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A: **Case series and case reports**

A1: Severe course of sarcoidosis with rare abdominal manifestation: a case report

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Introduction: Sarcoidosis normally affects the lungs and intrathoracic lymph nodes – an involvement of omentum in sarcoidosis is an extremely rare finding.
Case history: A 53-year old male with sarcoidosis was referred to our hospital at the end of 2010 for therapy assessment. His medical history was unremarkable except for a COPD GOLD stage II. He was road construction worker till 2000 and had massive exposure to dust. A sarcoidosis stage I was diagnosed in 2007. Hepatic impairment with cholestatic hepatitis was proven by liver biopsy showing noncaseating granuloma and incomplete cirrhosis with fibrosis stage III. Initially the patient received steroids that induced a severe diabetes. For this reason azathioprin was concomitantly administered, but in spite of this therapy cholestatic parameters increased again after three months. A sequential liver biopsy revealed a predominant drug-induced (azathioprin) liver damage. Therapy was switched to hydroxychloroquine and low dose steroids at the beginning of 2010. Six months later the therapy once again failed: The patient suffered from mild dyspnea under effort, arthralgia, abdominal pain (light tenderness by pressure) and splenomegaly; cholestatic parameters again increased. The chest X-ray showed at last bilateral hilar lymphadenopathy plus interstitial infiltrates. Spirometry demonstrated a moderate peripheral obstruction with significantly impaired diffusing capacity (51% of predicted). The laboratory results exhibited an increased GGT (866 IU/L), ALP (492 IU/L), CRP (19,6 mg/dL), a normal bilirubin, no leucocytosis and ACE with 80 U/L. The abdominal CT now displayed an unclear abscess-like lesion in the small intestine accompanied by enlarged mesenterial lymph nodes. Therefore this lesion in the omentum (3x3 cm in diameter) was localized and excised by a diagnostic laparoscopy. The histopathologic evaluation excluded cancerous cells and revealed a granulomatous infiltration of the omentum consistent with sarcoidosis.
Conclusions: This case report highlights a rare course of sarcoidosis with unusual multiorgan manifestation (lung, liver, mesenterium), and failure of usual immunosuppressive therapies. An alternative approach with biologic drugs (anti-TNFα) will be taken into consideration.

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A2: Churg-Strauss syndrome in a pediatric patient

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Background: The term 'vasculitis' denotes a pathological process of inflammation, vessel wall destruction and tissue necrosis. Vasculitis can occur secondary to drugs, infections and/or other disease like rheumatoid arthritis, and may also occur without known underling cause ('primary vasculitis')
Churg-Strauss syndrome (CSS) is an uncommon multisystem disorder characterized by this vasculitis and is associated with asthma and eosinophilia. CSS in childhood is rare and the clinical presentation can be quite diverse.

Methods: We report a 12-year-old boy with asthma and deterioration of general condition, who was eventually diagnosed with an ANCA-negative Churg-Strauss syndrome. Patient characteristics and clinical characteristics are summarized. He was treated with oral prednisone maintenance therapy, which was tapered down over 6 months.

Results: During the course of follow-up the patient improved dramatically. We documented the improvement with CT scans, laboratory and pulmonary function tests.

Conclusion: This case report describes the successful outcome of steroid treatment in a 12-year old boy. Despite the low incidence of CSS in children, physicians must be aware of this orphan disease in order to provide adequate therapy.

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A3: Fatal drug-induced interstitial pneumonitis associated with amitriptyline and clozapine drug-drug interactions

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Background: Drug-induced pulmonary toxicity is an increasingly common but often underestimated clinical problem (www.pneumotox.com). Often the connection with drug use and the development of related inflammatory damage or idiosyncratic toxicities is hard to recognize and objectify, especially in patients using multiple drugs. We describe a case of interstitial pneumonitis secondary to drug toxicity associated with a combination of amitriptyline and clozapine.

Methods and material: A 65-year-old female with a history of schizophrenia was admitted to our hospital because of a humerus fracture. Her daily medication consisted among others of amitriptyline (250 mg/day) and clozapine (300 mg/day). Surgery was performed, 11 days later she was admitted to the intensive care unit because of respiratory failure. High resolution computed tomography (HRCT) showed diffuse interstitial pneumonia with ground glass opacity. Bronchoalveolar lavage fluid (BALF) showed signs of inflammation (predominantly lymphocytosis), without any signs of infection. According to these findings, drug-induced pulmonary toxicity was suspected. Serum concentrations of amitriptyline and clozapine were 850 µg/L (therapeutic range: 100-250 µg/L) and 2657 µg/L (range: 350-1000 µg/L) respectively. Although both drugs were stopped immediately and high dose corticosteroids and N-acetylcysteine added, there was a fatal outcome.

Conclusion: The use of various drugs metabolized by the same enzymatic pathway (CYP2D6) should be avoided for this may result in significant accumulation of these drug(s) - in this case amitriptyline and clozapine - leading to toxic serum levels and severe side effects. Drug-drug interactions may even result in fatal drug-induced pulmonary toxicity.

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A4: Interstitial lung disease in common variable immunodeficiency associated systemic granulomatous disease: a comprehensive study on 21 cases

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Background: Interstitial lung disease (ILD) has not comprehensively been investigated in common variable immunodeficiency (CVID) associated systemic granulomatous disease (SGD). The aim of the study was to compare presentation and outcome of ILD in CVID associated SGD (ILD/CVID/SGD) compared to controls with pulmonary sarcoidosis (PS)
Methods: 21 patients with ILD/CVID/SGD were included in a retrospective study conducted by the Groupe Sarcoïdose Francophone. Clinical records, imaging, pulmonary function tests, bronchoalveolar lavage cell count, and lung pathology reports at presentation were centralized. Longitudinal evaluation of imaging and survival were studied. Patients with ILD/CVID/SGD were compared with 21 matched controls with PS.
Results: Differences in ILD/CVID/SGD vs controls were (i) recurrent infections and autoimmune diseases were more frequent (61.9% vs 4.8%, p< 0.001, and 38.1% vs 0, p=0.003, respectively); (ii) the rate of crackles, splenomegaly and hepatomegaly was significantly higher (47.6% vs 0, p<0.001; 71.4% vs 9.5%, p<0.001; 47.6% vs 9.5%, p=0.01, respectively); (iii) number of extrathoracic localizations was higher(p=0.006). At CT scan, the main patterns in ILD/CVID/SGD were nodules (33.3%), micronodules (23.8%) and lines (19%). Versus sarcoid controls nodules were more frequent in patients as primary lesion (p<0.001). Whereas micronodules had not a significantly different prevalence, they were more frequently the main CT pattern in sarcoid controls (p<0.001). Distribution of micronodules was perilymphatic in all sarcoid controls. Further ILD/CVID/SGD had more frequent nodules and bronchiectasis (81% vs 23.8%, p<0.001, and 66.7% vs 9.5%, p<0.001, respectively) and less frequent hilar lymph nodes (33.3% vs 66.7%, p=0.03). At the end of follow up (median 6,6 years) five patients with ILD/CVID/SGD were dead (23.8%). Three deaths were attributable to complications of CVID, none to granulomatous disease.
Conclusions: ILD/CVID/SGD makes up a specific picture markedly different from sarcoidosis particularly for clinical findings and CT imaging. Evolution is often severe and severity relied mainly on CVID complications.

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A5: Characteristics of inflammatory bowel diseases-associated interstitial lung diseases

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Background: Various interstitial lung diseases (ILD) have been reported in inflammatory bowel diseases (IBD) but most publications are limited to small series or predate the 2002 ATS/ERS statement on idiopathic interstitial pneumonias.
Aims of the study: To describe the epidemiological, clinical, radiological and pathological characteristics of IBD-associated ILD.
Methods: This is a monocentric retrospective study of 9 patients with IBD-associated ILD referred from 1990 to 2010 to the department of Pneumology of the Avicenne Hospital. Patients were classified according to HRCT and pathological patterns of ILD as recommended by 2002 ATS/ERS statement.
Results: There were 5 men and 4 women with a mean age of 49 ± 7 years (Crohn's disease: n=4, ulcerative colitis: n=3, undetermined colitis: n=2). ILD developed in the course of previously known IBD in 7 cases, with a median delay of 10 years, while IBD was diagnosed after ILD in 2 cases. Surgical lung biopsy was available in 4 patients. ILD final diagnoses were: "hypersensitivity pneumonitis" ("HSP"): n=4, nonspecific interstitial pneumonia (NSIP): n=2, NSIP secondary to "HSP": n=1, combined pulmonary fibrosis and emphysema: n=1, bronchiolitis: n=1. No patient had overt environmental exposure but 4 patients received mesalazine at the onset of ILD, of which only one with "HSP". One patient with NSIP also had dermatomyositis. Baseline FVC was 65 ± 19% of predicted value and DLCO was 34 ± 8% of predicted value. ILD worsened despite corticosteroids in 7 patients who required other immunosuppressive drugs. At the end of follow-up (4.3 ± 2.6 years), 2 patients died and 1 was transplanted.
Conclusions: Our study outlines the severity of IBD-associated ILD and the high frequency of "HSP", raising the possible role of a particular unrecognised antigen in such a context.

