantly worse prognosis than patients without dust exposure (n=58) ($p<0.001$, log-rank). During the follow-up period (more than 5 years), 22 patients changed or quitted their job. The patients who changed their environment experienced more spontaneous improvement ($p<0.05$). The survival time of the patients who changed their environment did not differ from the patients who did not changed. Smoking did not affect the prognosis and spontaneous improvement of the patients. Some patients had seasonal worsening in Winter.
Conclusion: Our data would provide a new environmental management of patients with aPAP.
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P1773

Adipose stem cell therapy in mice on repetitive intratracheal bleomycin induced pulmonary fibrosis

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Rationale: Adipose-derived stem cells are detectable in the parenchyma and large airways of lungs, and associated with reduced inflammatory infiltration and lung cell death.
Objectives: We hypothesized that adipose stem cells would ameliorate lung fibrosis induced repetitive intratracheal bleomycin instillation.
Methods: Male 8-week-old C57BL/6J mice (control group n=10, bleomycin only group n=20, bleomycin plus adipose stem cell group n=10) were used. Eighth biweekly dose of bleomycin was injected intratracheally via an intubation procedure at dose of 0.04 units in a total volume of 100 μl of sterile saline. Human ASC (6×10^5 cells) were administered systemically via intraperitoneal injection every other week during the last 2 months of the 4- month bleomycin exposure. Lungs were evaluated for fibrosis and collagen content. Bronchoalveolar lavage (BAL) was performed for cell counts.
Measurements and Main Results: Evaluation of lung histology from mice receiving repetitive dosing revealed patchy distributive lung fibrosis and extracellular matrix deposition based on trichrome blue collagen staining. Furthermore, alveolar ducts were increased in size and proliferated with several Clara cells and cuboidal epithelial cells hyperplasia with peri-alveolar ducts inflammatory infiltrations. These finding was ameliorated by adipose stem cells administration.
Conclusions: These results suggest a useful therapeutic effect of adipose stem cells on pulmonary fibrosis induced by repetitive bleomycin administration. Further studies are needed to evaluate the efficacy of adipose stem cells for the treatment of human IPF.

P1774

Diffuse alveolar hemorrhage caused by primary antiphospholipid syndrome

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Introduction: Diffuse alveolar hemorrhage (DAH) is a severe complication of primary antiphospholipid syndrome (PAPS). We describe treatment and outcomes of DAH due to PAPS.
Methods: Retrospective review of all adults evaluated at Mayo Clinic with DAH due to PAPS between 01/01/97 and 12/31/11. PAPS diagnosis met the revised Sapporo criteria. DAH was defined as bilateral pulmonary infiltrates with BAL documenting bloody return and/or >20% hemosiderin laden macrophages (HLM).
Results: Seventeen patients (men=12) were identified. Median age (IQR) was 43 years (36-47). Three patients underwent lung biopsy showing capillaritis. Median % of HLM was 87% (81-98), BAL differential was predominantly neutrophilic, median 30% (18-60). All patients were treated with high doses of glucocorticoids, 6 of whom did not respond. Sixteen patients were on anticoagulation at DAH diagnosis. Number of patients treated with immunosuppressants/number that achieved remission are described as follows: Mycophenolate mofetil 7/0, azathioprine 6/0, cyclophosphamide 7/3, plasma exchange 2/0, intravenous gamma-globulin 4/1, rituximab 6/3. Only 2 patients are off glucocorticoids(all treated with rituximab). Five patients died, 4 from complications of DAH and one from complications of autologous stem cell transplant conditioning regimen for treatment of refractory DAH/APS.
Conclusions: To the best of our knowledge, we present the largest series of DAH secondary to PAPS. This disease carries a poor prognosis with limited successful therapeutic options. Glucocorticoids are first line therapy and B-cell targeted immunosuppression with cyclophosphamide or rituximab may have the highest likelihood to induce remission and should be considered early.

P1775

Lung-limited IgG4-related disease: A new form of IgG4-related disease?