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A6: Characteristics of inflammatory bowel diseases-associated sarcoidosis

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Background: The association between sarcoidosis and inflammatory bowel diseases (IBD) has been reported, mainly in Crohn's disease which may share a common genetic susceptibility. However, the particularities of sarcoidosis phenotype in the setting of IBD remain unclear.
Aims of the study: To describe the epidemiological, clinical, radiological and pathological characteristics of IBD-associated sarcoidosis.
Methods: This is a monocentric retrospective study of 7 patients with IBD-associated sarcoidosis referred from 1990 to 2010 to the department of Pneumology of the Avicenne Hospital.
Results: There were 3 men and 4 women with a mean age of 33±7 years (Crohn's disease: n=6, undetermined colitis: n=1). Four patients were Caucasian, 2 were Afro-Caribbean and 1 was Indian. One patient had a familial history of sarcoidosis. Sarcoidosis preceded the diagnosis of IBD in 3 cases and was posterior in 4 cases. At the diagnosis of sarcoidosis, radiographic stages were as follows: stage 0: n=1, stage I: n=4, stage II: n=2. Mucosal bronchial biopsies were positive in 4/6 cases, with no apparent specificity in the pathologic aspect of granulomas. Extra-respiratory involvement was observed in 4 cases (heart: n=1, liver: n=1, joints: n=1, anterior uveitis with parotids: n=1) and one patient presented with erythema nodosa. Abnormalities in liver tests were noted in 4 cases. Serum angiotensin converting enzyme was increased in 4/6 cases. Four patients required a systemic treatment, because of extra-respiratory involvement in 3 cases. At the end of follow-up (6.8±5.2 years), 3 patients recovered from sarcoidosis.
Conclusions: Our study confirms that sarcoidosis is mainly associated with Crohn's disease and suggests that respiratory involvement is moderate in such a context.

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A7: Spinal cord neurosarcoidosis: A case series of 29 patients

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Background: Spinal cord neurosarcoidosis (SN) is problematic to diagnose because it mimics other inflammatory neurologic diseases. Treatment of this condition is not standardized. We report the clinical, radiographic, and laboratory features of 29 SN cases.
Methods: We retrospectively reviewed the charts of 29 patients with biopsy proven sarcoidosis and spinal cord involvement seen in three medical centers. Data collected from the medical record included patient demographics, neurological symptoms, plus biopsy, laboratory, and radiographic data.
Results: Histological diagnosis was established in all subjects: 4/29 from the spinal cord, and 25/29 from extra-neural organs. Demographics: 17/29 Men, 16/29 Black, age range 22-63 years (mean 43 years). The most common neurologic symptoms were: extremity weakness (19/29), paresthesia (18/29) and back/neck pain (8/29). Cerebrospinal fluid (CSF) studies in 20 patients revealed CSF angiotensin converting enzyme (ACE) level was elevated in only 1/11, and oligoclonal bands (OCB) were positive in 5/12. Common MRI findings were diffuse or nodular enhancing intramedullary lesions (20/29), and leptomeningeal (13/29) involvement. The most common levels of spinal sarcoidosis were thoracic (20/29), and cervical (16/29); less commonly, the conus medullalris /cauda equina (9/29). The number of involved spine segments ranged from 1-25 (mean 7). 24/29 had ≥3 spine segments involved. All patient received corticosteroids. Additional treatments included infliximab (20/29), methotrexate (18/29), and cyclophosphamide (8/29).
Conclusions: SN is typically involves over 3 or more spinal segments. Common spinal cord manifestations are diffuse or nodular enhancing intramedullary and leptomeningeal lesions. In most subjects, diagnosis was established by histological evidence obtained from extra-neural organs. CSF ACE level and OCB are not adequately sensitive or specific to diagnose SN.

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A8: Rapidly progressive pleural effusion as a rare manifestation of sarcoidosis

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A 65 year old male with no significant past medical history presented with cough, dyspnea and fever of one week duration. Chest X-ray showed left mid-lung infiltrate suggestive of pneumonia. The patient was treated with 10 days of antibiotics without improvement. Two months later, he developed worsening of his symptoms and a new oxygen desaturation. Chest computed tomography showed a left lower lobe (LLL) alveolar infiltrate, extensive mediastinal and hilar adenopathy and a newly developed moderate left sided pleural effusion. He underwent pleural fluid sampling which was consistent with a lymphocytic (85% lymphocytes) exudate. Acid-fast bacilli (AFB) and fungal cultures were negative. Bronchoscopic sampling failed to reveal a diagnosis. Given the suspicion for lymphoma he underwent a positron emission tomography (PET) scan which showed increased uptake in the region of the LLL infiltrate, the mediastinal and left occipital region lymph nodes. Surgical biopsy of the mediastinal lymph node, the LLL infiltrate and the pleura showed non-necrotizing granuloma. AFB and fungal stain and cultures on all the samples came back negative. Thus the diagnosis of sarcoidosis was made and the patient was treated with high dose steroids resulting in clinical and radiographic improvement.

Pleural effusions have remained a rare manifestation of sarcoidosis with a reported incidence of 1-10%. To our knowledge this is the first patient with sarcoidosis presenting with a rapidly progressive pleural effusion associated with an alveolar infiltrate.

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A: **Case series and case reports**

A9: Sarcoidosis and alopecia universalis: case report

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Introduction:
Sarcoidosis is a systemic granulomatous disease of unknown etiology. Although the skin is frequently involved in this disease, non-scarring alopecia is a rare manifestation. Alopecia areata is a cell mediated inflammatory form of hair loss with T-lymphocytes having a prominent role. When all the body hair is lost, diagnosis of alopecia universalis is made.

Case report:
The authors report a case of a 42-year old non-smoking male that presented with nonspecific chest pain in the last month. Past medical history was remarkable for dyslipidemia and alopecia universalis that developed at the age of 31 and did not respond to local steroids. Physical examination revealed only complete absence of body hair. A high resolution chest CT (HRCT) showed enlarged mediastinal lymph nodes and diffuse parenchymal micronodules. Lung function tests including diffusing capacity and serum ACE were normal. A bronchoscopy with bronchoalveolar lavage (BAL) and transbronchial biopsies was performed. Biopsies were inconclusive, but BAL showed moderate lymphocytosis (23%) and an elevated CD4/CD8 ratio (4,16). Bronchial aspirate microbiology was negative. Facing a diagnosis of thoracic stage II sarcoidosis, management included symptomatic treatment and observation. The patient became asymptomatic. Lung function tests and HRCT showed stability after one year of follow-up.

Conclusions:
Despite the large time-gap between both manifestations, the similar pathogenesis between sarcoidosis and alopecia areata and previous case reports suggest that this patient's alopecia was caused by sarcoidosis.

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A10: The Clinical and Histological Characteristics of Rapidly Progressive End-Stage Sarcoidosis; A Two Center Experience

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Background: A subset of pulmonary sarcoidosis patients appears to experience a rapid clinical deterioration despite treatment. There is paucity of information on the clinical, radiographic, and histological characterization of these patients.
Methods: Retrospective analyses of databases from 2 academic medical centers were reviewed for sarcoidosis patients who underwent lung transplantation. Clinical characteristics such as age at transplant, gender, years with diagnosis of sarcoidosis were examined. Microscopic slides from the explanted lungs and mediastinal/hilar regional lymph nodes were examined by a lung pathologist.
Results: Fourteen subjects were enrolled in the study that consisted of 7 men and 7 women. The mean (range) age at transplant was 49 (41 - 66) yrs. The years with diagnosis of sarcoidosis ranged between 3 to 33 years. Six of these patients were identified to have a clinical course with rapid deterioration. These patients were predominantly men and carried the diagnosis of sarcoidosis on average of 4.2 (3-7) yrs. Histopathological examination revealed moderate to severe chronic interstitial pneumonitis previously described as atypical in end-stage sarcoidosis. Furthermore, 3 of these patients had a pattern resembling usual interstitial pneumonitis (UIP) with fibroblastic foci in presence of granuloma in lymph nodes and lung parenchyma.
Conclusions: We identified a subset of end-stage sarcoidosis that appeared to have a distinctly unique clinical course with rapidly progressive lung impairment requiring lung transplantation. These patients were mostly men and many of them have chronic interstitial pneumonitis, in some cases UIP pattern, hitherto considered atypical of end-stage sarcoidosis. Further studies are needed to elucidate the histological progression of sarcoidosis to end-stage fibrosis and the significance of chronic interstitial pneumonitis such as UIP pattern as a marker for rapidly progressive sarcoidosis.

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A11: A child with idiopathic pulmonary hemosiderosis and celiac disease: a causative mechanism?

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Background: We present a case of a child with idiopathic pulmonary hemosiderosis and celiac disease, also known a Lane-Hamilton syndrome.
Case Report: A boy presented at the age of 3 years with failure to thrive and recurrent respiratory infections, for which he received several courses of antibiotics. The chest X-ray demonstrated a diffuse interstitial pattern, confirmed with HRCT. After starting a gluten free diet for celiac disease, all gastrointestinal and respiratory symptoms have resolved. His pulmonary function and imaging improved to virtually normal. Based on the clinical picture and imaging the combination of celiac disease and idiopathic pulmonary hemosiderosis was diagnosed, also known as Lane-Hamilton syndrome. Additional genotyping revealed that the patient was a *1/*3 heterozygote for cytochrome P450 (CYP) 2C9 and beared a vitamin K epoxide complex 1 (VKORC1) wildtype (*1/*1, functional enzyme).
Discussion: The pathophysiology of the Lane-Hamilton syndrome is still unknown. In this case of celiac disease and pulmonary hemosiderosis a vitamin K deficiency was considered. Malnutrition and malabsorption due to celiac disease may cause vitamin K deficiency. This vitamin K malabsorption can be further negatively influenced by repeated antibiotic treatment. Low serum vitamin K can lead to hypoprothrombinaemia, resulting in diffuse alveolar haemorrhage. Moreover, patients with VKORC1 and/or CYP2C9 polymorphisms have an additional risk of hypoprothrombinaemia and subsequent bleeding diathesis. This added risk and subsequent symptoms can be the result from inhaled or ingested substances with coumarin(-like) properties (such as certain dyes and fragrances, cinnamon, strawberries, and licorice for example).
Conclusion: In patients with celiac disease vitamin K deficiency should be avoided to lower the risk of idiopathic pulmonary hemosiderosis. This case strengthened that screening for VKORC1 and CYP2C9 polymorphisms should be considered as well as adding vitamin K supplementation to the therapeutic management.

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B: **Genetics and mechanisms in sarcoidosis**

B1: Upregulation of IL-23R is associated with progressing sarcoidosis

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Sarcoidosis is a systemic inflammatory disease with unknown aetiology characterised by alveolitis and granuloma formation in involved organs, including the lung. Although the recent genetic study in Germans highlighted the importance of interleukin-23 receptor (IL-23R) for the development of chronic sarcoidosis, there is limited information about the IL-23R expression profiles in sarcoidosis patients with various disease outcomes.
We, therefore, investigated mRNA expression profiles of IL23-R in bronchoalveolar (BAL) cells from 74 Czech patients with sarcoidosis (S) and 17 control subjects (C) by quantitative RT-PCR. PSMB2 was used as a reference gene. Analysis was performed in patient subgroups as assessed by the 2 years disease outcome: patients with progressing (n=40) and remitting sarcoidosis (n=26).
When compared to control subjects, sarcoid BAL cells expressed higher mRNA levels of IL-23R, a specific receptor for the p19 chain of IL-23 (mean relative expression IL-23R/PSMB2: S/C, 0.021/0.005; p=0.0000001). The expression profiling in sarcoid phenotypes revealed upregulation of IL-23R in patients with progressing sarcodosis in comparison to those where remission was achieved within 2 years of follow-up (0.025/0.017; p=0.01).
In conclusion, IL-23R is upregulated in BAL cells from patients with pulmonary sarcoidosis. Moreover, patients with progressing disease express more IL-23R transcripts than those with remitting sarcoidosis. Functional studies are needed to prove the role of IL-23R in the termination of inflammation.
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B2: HLA-DRB1* alleles and symptoms associated with Heerfordt´s syndrome in sarcoidosis

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Background: Heerfordt´s syndrome (HS), consists in its complete syndrome of uveitis, parotid or salivary gland enlargement and cranial nerve palsy. The objective of the present study was to analyze if there are links between HLA-DRB1* alleles and HS, with its specific phenotype of sarcoidosis, in a homogenous population of Scandinavian sarcoidosis patients.
Methods: 1000 patients with sarcoidosis, out of which 83 had symptoms associated with HS, were included in the study together with a group of 2000 healthy individuals from the same population, matched for gender and age. HLA-DRB1* allelic groups were determined for all individuals, and comparison were made between different diseases subgroups and between patients and healthy controls.
Results: We found that the HLA-DRB1*04 allele was overrepresented in patients with uveitis, enlargement of parotid or salivary glands and cranial nerve palsy. Eighty-three (8.3%) of all patients had one or more of these symptoms and forty-six (55%) of them were HLA-DRB1*04 positive. Forty-four (55%) of the patients with ocular sarcoidosis were HLA-DRB1*04 positive, compared to 35.9% of healthy controls (p=0.0008) and only 26.6% of the hole group of sarcoidosis patients (p<0.0001).
Conclusions: HLA-DRB1*04 seems to protect against contracting sarcoidosis, but be a significant risk factor for symptoms associated with HS. Therefore, we find it reasonable to suggest that HLA-DRB1*04 positive patients should be closely monitored for particularly uveitis, but also parotid or salivary gland enlargement and cranial nerve palsy.

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B3: Cytokine gene polymorphisms in sarcoidosis

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Background: Sarcoidosis is a disease characterized by granuloma formation in many organs, but mostly in lung and lymph nodes. The immunopathogenic background of the disease is probably based on disregulation of immune response to different antigens. The imbalance of immune reactivity might be influenced by genetic background. In our study, we have investigated cytokine genetic polymorphisms in sarcoidosis group and compared the results with that of a group of healthy volunteers.
Methods: 31 sarcoidosis patients, Caucasians from the Czech Republic (mean age 47.5 yr, 14 M, 17 F), were enrolled to our study. The control population of 145 unrelated individuals (mean age 43.1 yr; 24 M, 121 F) were also all Caucasians from the Czech Republic with no previous history of interstitial lung disease. Basic demographic data were collected in both groups. The samples of peripheral blood were taken after obtaining informed consent and DNA was isolated. Polymorphisms in the promoter regions of the IL-1alpha, IL-1beta, IL-1R, IL-1RA, IL-2, IL-4, IL-6, IL-10, IL-12, TNF-alpha, IFN-gamma and in the translated regions of the TGF-beta, IL-1 beta, IL-2, IL-4 and IL-4RA genes were characterized utilizing PCR- SSP method.
Results: For IL-10, the (-819) and (-592) CC homozygosity was statistically more frequent in the sarcoidosis group compared to healthy controls. According to the haplotypes, the majority of sarcoidosis patients had IL-10 (-1082)(-819)(-592) ACC haplotype 2 compared to controls with ATA in most of the cases.
Conclusions: The results of our study support the hypothesis of a genetically encoded immune regulation imbalance in sarcoidosis. The high-producer IL-10 (-819) and (-592) CC genotypes and intermediate- producer IL-10 (−1082) (−819)(−592) ACC haplotype 2 present in the majority of our sarcoidosis patients could support the role of genetically encoded disregulation of cell- mediated immune response to an unknown antigen.

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B4: HLA-DRB1 and C4 allele frequencies in Finnish sarcoidosis patients and associations with disease prognosis

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Background: Sarcoidosis is a complex disease with unknown aetiology and varying clinical picture. In Finland the annual incidence is approximately 10 per 100,000. Previous studies show that genes in the MHC region are involved in sarcoidosis. Our primary aim was to detect genetic differences at the MHC and to correlate the results with prognosis of sarcoidosis.
Methods: We studied three genes in the MHC region (HLA-DRB1, complement C4A and C4B) and compared the allele and haplotype frequencies and copy number variation (CNV) between sarcoidosis patients (n=188) and controls (n=150/224). According to the presence of disease activity after a 2-year follow-up, the patients were divided into two subgroups: with resolved (n=90) or persistent (n=98) disease. We included only patients with a firm diagnosis of sarcoidosis and required at least a 5-year follow-up.
Results: We found that the main susceptibility allele for sarcoidosis was HLA-DRB1*1501 (p=0.011; OR=1.67) and the main protective allele was HLA-DRB1*0101 (p=0.001; OR=0.43). HLA-DRB1*0301 was associated with resolving disease when compared with the persistent group (p=0.01; OR=2.2). In the phenotypic level, 34.4 % of the resolved group had at least one copy of DRB1*0301 whereas in the persistent group the frequency was 16.6 % (p=0.007, OR=2.7). The C4A and C4B CNV differed between patients and controls but based on the haplotype analysis we suggest that the association is due to the tight linkage disequilibrium in the MHC region.
Conclusion: Our results support the importance of the HLA-DRB1 gene as a predisposing gene to sarcoidosis. Especially HLA-DRB1*0301 was associated with a favourable prognosis. Thus, the accurate categorisation of disease phenotype and HLA-DRB1 sequencing offers the basis for disease course estimation of sarcoidosis.

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B: **Genetics and mechanisms in sarcoidosis**

B5: Effect of smoking on development and clinical manifestations of sarcoidosis in a Japanese population

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Background: Several studies have shown that individuals with sarcoidosis are less likely to have smoked before diagnosis in Western populations. Epidemiological characteristics of sarcoidosis are known to be different between Japanese and Westerners. Thus, we wondered how smoking might be related with sarcoidosis in the Japanese population.
Methods: We retrospectively identified 605 patients newly diagnosed with sarcoidosis between 2000 and 2008 based on the Japanese guideline, using a digital data system and medical records. We evaluated smoking status at an initial visit and compared clinical manifestations between current smokers and never smokers, while excluding former smokers.
Results: The prevalence of current smokers when diagnosed as sarcoidosis was 59.6% in males and 25.1% in females. This prevalence was surprisingly similar to the result of Japan smoking rate survey, the largest survey of smoking rate in the Japanese general population. Current smokers were younger and had a higher male-to-female ratio compared with never smokers ($P<0.001$, $P<0.001$). The prevalence of lung parenchymal involvement was higher in current smokers compared with never smokers ($P=0.001$).
Conclusions: Unlike in Western populations, smoking status may not be related with development of sarcoidosis in the Japanese population. Smoking may be linked with likeliness of lung parenchymal involvement in sarcoidosis.

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B6: Glicoregulation in patients with sarcoidosis

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Sarcoidosis is granulomatous disorder than can affect any organ. The usual course is chronic. As etiological moment is still behind the door, usual therapy remains as corticosteroid. Different option can be notified regardless administering corticosteroid especially when we face to young adults.
Aim of the work: to obtain whether corticosteroid can change the Glico regulation in patients in which sarcoidosis had been previously histologically prove.
Method: Analysis is prospective. Incoming patients to the Clinic for lung diseases and tuberculosis were obtained. Medical history and examination, blood serum analyses, chest radiograph, profiles go serum blood glycaemia, BM, blood serum activity of diseases.
Results: are from the medical documentation of 27 (20F/7M, average ages of 47.8 years: mean time of sarcoidosis duration were 2.87 years; In time of analysis had been obtained 13 patients were treated with prednisone, 8 were without therapies, 6 were on therapy methotrexate + prednisone; previously all were treated with corticosteroid therapy). Body mass index were normal in 7 patients, in 10- high and in 10 patients-extreme high. Elevated level of triglycerides and cholesterol were obtained in 20 patients. At the time of analysis had been taken in 2 patients high levels of morning glycaemia were obtained in 2 patients (8.2; 8.4 moll/L) and after 120min high elevated levels of glycaemia were obtained in 7 patients while in only 2 of them glycaemia were 11 mmo/L and 12.1 moll/L. Additional analyses had been obtained in Clinic for endocrinology due to Homa b and homa ir. The final results of this group were obtain diabetes mellitus in 2 patients, while in 18 patients no impairments were notified. In the rest of patients, 7 of them, the impairment of glicoregualtion had been obtained (the maximum levels for glycaemia were 10.3 mmol/L). Another surprisingly matter had been obtained: small number of patients had as the obvious routine physical activity along with low intake of water.
Conclusion: Controlling glycaemia levels in patients who are on corticosteroid therapy is obligatory along with information's due to changing habits in to more healthier level.