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Background: 'Immunoglobulin G4 (IgG4)-related disease (IgG4-RD)' comprises multi-organ diseases including pulmonary disorders. Typical patients show extrathoracic lesions. We compared cases with or without extrathoracic lesions.
Method: Tokyo Diffuse Lung Diseases Study Group retrospectively examined data from 44 patients suspected of IgG4-RD. Diagnostic criteria included high serum IgG4 level (>135mg/dL). Lung biopsy specimens showed massive IgG4+ plasma cell infiltrations (IgG4+/IgG+ >40% and >10/high power fields). Computed tomography and pathological findings were evaluated by diagnostic radiologists and pathologists independently. Final diagnoses were made by open panel conference.
Result: Of 44 patients, 20 had extrathoracic lesions and 24 had intrathoracic lesions alone. We classified 20 extrathoracic lesion cases as IgG4-related RD (A group) and 15/24 cases without extrathoracic lesions as suspected disease entity such as non-specific interstitial pneumonia (B group). In A, radiological findings included hilomediastinal lymphadenopathy, bronchial wall and bronchovascular (BV) bundles thickening, interlobular septal thickening and/or periBV consolidation. Pathological findings showed abundant lymphoplasmacytic inflammation in interlobular septa, periBV interstitium, bronchus and pleura. Phlebitis, angiitis, granulation tissue, and/or fibrosis were also observed. The remaining 9 (C group) showed similar pulmonary involvement as A excluding extrathoracic lesions.
Conclusions: There is C group with similar radiological and pathological features

as A excluding extrathoracic lesions, and it might be "lung-limited IgG4-RD". Further discussion is necessary for diagnostic consensus of lung-limited disease.

P1776

Prolonged sirolimus therapy in advanced pulmonary lymphangioleiomyomatosis; a multicenter French experience

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Introduction: Lymphangioleiomyomatosis (LAM) and tuberous sclerosis (TS) are orphan diseases associated with TSC1/2 gene mutation and dysregulated mTOR/Akt signaling. Usefulness of the mTOR inhibitor sirolimus (SIR) in slowing the decline of lung function in LAM has been shown.
Aims and objectives: Evaluate efficacy and safety of prolonged treatment with Sir in LAM patients.
Methods: An observational retrospective study of 14 patients with LAM and declining lung function treated with Sir and follow-up over 18 months of SIR.
Results: All patients included in the study were female with sporadic LAM (n=9) or TSC-LAM (n=5). Six patients had chylous effusion and 3 had pulmonary hypertension. The median dosage of SIR was 2.5 mg/d and the blood T0 was 5.5 mg/mL (1.8-10). Clinical and functional improvement from baseline was observed in all after 6 months of SIR therapy. At M18 and M24, the functional benefit remained for all except one, 5 of the 6 patients with chylous effusion experienced complete resolution of this condition and pulmonary hemodynamic normalized in two. Progression of the disease after routine discontinuation of SIR after 2 years in 3 patients encouraged rechallenge with SIR, with again restoration of disease control. Tolerance of SIR was good in 12 patients, and therapy was stopped for moderate adverse events in 2 cases but restarted at lower dosage in one without complication.
Conclusion: This study demonstrates that mTOR inhibition with SIR is useful in advanced LAM. Decline in lung function was observed after discontinuation of SIR with benefit of subsequent rechallenge in uncontrolled disease. Therapeutic scheme of SIR over time in LAM should be defined.