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B7: Different models of sarcoidosis - one sarcoid granuloma

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Sarcoidosis (SA) is a granulomatous disease with unknown etiology. Infectious, inorganic, genetic and autoimmunity factors have been considered as potential causes of the disease. Recently some animal models of SA were conducted using Mycobacteria, Propionibacteria and Corynobacteria. It's reported that low-virulence strains of bacteria, e.g., Mycobacteria, Propionibacteria, Corynobacteria, with capacity to persist in host phagocytes for prolonged periods may generate production of abundant quantities of antigens, eg., evolutionary conserved host heat shock proteins (hsp) as well as microbial hsp. Both host/human and microbial hsp are immunodominant antigens for both T- and B-cell responses. Therefore, increased epitopes' load of persistently stimulating microbial antigen(s), e.g., mycobacterial hsp (Mtb-hsp), propionibacterial hsp, corynobacterial hsp to target cells causes increased proliferation of CD4+T and saroid granuloma formation in predisposed host. Moreover, T-cells cross-reactivity between M.avium paratuberculosis (MAP-hsp) and Mtb-hsp and human hsp70, hsp65, hsp16 due to high homology (95%, 46%, 60%, 18%, respectively) may also induce autoimmunity. It has been also revealed that Propionibacterium acnes-hsp demonstrate 45-50% identity to their human and 78% identity to Mtb-hsp60 and 67% for Mtb-hsp70 homologues at the amino acid level. It is known that phylogenetically closest genera Mycobacterium, Corynebacterium, and Streptomyces share an extended set of genes with P.acnes. Thus, different animal models of SA may suggest that phagocyted Mycobacteria, Propionibacteria or Corynobacteria and phagocytes produce immunogenous bacterial and host/human hsp with high degree of homology and functions may induce autoimmunity and sarcoid granuloma, depending on sarcoid genetic background of host/human. Therefore, sarcoid granuloma in different animal models of SA may results from high homology among Mtb-hsp, MAP-hsp, P.acne-hsp, corynobacterial hsp and/or to host/human hsp. It is also possible that only host/human hsp produced independently from infectious and non-infectious stimuli of phagocytes, e.g., phagocytosis of different microbes, katG, nitric oxide, redox radicals, metals may induce sarcoid granuloma in organic and inorganic models of SA depending on host's sarcoid genetic background.

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B8: Are B-cells emerging as key players in the pathogenesis of sarcoidosis?

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Background: Previous pathophysiological studies in sarcoidosis are predominantly focused on the role of T-cells. However, non-caseating granulomas, the hallmark of sarcoidosis, also occur in a subgroup of a typical "B-cell-related disease", common variable immunodeficiency (CVID), described "a sarcoid-like syndrome". In addition to this, in patients with a total lack of B-cells (X-linked agammaglobulinemia), granulomas are never observed indicating the necessity of B-cells in the granulomas development. A maturation arrest in the B-cell development causes humoral disturbances. Whether defects in B-cell differentiation play a role in the pathogenesis of sarcoidosis is unknown. We therefore investigated the maturation of B-cells and their functionality in sarcoidosis patients.
Methods: 23 therapy naïve biopsy-confirmed sarcoidosis patients and 23 healthy controls were included. Tissues of 17 patients were stained with B-cell markers (CD20, CD79a, C138, PAX5, IgG, IgA and IgM). Immunophenotyping analysis on peripheral blood B-cell subsets was performed in all patients and healthy controls and stained with antibodies against CD19, CD24, CD27, CD38, IgD and IgM, analyzed by flowcytometry. T-cell independent and T-cell dependent vaccinations (Mexican flu, seasonal flu 2009-2010 and Haemophilus influenzae type B (HiB), pneumococcal) were given to 13 of these patients.
Results: In all tissues B-cells were observed, predominantly in the granulomatous regions, but in 5 patients also inside the granuloma itself. Frequencies of peripheral blood natural effector cells, IgM+ and IgG+ memory B-cells were significantly lower than those of controls (all $P<0.0001$), whilst transitional cells and IgA+ memory B-cells were increased ($P<0.05$ and $P<0.0001$, respectively). All patients showed normal vaccination responses.
Conclusions: We demonstrated numerous B-cells in granulomas of sarcoidosis patients. Furthermore, the distinctive pattern of peripheral B-cell differentiation, decreased numbers of peripheral memory B-cells, but intact vaccination response might indicate a specific role for B-cells in sarcoidosis that never has been shown before. As a consequence B-cell ablative therapy might become a new strategy in the treatment of sarcoidosis patients. Further investigations on the role of the B-cells as antigen presenting cells or producers of cytokines in the pathogenesis of sarcoidosis are currently in progress.

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B: **Genetics and mechanisms in sarcoidosis**

B9: Polymorphisms in CCR5 confer susceptibility to Löfgren's syndrome and may regulate the immune response

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Background:
Löfgren's syndrome is an acute and usually self-remitting phenotype of sarcoidosis. Several studies have found associations between specific gene polymorphisms and susceptibility to sarcoidosis.
Chemokines are small peptides that mediate monocyte, lymphocyte and neutrophil chemotactic activity by binding to specific G-protein coupled receptors, such as CCR5. A study showed that the HHC haplotype of CCR5, with single nucleotide polymorphism (SNP) rs1799987, was associated with Löfgren's syndrome.
Objective:
We investigated if SNPs of the CCR5 gene were associated with Löfgren's syndrome and had an effect on the B-lymphocyte response of patients.
Methods: Hundred and twenty patients with Löfgren's syndrome were characterized and genotyped for 4 SNPs in CCR5. Our control cohort consisted of 313 self-reported healthy individuals.
Calcium mobilization response to MIP-1a, a ligand of CCR5, was measured in peripheral blood B-lymphocytes of 18 Löfgren's syndrome patients (3 male and 15 female) and 3 controls.
Results:
Carriage of the G-allele was significantly higher in patients with Löfgren's syndrome than in healthy controls (p=0.00557, CI 1.13-2.01, OR 1.505).
Twelve out of 18 patients with Löfgren's syndrome showed no calcium response, of which 11 were carriers of the G-allele: 7 GG, 4 GA, 1 AA. Nine of these 12 patients where women. All controls showed a calcium mobilization response upon stimulation with MIP-1a.
Conclusions:
The SNP rs1799987 in the CCR5 genes is associated with Löfgren's syndrome. Functionality assays showed that polymorphisms of the CCR5 have an impact on cellular processes that may regulate the response of B-lymphocytes.

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B10: DRB1*03-DQB1*02 haplotype is associated with chronicity in Löfgren's syndrome

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Background: Löfgren's syndrome (LS) is a distinct form of sarcoidosis usually associated with a favorable prognosis. However there are a small number of patients that evolutes to a chronic disease. A strong association between HLA-DRB1* 03 and disease course has been described, namely the association between DRB1*03-negative patients and chronicity.
Aim: Study of HLA – class II influence on LS disease course in a Portuguese population.
Material and Methods: Fifty four sarcoidosis patients with LS were included and typed to HLA-DRB1*, - DQB1*. The diagnosis was made according with ERS/ATS/WASOG statement. A chronic course was defined as disease over at least 2 years and disease resolution was considered when a disappearance of symptoms, normalization of chest x-ray and pulmonary function tests occurred within 2 years after diagnosis. HLA typing was performed by PCR-SSO. HLA allele frequencies where determined by direct counting. After confirm Hardy-Weinberg expected proportions, HLA-DRB1*-DQB1* haplotypes frequencies for resolution and chronicity were estimated with the Expectation-Maximization algorithm. $\chi 2$ test (or Fisher exact test, when appropriated) was used to assess association of HLA alleles and haplotypes with disease course. Odds ratios (OR) and their 95% confidence intervals (95% CI) were also calculated as association measures.
Results: In comparison with LS patients with disease resolution, chronic patients have increased frequencies for DRB1*15 and DQB1*06 alleles, while DRB1*03 and DQB1*02 frequencies are decreased. DRB1*03-DQB1*02 haplotype frequency is also significantly lower in chronic patients when compared with disease resolution ($p< 0.01$; OR = 0.22; 95%CI = [0.04;0.84]). We also found a risk effect for chronic evolution related with DRB1*15-DQB1*06 haplotype ($p<0.01$; OR= 7.6; 95%CI = [1.13;82.58]).
Conclusions: This study identifies two HLA-DRB1*-DQB1* haplotypes associated with LS disease course. While DRB1*15-DQB1*06 haplotype have a risk effect for chronicity, DRB1*03-DQB1*02 haplotype is associated with disease resolution. This results also confirm the association between HLA-DRB1*03 and LS.

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B11: Honeycombing pattern: a particular form of sarcoidosis-related pulmonary fibrosis

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Background: Pulmonary fibrosis, as defined by radiographic stage 4, is the major cause of morbidity and mortality in sarcoidosis. Three main patterns of pulmonary fibrosis have been described on HRCT: bronchial distorsion, hilo-peripheral linear opacities, and honeycombing (HC).
Aims of the study: To determine whether patients with HC pattern have a particular phenotype of sarcoidosis.
Methods: This is a retrospective and monocenter study, comparing 34 patients with HC pattern (men: 62%, age: 56±14 years) with 34 controls with other HRCT pattern. Controls were matched with patients for the date of the first available workup with stage 4.
Results: HC predominated in the upper lobes, but 5 cases (15%) had evidence of basal and peripheral predilection close to that seen in idiopathic pulmonary fibrosis. Patients differed from controls for a higher frequency of environment exposure (39% versus 15%, p=0.045), more altered gas exchanges (PaO2: 77±10 mmHg versus 82±11 mmHg, p=0.04 and DLCO: 38 ± 16% versus 60 ± 16%, p<0.0001) and reduced lung volumes (FVC: 62 ± 2% versus 75 ± 20%, p=0.017) and an increased occurrence of pulmonary hypertension (62% versus 32%, p=0.029) and oxygen requirement (56% versus 15%, p=0.001). Extra-respiratory involvement of sarcoidosis was less frequent (21% versus 47%, p=0.04) in patients as well as residual granulomatous activity, as judged by serum angiotensin converting enzyme and HRCT signs. However, mortality was similar between the two groups.
Conclusions: The phenotype of patients with HC is original. An exposure to inhaled particles may play a role in the development of this particular fibrosis evolution and explain the local respiratory severity of sarcoidosis while the disease seems to be less active and severe from a systemic point of view.

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B12: Patients with ANCA-associated vasculitis in long-term remission have increased numbers of circulating IL-10 producing Th17 cells

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Background: IL-17 producing T-cells (Th17) are a pro-inflammatory subset of T-cells which are pivotal in autoimmunity. Importantly, anti-inflammatory regulatory T-cells (Treg) are able to convert to Th17 cells (“Plasticity”) and vice versa. Little is known about the Th17 response or plasticity in ANCA-associated vasculitis (AAV). Therefore, we investigated Th17 subsets in AAV.
Methods: 30 AAV patients in remission (AAV-r), 22 with active disease (AAV-a) and 14 healthy controls (HC) were enrolled. PBMC were isolated, cytokine-producing T-helper-cells were detected by intracellular FACS. Renal biopsies with necrotizing-crescentic-glomerulonephritis (NCGN) were stained by immuno histochemistry. Data is given as mean ±SD and percentage of total T-helper-cells.
Results: AAV-r and AAV-a patients had more IL-17A+ T-helper-cells than HC (1.99 ±1.6% vs. 0.71 ±0.3% and 1.79 ±2.1% vs. 0.71 ±0.3%, all $p<0.05$). IL-17+ cells were also present in renal biopsies with NCGN. AAV-r patients showed a higher percentage of IL-10+/IL-17A+ double-positive T-cells than HC (0.052 ±0.05% vs. 0.028 ±0.016%, p=0.07). AAV-r patients in stable long-term remission (>4 years) had significantly more IL-10+/IL-17A+ T-cells than AAV-r patients with relapsing disease course or HC (0.053 ±0.026% vs. 0.040 ± 0.051%, $p<0.05$ and 0.053 ±0.026% vs. 0.028 ±0.016%, p=0.007). The amount of IL-10+/IL-17A+ T-cells was strongly associated with the number of Tregs in AAV-r (r=0.83, $p<0.0001$) but not in HC (r=-0.09, p=0.76).
Conclusion: Th17 cells are expanded during active and quiescent phase of AAV. Elevated numbers of IL-10 producing Th17-cells are demonstrated for the first time in AAV and might point at enhanced plasticity. Since these cells are elevated in patients with long-lasting remission we postulate that these cells are protective.

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C: **Diagnostic tools in sarcoidosis: What is new?**

C1: The Reproducibility of Serum Vitamin D 1,25 Measurement in Sarcoidosis

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Background:
Hypercalcemia and hypercalciuria are encountered in about ten percent of sarcoidosis patients. Vitamin D-1,25 (D-1,25) is the metabolically active form of vitamin D-25 (D-25). Increased D-1,25 is a common mechanism provided for the hypercalcemia and hypercalcuria experienced by sarcoidosis patients. Bone health guidelines recommend that only serum vitamin D-25 (D-25) be measured in non renal failure patients; however, these guidelines have not been adapted for sarcoidosis patients. Although a correlation between D-25 and D-1,25 levels usually exists, investigators have reported high levels of D-1,25 with concurrent low levels of D-25 in some sarcoidosis patients.
Methods:
In order to test the reproducibility and concordance between concentrations of D-1,25 and D-25 levels in sarcoidosis patients, we compared serial serum levels of calcium with vitamins D-1,25 and D-25 levels. These measurements were obtained at least three months apart.
Results:
A total of 49 sarcoidosis patients was studied. Nine patients discontinued supplemental vitamin D between the two measurements because of elevated levels of D-1,25. In all nine cases, the D-1,25 levels decreased to normal and were not further studied. In the remaining 40 cases, a significant correlation was noted between the first and second D-1,25 levels ($R=0.798$, $p<0.0001$). There was also a significant correlation between the first and second measurements for D-25 ($R=0.658$, $p<0.0001$) and serum calcium ($R=0.660$, $p<0.0001$).
Conclusion:
Evaluation of random serum D-1,25 provides a reproducible measure of the active form of vitamin D metabolism and should replace measurement of serum D-25 in patients with sarcoidosis.

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C2: Association of HRCT findings with pulmonary PET activity in sarcoidosis

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Background: The value of high-resolution computed tomography (HRCT) and whole body F18-FDG PET/CT scan (PET) to assess inflammatory activity in sarcoidosis is well established. The aim of this retrospective study was to address the association of HRCT findings with pulmonary PET activity.
Methods: The clinical records of 96 (age: 46.1±10.7; female: 40) known sarcoidosis patients visiting the outpatient clinic between June 2005 and June 2010, who underwent a PET as well as a HRCT, were reviewed. All PET scans were interpreted by an experienced nuclear medicine physician, and all HRCT scans were classified by an experienced radiologist according to the Oberstein scoring system.
Results: In 78/96 of the studied patients PET was positive. The HRCT score according to Oberstein of the PET positive patients was high (6.04±3.84) compared to the PET negatives (2.89±3.00; $p=0.002$). In 56/78 of the PET positive patients, PET positivity in the pulmonary parenchyma was found. In these patients, Oberstein scores (mean 7.14±3.16) were high compared with the 22/78 patients without PET positivity in the pulmonary parenchyma (3.23±2.90; $p<0.0001$). Furthermore, the diffusing capacity for carbon monoxide (DLCO) was lower in patients with parenchymal PET positivity (64.8±20.1 % predicted) versus the patients without parenchymal PET positivity (80.5±14.2 % predicted; $p<0.01$). Moreover, in 59/78 of the PET positive patients the mediastinal lymph nodes were PET positive. No differences in the HRCT score or DLCO were found between patients with or without PET positive mediastinal lymph nodes.
Conclusion: Abnormal changes of lung parenchyma, established by HRCT features, were associated with parenchymal PET positivity and abnormal gas exchange.

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C3: Bone and bone marrow involvement in sarcoidosis as detected by F18 FDG-PET/CT

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Background: The incidence of bone involvement in sarcoidosis is not clear. Previous studies, based on morphologic imaging, suggest a prevalence of 3-5%, most frequently affecting the phalanges. Till now the usefulness of PET/CT in detection of osseous involvement has not been evaluated. The aim of this study was to determine the frequency and distribution pattern of bone and bone marrow involvement as detected by PET/CT in sarcoidosis patients.
Methods: Between June 2006 and September 2010, 122 patients suffering from severe sarcoidosis underwent a PET/CT scan. Of these 122 patients 94 (77%) had PET-positive findings associated with sarcoidosis. Subsequently, these 94 PET/CT scans were screened for the presence of bone or bone marrow localisations. All low-dose CT scans were examined by an experienced musculoskeletal radiologist to exclude other causes of enlarged bone uptake.
Results: In 32 (34%) of the 94 patients, PET positive bone or bone marrow localisations were present. Sixty % (19/32) showed obvious focal bone lesions at various locations: axial skeleton (47%), pelvis (40%), extremities (34%), and skull (2%)). In 40% (13/32) diffuse enlarged uptake in both the axial and peripheral bone marrow, without focal lesions were found. Both diffuse and focal uptake was seen in 34% (11/32), whereas in 25% (8/32) only focal lesions. In all but 2 (6%) patients no bone-abnormalities on low dose CT were found.
Conclusions: More than one third of PET/CT positive sarcoidosis patients had osseous sarcoidosis features on PET/CT, 26% (32/122) of all studied patients. The majority of these lesions (94%) could not be detected on low dose CT. There was no single location of preference. These preliminary results stress the importance of functional PET-imaging as a sensitive tool to investigate bone involvement in sarcoidosis.

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C4: Localization of cardiac involvement in sarcoidosis by FDG-PET

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To assess localization of cardiac involvement in sarcoidosis, all FDG PET scans performed on patients were reviewed between December 2004 and December 2009. The records associated with the scans were reviewed to be sure that the patients followed a carbohydrate free diet for 24hours prior to the scan. The records of these patients were then reviewed to be certain that they had a tissue diagnosis of sarcoidosis confirmed. Finally the scans were assessed for localization of cardiac involvement as well as extracardiac involvement. Cardiac involvement was graded as lateral wall (LW), Inferior wall (IW), Septal (S), Anterior Wall (AW), Apex (A) or right ventricle (RV). Extra cardiac involvement was graded as lungs (L), pleura (P), spleen (Sp), bone (B), liver (Li), right supraclavicular (RS), left suprclavicular (LS), right paratracheal (RP), left paratracheal (LP), precarinal (PC), subcarinal (SC), left pericardial (LC), right hilar (RH), left hilar, prevascular (PV), right axillary (RA), left axillary (LA), paraesophageal (PE), peripancreatic (PP), and porta hepatis (PH). Fifty-two individuals had at least one FDG-PET scan with a confirmed diagnosis of sarcoidosis. The male/female ratio was 28/24 and the number of positive scans were 23. 9/24 (38%) females had positive scans while 14/28 (50%) of males had positive scans. A single cardiac lesion was noted in only 4/23 (17%) patients and involved the apex in 2 and the inferior wall and septum in the other 2. Specific involvement in the 23 patients was 17/23 (74%) LW, 12/23 (52%0 IW, 16/23 (70%) S, 14/23 (61%) AW, 13/23 (56%) A, and 3/23 (13%) RV. Thus all portions of the heart can be involved with the LW most common and the RV least common. Five of twenty-three (22%) with cardiac involvement had no evidence of extracardiac involvement at the time of the PET scan. Of the twenty-nine individuals with a negative PET scan 15 did not have cardiac sarcoidosis. Of the 14 patients with cardiac sarcoidosis with a negative PET scan, 10 were on Rx at the time of the PET scan. The 4 patients with cardiac sarcoidosis with negative PET scan and not on treatment all had stable long standing disease. Thus, the PET scan with a proper diet appears to be sensitive for the diagnosis of active cardiac sarcoidosis. In addition, most cases of cardiac sarcoidosis appear to involve multiple areas of the heart.