P1777

A retrospective clinical and radiological review of 20 Castleman's disease cases

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Rationale: Castleman's disease is one of the rare lymph proliferative diseases, which usually involves lung. There is still no established standard therapy at the moment. We have examined the clinical findings including therapies and prognoses.
Methods: On the cases of Castleman's disease consulted in our hospital in the past 10 years until 2011, we retrospectively examined based on the medical records.
Results: The study comprised 20 patients (70% male) with a mean age of 55 (25-70) years old. As complications, 6 cases had POEMS syndrome (2 with PH), 3 had hematologic diseases (DLBCL, ITP, MDS). By chest CT, mediastinal adenopathy was confirmed in all cases, 10 were confirmed with lung involvement (5: nodular shadow; 2: reticulonodular shadow; 4: GGO; 5: interlobular septal thickening), 6 with pleural effusion, and 3 with cardiac effusion. The diagnostic methods were 13 cases of surgical lymph node biopsy, 6 cases of lung biopsy (2: TBLB; 2: VATS lung biopsy; 1: open lung biopsy; 1: CT-guided lung biopsy), 1 case of lachrymal gland biopsy. Histopathologically, there were 4 cases of hyaline vascular type and 16 cases of plasma cell type. 4 cases were prioritized on the treatment for complications (3: POEMS syndrome; 1: ITP), and 2 were left untreated for a follow-up. Treatment was started for 14 cases using PSL at the time of diagnosis or exacerbation of symptom.
Conclusions: In this study, lung biopsies are useful as diagnostic method in the cases with pulmonary involvement. Few cases were confirmed with reduction of lymph node and improvement of lung involvement by treatment. But in many cases symptomatic improvement or suppression of the progress of the disease by the treatment were observed.

P1778

MUSIC: Efficacy and safety of macitentan in idiopathic pulmonary fibrosis (IPF)

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Endothelin-1 may contribute to IPF pathophysiology. The prospective double-blind Macitentan USe in an IPF Clinical (MUSIC) trial (NCT00903331) investigated the endothelin receptor antagonist macitentan in adults with IPF of <3 years duration and a histological pattern of usual interstitial pneumonia on surgical lung biopsy. Patients were randomised 2:1 to macitentan (10 mg once daily) or placebo. The primary objective was to show that from baseline up to Month 12 macitentan positively affects forced vital capacity (FVC) versus placebo. Baseline demographics and characteristics were comparable between treatment arms.

	Macitentan, n=119	Placebo, n=59
Males, n (%)	84 (70.6)	37 (62.7)
Mean age±SD, years	65.1±7.9	64.5±6.3
Mean FVC±SD, % predicted	76.5±15.6	74.8±14.6
Mean corrected DLco±SD, % predicted	47.8±13.4*	45.6±11.2

*n=115; DLco, carbon monoxide diffusing capacity; SD, standard deviation.

Mean (range) exposure to macitentan was 14.3 (0.0–24.6) months and to placebo was 15.4 (6.3–24.3) months. Median (95% confidence limit) change in FVC was −0.20 L (−0.29 to −0.16) for macitentan and −0.20 L (−0.28 to −0.13) for placebo; ie. no treatment effect on FVC was observed. No difference in time to IPF worsening or death was observed. Adverse events in ≥10% of macitentan-treated patients with a ≥5% difference versus placebo comprised peripheral oedema, anaemia (both favoured placebo), cough (favoured macitentan). Hepatic aminotransferase elevations >3× upper limit of normal occurred in 3.4% of macitentan recipients and 5.1% of placebo recipients. In summary, the primary objective was not met. Long-term macitentan exposure was well tolerated with rates of elevated hepatic aminotransferases comparable to placebo.

P1779

Efficacy of rituximab in patients with connective tissue disease associated interstitial lung disease: Preliminary results in safety and clinical response

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Objectives: We present safety and clinical outcomes of Rituximab (RTX) in connective tissue diseases-associated interstitial lung disease (CTD-ILD) in a real-life clinical setting.
Methods: Efficacy was assessed by lung function tests (LFT) and high-resolution computed tomography (HRCT). ILD exacerbations and safety were assessed.
Results: 14 patients with CTD-ILD (29% rheumatoid arthritis, 21% Sjögren, 21% unclassifiable CTD, 14% systemic sclerosis and 14% myopathies) received 4000mg (2000 – 6000) RTX (observation period 161 patient-year). ILD patterns were: 57% usual interstitial pneumonia, 21% unclassifiable ILD, 7% nonspecific interstitial pneumonia, 7% cryptogenic organizing pneumonia and 7% lymphoid interstitial pneumonia. At baseline, IgG levels and leukocyte subset counts were within normal range, with reduced numbers of unswitched memory B cells. Incidence infection rate during RTX therapy was 4.35/100 patient-month (p/m) with one case severe. There was 1 death, due to neutropenia with a disseminated fungal infection. LFT available in 12 patients showed an overall improvement in FVC (85%±19 vs. 73%±18) and DLCO (58%±18 vs. 45%±19). Radiographic progression could be evaluated in 6 patients, with 5 stabilized and 1 improved. ILD incidence relapse rate during RTX therapy was 0.745/100 p/m compared to 5.56/100 p/m in the pre-treatment period.
Conclusions: Our preliminary data indicate that RTX is safe and shows a low exacerbation rate. Although optimal outcome measures in the short term are difficult to establish, we could confirm disease stabilization in most patients.