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C: **Diagnostic tools in sarcoidosis: What is new?**

C5: Whole-body FDG-PET/CT in the assessment of patients with sarcoidosis. A preliminary report

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Background:
FDG-PET has been reported to be useful in the assessment of residual activity in patients with fibrotic pulmonary sarcoidosis and in the detection of occult granuloma sites. We report our preliminary experience in the application of this technique in a short series of patients with sarcoidosis.
Methods:
Thirteen whole-body FDG-PET/CT were performed in 11 patients with biopsy-proved sarcoidosis (3 under corticosteroid treatment). Indications included: 1) assessment of presence of pulmonary activity in chronic fibrotic pulmonary sarcoidosis (5 patients), 2) detection of occult activity sites in systemic sarcoidosis (3 patients), 3) characterization of mediastinal lymph nodes (2 patients) and 4) staging of primary lung cancer in patients with previously known sarcoidosis (1 patient).
Results:
FDG-PET/CT showed pulmonary activity in 3 out of 5 patients with chronic fibrotic pulmonary sarcoidosis (SUV ranged from 3.5 to 12.1). The other 2 patients did not show any uptake. Mediastinal uptake was present in 6 patients (SUV 6.9-44.3). Four patients showed 4 occult inflammatory sites not suspected clinically (SUV 3.4-11): salivary glands, subcutaneous, muscle and supraclavicular lymph node. Two patients had a second FDG-PET/CT: one patient showed a significant reduction in pulmonary uptake after corticosteroid therapy, and the patient with lung cancer did not show any metabolic change in sarcoid mediastinal lymph nodes uptake after radiochemotherapy.
Conclusions:
Our preliminary results show that whole-body FDG-PET/CT is useful to detect residual activity in patients with chronic fibrotic pulmonary sarcoidosis and may help to therapeutic decisions. In addition, it is also useful in detecting occult activity sites in the evaluation of patients with multisystemic disease. Longer series are needed to better define the role of FDG-PET/CT in the assessment and follow-up of patients with sarcoidosis.

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C6: Fluorodeoxyglucosae positron emission tomography scans in sarcoidosis

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Introduction: Sarcoidosis is granulomatous disorders which can affect any organ. Chronic course of diseases is the main characterics in most patients so the visiting doctors with every new complaint are obvious. As the etiological diagnosis is unknown despite the efforts, corticosteroid therapy is still the leading medication. The relative new, for us, diagnostic procedures, 18 F-FDG PET is find place for it self in malignancies. Can we re rely on this findings during the course of sarcoidosis. Aim of work: is to analyse the findings during the 18F FDG PET scan in patients with course of sarcoidosis. Results: Analysis is prospective. We obtained the results from 50 patients who were referred to the Clinic for lung diseases and tuberculosis either ambulatory. Chronic Course of sarcoidosis were obtained in 41 patients (27 F/ 14 M, mean age of 47.7 years) and in 9 patients with acute onset of sarcoidosis (5 F/4M, mean age of 35 .7 years). The main findings in all patients were enlarged lymph nodes predominantly mediastinal (11 patients), lung involvement with positive scan in 11 patients, positive lymph nodes with positive pulmonary scan in 7 patients. Non pulmonary positive scan were obtained in 1 female, cortex of central nervous system, muscles in 2 patients, myocardial in 2 patients, glandula thyreoidea in 2 patients, skeletal in 3 patients. Comparative analysis were performed due to CT thoracic scan and in 3 patients with fibrous findings positive scan were obtained. Levels of SUV were from 3.2 do 21. In two patients despite the therapy, high levels of biochemical marker activities, clinical symptoms and negative scan were obtained. Activity markers levels for sarcoidosis were obtained and only in acute sarcoidosis levels were high (mean values 83.3 U/L ACE) while in sarcoidosis of chronic course mean values for ACE were 52.2 U/L. Another correlation were obtained due to spirometry and diffusion capacity for CO but no statistical results were obtained. Since the main leading symptoms were fatigue in chronic sarcoidosis, fatigue scale performed and no statistical findings were obtained. Conclusion: Sarcoidosis is chronic disorders with phase of reactivations that wasting for additional therapy. Where is the final point for delivering therapy with positive outcome we don't known, for sure. In that name, could this expansive method, take place as the potential marker of disorders activity.

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C7: Neurosarcoidosis : MRI and CSF findings in our patients

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Establishing the diagnosis of neurosarcoidosis may be difficult, so the goal of this study was to elucidate the relation between clinical symptoms, MRI findings, and cerebrospinal fluid (CSF) findings.
Methods: 73 patients with probable diagnosis of neurosarcoidosis were analyzed (51 female/22 male), mean age 51.73 years; the mean follow - up period 7 years. 70 patients (95.9%) had chronic biopsy positive sarcoidosis, only 3patients had acute sarcoidosis at the time they experienced symptoms of possible neurosarcoidosis MRI findings were classified according to Zajicek J.P.Scolding N.J et al. Central nervous system sarcoidosis-diagnosis and management Q J Med 1999; 92:103-117. Out of the whole group in 63 (87.7%) patients CSF was taken for ACE analyze.
Results: in 13/63 patients (20%) ACE in CSF was not detected (0 U/L). 51/63 patients (80%) had different CSF ACE levels ranging from 1-15 U/L. All patents were symptomatic and besides constitutional symptoms of sarcoidosis they have: headache 47 (92.2%), vertigo 24 (47.1%), cranial nerve involvement (facial palsy) 7 patients (13.7%), blurred vision 10 (19.6%), and seizures 2 patients (3.9%). Comparing the MRI appearances in patients with positive ACE in CSF we found:
(1).spinal cord lesions - 3 patients (5.9%);
(2).hydrocephalus - 2 patients (3.9%);
(3).meningeal enhancement -18 patients (35.3%);
(4).parenchymal lesions - 31 patients (66%);
(5).white matter lesions -11 patients (23. 4%);
(6).normal MRI findings had 2 patients (3.9%).
Conclusion: In this study we intended to show a certain relationship between the MRI findings and CSF analyses emphasizing the significance of the CSF ACE levels. In patients with biopsy positive sarcoidosis (different organs involvement) and probable diagnosis of neurosarcoidosis CSF must be obtained for further analyses among which the ACE level with MRI finding can indicate the diagnosis, because these two examinations correlated in 96% of our patients.

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C8: Chitotriosidase – reliable test for sarcoidosis activity

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Background:
Chitotriosidase is an enzyme secreted by activated macrophages able to catalyze the hydrolysis of both chitin and chitin-like substrates. Patients with sarcoidosis have elevated levels of chitotriosidase as the result of massive production of chitotriosidase by sarcoid macrophages. The aim of the study was to analyze serum chitotriosidase concentrations in patients with active vs inactive sarcoidosis. We also wanted to compare the reliability of these results with serum angiotensin-converting enzyme (ACE) in sarcoidosis patients.
Methods:
53 biopsy positive sarcoidosis patients were enrolled in this study (39 female and 14 male) mean age 50.4 years. 37 patients had active and 16 patients had inactive sarcoiodosis. Level of serum chitotriosidase and serum ACE were analyzed in relation to the clinical activity of sarcoidosis.
Results: Considering the small investigated sample of our group of patients we performed discriminant analyses. Discriminant analysis showed that cut point for chitotriosidase in the group of patients with active diseases is highly statistically significant (Lamda=0.654; Chi square=21.417; Canonical Correlation=0.588 $p<0.05$) than in the group with inactive disease. Difference for serum ACE levels were not statistically significant to coexist with the disease activity. (Lamda=0.896; Chi square=5.525; Canonical Correlation=0.322 $p<0.05$)
Conclusion:
Although the data need to be validated by further investigations, the observations made in this study seem to indicate that serum chitotriosidase concentrations may be a useful marker for monitoring sarcoidosis disease activity and prognosis.

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C: **Diagnostic tools in sarcoidosis: What is new?**

C9: Somatostatin receptor scintigraphy in sarcoidosis

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Background: Somatostatin receptor scintigraphy (SRS) localizes granulomas by binding to somatostatin receptors that are expressed in sarcoidosis, a granulomatous disease frequently involving lungs, skin or eyes. We correlated uptake patterns on SRS and to disease parameters.
Methods: The degree of intensity (DoI) of uptake and localization of sarcoidosis associated lesion (SAL) in 218 patients were determined. DoI was compared with serum angiotensin converting enzyme (ACE) and serum soluble interleukin-2 receptor (sIL-2R). Typical patterns on SRS were compared to conventional chest CT and -X-ray.
Results: SRS was negative in 28 patients, 10 patients had one -and 180 patients had more SAL. The DoI correlated significantly with ACE ($P < 0.001$) sIL-2R levels ($P < 0.01$). Mediastinal lesions together with either eye, salivary glands, clavicular or hilar localizations on SRS demonstrated a significant characteristic pattern. All patients with abnormal conventional tests had SRS uptake. Moreover, of 94 patients with normal radiological findings 49 expressed pathological SRS uptake. In 36 of these 49 patients a lung biopsy was taken, which revealed sarcoidosis in 31 patients.
Conclusions: The DoI in SRS correlates with sarcoidosis activity. SRS is more sensitive in diagnosing sarcoidosis, even in patients with normal chest radiology. SRS therefore provides a useful and sensitive imaging technique to monitor organ involvement and therapy in patients with sarcoidosis.

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C10: The significance of high resolution computerized tomography in the diagnosis of pulmonary sarcoidosis

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Background: A radiological chest finding is relevant for diagnosing a disease, deciding about the treatment to be applied, as well as for predicting and monitoring the course of the disease and its response to the applied treatment.
Objective: To analyse the high resolution computerized tomography (HRCT) findings in patients with histological confirmed pulmonary sarcoidosis (without the other pulmonary diseases).
Methods: We analysed the presence and frequency of HRCT signs characteristic of pulmonary sarcoidosis, their distribution in the craniocaudal and centro-peripheral direction, as well as the symmetry of the lesions in 52 patients with histologically confirmed pulmonary sarcoidosis.
Results: The following HRCT findings were registered: enlarged lymph nodes – 41 (78.8%) pts, nodular lesions – 21 (40.43%) pts, linear lesions – 26 (50%) pts, increased density areas in the pulmonary parenchyma – 16 (30.77%) pts, and deteriorated pulmonary parenchyma architecture (the so called definite lesions) – 15 (28.85%) pts. Predilection of the detected pulmonary lesions towards the craniocaudal lung regions, centro-peripheral direction and a symmetric distribution of the lesions were registered in 19 (36.54%), 2(3.85%) and 35 (67.31%) of the patients.
Conclusion: Providing an insight into the extent, severity, and activity of the pulmonary parenchyma lesions the HRCT has for a clinician very significant diagnostic value.

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C11: Interferon γ release assay are useful to exclude M. tuberculosis infection in sarcoidosis patients with positive tuberculin skin test

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Introduction:
Both sarcoidosis and tuberculosis are similar in their radiological and histological pictures. Sometimes it is difficult to distinguish between these two diseases. Tuberculin skin test (TST) is helpful in differential diagnosis. There is tuberculin anergy in sarcoidosis, and most patients have negative TST. There is a group of sarcoidosis patients with positive TST. In countries with high incidence of tuberculosis, positive TST in sarcoidosis- suspected patients indicates rather for tuberculosis. This is more complicated in BCG vaccinated populations, because of BCG vaccination might be the cause of TST positivity.
Object and rationale: BCG vaccination is obligatory in Poland since early 50-ties. Over 90% of the population is vaccinated. We used two commercially available interferon γ release assays (IGRAs) to verify the value of positive TST in BCG vaccinated patients with pulmonary sarcoidosis.
Material and methods:
34 patients (12 female, 22 male; mean aged 36.6±9.5) with newly diagnosed sarcoidosis were included in this study. 7/8 (87.5%) of TST positive patients were BCG vaccinated as was proven by the presence of the scar. The blood samples for both commercial IGRA (Quantiferon TB Gold and T-SPOT-TB) were collected directly before tuberculin testing.
Results:
TST was negative in 26 (76.5%) and positive in 8 (23.5%) of 34 patients. There were no differences in age and sex between these two groups. Both tests: QFT and T-SPOT-TB were negative in all TST positive as well as TST negative sarcoidosis cases.
Conclusions:
Positive TST in sarcoidosis patients is not related to M. tuberculosis infection. There was excellent agreement between two IGRAs.

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C12: Assessment of prevalence and severity of hepatic involvement in sarcoidosis

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Backgrounds: Sarcoidosis is a multisystemic inflammatory granulomatous disease, with rare symptomatic hepatic involvement. Diagnosis of hepatic sarcoidosis is a clinical challenge, because of the wide spectrum of disease presentation and course. The aim of this study is to evaluate the association between severity of liver test abnormalities and histopathological characteristics.
Methods: In this retrospective analysis patients with confirmed extrahepatic sarcoidosis presenting with liver test abnormalities (alkaline phosphatase, γ-glutamyl transaminase, alanine aminotransferase and/or aspartate aminotransferase >1.5 times the upper limit of normal (ULN)) classified according to severity into mild (0 liver tests ≥3x ULN), moderate (1 or 2 liver tests ≥3x ULN) and severe (3 or 4 liver tests ≥3x ULN) were studied. The association between severity of liver tests and histology was examined using non-parametric statistics and multiple regression analysis (p-value<0.05 statistically significant).
Results: Liver test abnormalities were found in 204 out of 837 chronic sarcoidosis patients (24.4%), 127 of which (15.2%) were suspected of having hepatic sarcoidosis (79/127 male, 111 Caucasian, 16 other races). In 22/127 (17.3%) a liver biopsy was obtained; 21 compatible with hepatic sarcoidosis. Severity of liver test abnormalities was significantly associated with extensiveness of granulomatous inflammation ($\rho=0.582$, $p=0.006$) and degree of fibrosis ($\rho=0.643$, $p=0.002$). These results persisted after multiple regression analysis for treatment status, gender, genetics, ethnicity and age.
Conclusions: This was the first study indicating that in hepatic sarcoidosis severe and moderate liver test abnormalities are associated with more advanced histopathological disease. Therefore, in the management of sarcoidosis patients with moderate and severe liver test abnormalities a liver biopsy is recommended. Future studies are needed to assess the effect of treatment on disease progression and complications.

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D: **Treatment options in sarcoidosis**

D1: Rituximab for granulomatous ocular disease

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Background:
The etiology of granulomatous ocular disease includes both sarcoidosis and ANCA-associated vasculitis. Inflammation can present as episcleritis, iritis, or uveitis. Both local and systemic administration of corticosteroids can be effective in controlling acute inflammation. However, long term use of corticosteroids may lead to significant ocular toxicity, including cataract formation and glaucoma. Alternative corticosteroid-sparing treatments include cytotoxic agents such as methotrexate. Although the use of anti-tumor necrosis factor (TNF) agents may be useful for refractory eye disease, these agents are associated with significant toxicity.
Methods:
We performed a retrospective review of a single institution experience of rituximab for the treatment of refractory granulomatous ocular disease.
Results:
To date, we have administered for at least six months rituximab for refractory ocular inflammation in six patients. Three patients were diagnosed with ANCA-associated vasculitis and three patients with sarcoidosis. All patients had persistent disease despite prior prednisone therapy and at least one cytotoxic agent including cyclophosphamide (4 patients), methotrexate (4 patients), and leflunomide (2 patients). Additionally one patient failed to respond to infliximab. Two sarcoidosis patients with persistent disease declined infliximab because of toxicity concerns. All patients received at least six months of rituximab. Five of six patients experienced clinical responses with improvement in ocular inflammation and reduction in corticosteroid dosage. Four of six patients had co-existing lung disease, and all four patients experienced improvement in lung lesions. Transient leukopenia developed in two patients. However, leukopenia reversed with reduction of concurrent cytotoxic treatment. No other significant toxicities were encountered.
Conclusion:
We propose that rituximab may be a safe and effective treatment for refractory ocular granulomatous disease.

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D2: Successful treatment with adalimumab of sarcoidosis patients with chronic non-infectious uveitis

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Background: Adalimumab, a humanized monoclonal antibody targeted against TNF-α, has proved to be successful in the treatment of uveitis. Another anti-TNF-α agent, i.e. infliximab, has been reported of benefit in the treatment of refractory sarcoidosis. The aim of this study was to evaluate the effect of adalimumab on refractory uveitis and other disabling sarcoidosis symptoms.
Methods: In this retrospective, institutional study sarcoidosis patients with refractory posterior uveitis (n=26, 17 females, 41 eyes in total) were followed for twelve months after initiation of adalimumab. Inclusion criteria were non-responsiveness or intolerance to prednisone or other immunosuppressive drugs (mainly methotrexate). Main outcome measures were visual acuity, vasculitis, papillitis, and/or macular edema improvement, stabilization or no recurrence of the uveitis. Furthermore, other clinical manifestations of sarcoidosis (fatigue, lung function, laboratory parameters) were followed.
Results: Visual acuity improved in at least one affected eye in 15/26 patients (58%), while remaining stable in 10 patients (38%), 6 of these latter patients had a decreased visual acuity. In one patient (4%), visual acuity decreased in one eye and remained stable in the other affected eye. Choroidal involvement resolved in 9/14 patients, 5 had partial improvement; vasculitis resolved in 1/1 patient; papillitis resolved in 7/8 patients, 1 had partial response; macular edema resolved in 5/8 patients, 3 had partial response; vitreous cleared complete in 5/5 patients. Final outcome regarding inflammatory signs showed improvement in 22 patients (85%) and stabilisation in four patients (15%). At 12 months no recurrences were reported in those successfully treated. Fatigue improved in 14/21 (67%) of the patients suffering from fatigue and 7/8 (88%) patients suffering from a diffusing capacity problem (DLCO <80% predicted) demonstrated an improvement ($p<0.01$). Moreover, laboratory parameters of inflammatory activity (C-reactive protein; serum angiotensine converting enzyme and soluble interleukin-2 Receptor) improved as well.
Conclusion: Adalimumab appeared to be a successful treatment of chronic non-infectious uveitis in sarcoidosis patients, demonstrating a favourable outcome.

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D3: Successful treatment of multi-organ refractory sarcoidosis with adalimumab

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Background: Sarcoidosis is an granulomatous inflammatory disease in which Tumor necrosis factor-alpha plays an important role. Refractory sarcoidosis can be very difficult to treat and inhibition of TNF-α with Infliximab has been reported successful in such patients. Nevertheless, Infliximab has several disadvantages above another TNF-α inhibiting agent, namely Adalimumab, which is now only reimbursed in the treatment of uveitis. To emphasize the clinical usefulness of anti-TNF-α therapy and propose a good alternative to Infliximab, we here report two cases of refractory multi-organ sarcoidosis responding well to treatment with Adalimumab.
Methods: Two patients, one male and one female, respectively 55 and 69 years old, both suffered from pulmonary as well as extrapulmonary manifestations of sarcoidosis, with involvement of the skin (biopsy proved granuloma annulare) and eyes (iridocyclitis and posterior uveitis respectively). Both patients did not respond to initial therapy with methotrexate, so that Adalimumab 40mg weekly was added.
Results: In both patients all manifestations, both pulmonary and extrapulmonary, improved dramatically within six months of treatment. We documented the improvement with photo's of the skin lesions, laboratory tests and CT scans of the thorax.
Conclusion: Our case report indicate the usefulness of Adalimumab in chronic refractory sarcoidosis with granuloma annulare, uveitis, as well as pulmonary involvement, thereby emphasizing its potential clinical usefulness in treating sarcoidosis and proposing it as a suitable alternative to Infliximab. This finding is concordant with other case reports. To make future registration for the use of Adalimumab in this indication possible, additional randomized controlled trials need to be performed to evaluate efficacy, safety and appropriate dose.