P1780

The death receptors (DRs) expressed on alveolar lymphocytes (AL) in interstitial lung diseases (ILD) participate in apoptosis regulation

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Background: The number of AL, regulated by their local proliferation and apoptosis, contributes to the activity of immune process in ILD. Ligation of death receptors (DRs) by specific ligands on AL seems to be a potent mechanism of apoptosis induction.
Aim: Evaluation of DRs expression on AL. The assessment of DR role in AL apoptosis in ILD.
Methods: Bronchoalvolar lavage (BAL) carried out in sarcoidosis (PS), extrinsic allergic alveolitis (EAA), idiopathic interstitial pneumonias (IIP) and controls (n=46,7,23,13 resp.). AL staining for Fas, Fas Ligand (FasL), DR3, DR4 and

TNFα receptors (CD120A and B). BAL supernatant soluble Fas (sFas), sFasL, TRAIL and TNFα levels measured by ELISA.
Results: In general, common Fas and CD120B apearance on AL coexists with low expression of DR3, DR4 and CD120A. AL apoptosis rate was sign. positively correlated with TNFα level as well as with DR4, FasL, CD120A expression and ($p<0.00001$) CD120A/CD120B ratio; sign. negative correlation was found for sFas. Remarkably declined FasL and CD120A expression was shown in ILD with low AL apoptosis rate, as Loefgren syndrome, progressive PS and EAA (e.g. CD120A+: 5.6±1.5% in PS and 3.3±2.1% in EAA vs 11.0±6.7% in controls, $p<0.01$, median±SEM), Increased percentage of AL FasL+, high TRAIL and TNFα levels were characteristic for IIP, the disorder with frequent AL apoptosis.
Conclusions: DRs participate in AL number regulation, however different mechanisms may drive the process in specific ILD. TNFα proapoptotic effect on AL is probably dependent on the imbalance of its receptors (CD120A and B). Fas/FasL system seems to be active by FasL membrane-bound form, but not soluble one.

P1781
Distinct clinical characteristics of idiopathic interstitial pneumonia predominant in upper lobes
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Objectives: Idiopathic interstitial pneumonia (IIP) predominant in upper lobes is not common but present in clinical practice with certain features such as severe breathlessness and poor outcome. In this study, we aimed to define clinical features in patients with this type of disease.
Methods: We reviewed the medical records of all 301 patients with surgically proven IIP in our institute between 2001 and 2011. HRCT was employed to specify the distribution of the lesion. Pulmonary function, 6-minute walk test, clinical background, and outcome in patients with IIP predominant in upper lobes with pathological UIP (upper lobe UIP) were especially analyzed. The data were compared with those in UIP predominant in lower lobes (usual UIP).
Results: Nine patients of upper lobe UIP and 111 patients of usual UIP were identified in 301 patients. There was no significant difference in 6-minute walk test, smoking history or other clinical background between upper lobe UIP and usual UIP. However, significant difference between the two groups was observed in KL-6 (600 vs 1166 IU/ml), %DLCO (108.7 vs 78.0%), RV/TLC% (42.6 vs 33.6%), $PaCO_2$ (44.6 vs 41.7 torr), and $AaDO_2$ (7.1 vs 16.8 torr). The increase of residual volume and $PaCO_2$ may indicate alveolar hypoventilation and contribute to the respiratory distress in patients with upper lobe UIP, even though pulmonary diffusing capacity were more likely retained. The survival time tended to be shorter in patients with upper lobe UIP.
Conclusions: Our results delineate the characteristics of upper lobe UIP and support the view that upper lobe UIP is a distinct category of IIP.