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D4: Effect of infliximab on lung function and well-being in patients with refractory sarcoidosis

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Background: Infliximab improves the lung function in refractory sarcoidosis. Changes in other clinical relevant parameters are still unclear.
The purpose of this study is to assess the effectiveness of treatment with infliximab on change in pulmonary function tests, fatigue severity and physical functioning, serum parameters and uptake on (18)F-fluorodeoxyglucose positron emission tomography (FDG-PET) in patients with refractory sarcoidosis.
Methods: Retrospectively, 45 patients with refractory sarcoidosis were evaluated. All patients received 6 infusions of infliximab (5mg/kg bodyweight).
Main endpoints were the change in percentage of predicted vital capacity (VC), forced expiratory volume in 1 second (FEV1) and diffusing capacity of the lung for carbon monoxide corrected for haemoglobin concentration (DLCOc) from baseline till after dose 6. Other endpoints were the changes in fatigue (Checklist Individual Strength) and physical functioning (Medical Outcome Score-short form), serum soluble interleukin-2 receptor (sIL-2R), angiotensin converting enzyme (ACE) and change demonstrated by FDG-PET, expressed as maximum standardized uptake value (SUVmax).
Results: VC showed an increase of 5,4% ($p < 0.0001$), FEV1 an increase of 5,3% ($p < 0.001$) and DLCOc an increase of 3,1% ($p = 0.012$). Examining the subgroup of patients that had a pulmonary indication for treatment, these percentages were even higher (VC: 7,6%, FEV1: 7,9%, DCLOc: 3,5%).
In the total group fatigue severity scores and physical functioning scores significantly changed over time, indicating clinically relevant improvement. Additionally, a significant decrease in ACE, sIL-2R and SUVmax was observed.
Conclusions: Infliximab improves lung function as well as well-being and serum and PET-parameters in patients with refractory sarcoidosis.

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D: **Treatment options in sarcoidosis**

D5: Infliximab in refractory sarcoidosis: the ild care experience

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Background:
Only one double blind randomized study evaluating the effect of the anti-TNF-α drug Infliximab in sarcoidosis was performed. The aim of this study was to report our experience with Infliximab in refractory sarcoidosis patients.
Methods:
From 2003 till 2010 in 77 refractory sarcoidosis patients (55 male; Chest X-ray stage 0-I: n=28; stage II-II: n=31 and stage IV: n=18) treatment with Infliximab was initiated as they did not respond to corticosteroids and/or methotrexate (MTX), or suffered from severe side effects. The indication varied from respiratory functional impairment, small fiber neuropathy (SFN) to neurosarcoidosis. The dose was 402±54 mg; the dose interval 4.5±0.6 weeks (range 3-5). Additionally, they used prednisone (4.5±6.5; range 0-25mg daily) and MTX 4.8±4.4; range 0-15mg once a week). Clinical data were gathered and they all completed the Fatigue Assessment Scale (FAS) and the small fiber neuropathy screening list (SFNSL). Retrospectively, the data were analysed after a follow-up period of one year.
Results:
The treatment period was 1.8±1.3 (range: 0-7yrs). After one year follow-up the signs of inflammation in the peripheral blood improved, as well as the radiological features. Fatigue was less prominent (FAS score: 29.9±7.8 vs 34.1±7.5; p<0.001), and the SFN related symptoms improved (SFNSL 23.1±14.7 vs 28.5±15.0; p<0.001). The exercise capacity and fatigue improved in >70% of the patients, 20% were stable. Seven cases (9%) had to stop due to antibody formation, 31 are still on treatment.
Conclusion:
Infliximab appeared successful in 70% of the treated refractory sarcoidosis patients, demonstrating improvement of symptoms including fatigue and those related to SFN. The interval of 4-5 weeks seemed to be appropriate.

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D6: Steroid therapy and metabolic syndrome in patients with sarcoidosis

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Background: The aim of this study is to analyze the influence of steroid therapy on metabolic impairments - metabolic syndrome in sarcoidosis patients. 88 biopsy positive sarcoidosis patients (69 female/19 male) were enrolled in this study. Methods: 39 patients from the analyzed group had metabolic syndrome as defined by the Third National Health and Nutrition Examination Survey (ADPIII)[1]. 129 patients were on high doses of steroid therapy (20mg/ daily), 32patients were on morbostatic doses (5-10mg daily) and 27 patients were without steroid therapy. Results: Statistically significant difference was found in lipid metabolism between patients with metabolic syndrome and sarcoidosis patients without metabolic syndrome. (F=2629.336;df1=4;df2=80; p<0.01). Multivariant analyses revealed significant link between metabolic syndrome and dose of steroid therapy (F=4.911;df1=4;df2=82;p<0.01).Patients on low doses of steroid therapy with metabolic syndrome had significantly higher TrgHDL ratio compared with the same therapy regime patients without metabolic syndrome (F=2.672;df=4;p<0.05). However, in patients on high doses of steroid therapy with metabolic syndrome Trg:HDL ratio (1.22±0.807) compared with patients without metabolic syndrome (1.13±1.063) did not show significant difference. Statistically significant interaction was found between the doses of steroid therapy on glucose fasting. (F=52.743; df=6;df2=82;p<0.01). Patients without metabolic syndrome, on low doses of steroid therapy had significantly lower(5.20±0.88) glucose fasting, than patients with metabolic syndrome(7.92±4.30) on same dose of steroid therapy. No significant difference in glucose fasting between patients on high doses of steroid therapy and metabolic syndrome and patients without metabolic syndrome was found. Conclusion: The surprising fact from this analyses is that high doses steroid therapy did not influence metabolism to the degree we expected towards the developing of metabolic syndrome. The possible explanation is that high doses are more efficient in strengthening physical activity and therefore seemed even safer towards metabolic syndrome which is usually generated by insulin resistance under conditions of low physical activity During physical activity corticosteroids redirect the glucose metabolism towards the loss of lipids. JAMA,2002;287(3):356-9

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D7: Efficacy of adalimumab in sarcoidosis

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Background: Adverse effects and lack of specificity often hamper the use of conventional immunosuppressive drugs in patients with systemic sarcoidosis. Adalimumab, a monoclonal antibody directed against the key cytokine involved in sarcoidosis, tumor necrosis factor (TNF)-α, is used in patients with various immunological disorders. Improved specificity, hence less adverse effects are the major advantages of this new class of drugs leading to an exponentially increasing role in their clinical use. However, few reports involving adalimumab in patients with sarcoidosis have been published so far and are restricted to case reports. We in investigated the clinical and biochemical effects of adalimumab therapy in chronically active systemic sarcoidosis patients.
Methods: Five patients with active, symptomatic and biopsy-proven systemic sarcoidosis received adalimumab with an induction scheme of 160mg at week 0, 80mg at week 2 and 40mg every other week for the observational period of 12 weeks. Therapeutic efficacy was monitored by computered tomography (CT)-scan, somatostatin receptor scintigraphy (SRS), pulmonary function tests, physical examination and various inflammatory parameters.
Results: Therapeutic and symptomatic response was seen in four out of five (80%) patients in the observational period of 12 weeks. This was accompanied by decreased uptake on SRS and more than 15% reduction of pathological lymph node volumes on the CT-scan. Clinical symptoms improved followed by a fall of the inflammatory cytokines interferon-γ (IFN-γ) and interleukin-8 (IL-8).
Conclusions: Four out of five patients (80%) patients were considered to benefit from the adalimumab treatment within 12 weeks. The clinical improvement was accompanied by more than 18% decrease of initially elevated serum IFN-γ en IL-8 levels. This is the first study demonstrating both a trend in clinical and biochemical improvement after adalimumab treatment in chronic sarcoidosis.

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D8: Calcium and vitamin D suppletion in sarcoidosis

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Background: Granulomas in sarcoidosis express high levels of 1α-hydroxylase, an enzyme that catalyzes the hydroxylation of 25-OH vitamin D to its active form, 1,25(OH)2 vitamin D. Overproduction of 1α-hydroxylase is held responsible for the development of hypercalcemia in sarcoidosis patients. Corticosteroids are used as the first-line treatment in organ-threatening sarcoidosis. Indeed osteoporosis prevention with calcium and vitamin D (CAD) is needed. It is believed that additional suppletion of vitamin D might provoke a clinical significant hypercalcemia. We studied this hypothesis in a cohort of patients with sarcoidosis.
Methods: We retrospectively analyzed data of 392 sarcoidosis patients seen between July 1986 and October 2010 at the outpatient clinic of immunology at Erasmus MC, for the use of CAD, levels of serum calcium, 25-OH vitamin D and 1,25(OH)2 vitamin D. Hypercalcemia was defined as a serum calcium >2.65 mmol/l adjusted to serum albumin. Statistical analysis included a Pearson correlation method using the SPSS 17.0 software package. Results: In total, 104 patients were on CAD. Five patients developed hypercalcemia. In three of them this was associated with worsening of their sarcoidosis. The other two had primary hyperparathyroidism that was treated accordingly. In all sarcoidosis patients hypercalcemia occurred in 23 (5.9%) and was associated with sarcoidosis activity in 21 patients.
No statistically significant correlation was found between on the one hand serum levels of 25-OH vitamin D and 1,25(OH)2 vitamin D and on the other hand serum level of calcium (respectively r = 0.039; P = 0.544 and r = 0.117; P = 0.066). Serum 25-OH vitamin D correlated significantly (r = 0.361; P < 0.001) with levels of 1,25(OH)2 vitamin D. Remarkably however, hypercalcemia never occurred when both values were elevated. Conclusion: In this study 5.9% of the sarcoidosis patients developed hypercalcemia during their illness, which is comparable to previously described prevalences. Suppletion of CAD to prevent osteoporosis appears safe in patients with sarcoidosis, but hypercalcemia is an obvious contraindication.

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D: **Treatment options in sarcoidosis**

D9: Rapid response to infliximab in refractory sarcoid with osseous lesions

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Background:
Lupus pernio and plaque are associated with more severe systemic involvement of sarcoid, while erythema nodosum is the hallmark of acute and benign disease. Treatment of sarcoidosis is unsatisfactory. The conventional therapeutic approach has been with systemic corticosteroids followed by anti-malarial drugs and or immunosuppression using methotrexate or azathioprine. Many patients are unable to tolerate these agents due to side effects. Refractory cutaneous sarcoid and lupus pernio have been shown to respond to the anti-tumour necrosis (TNF) factor, infliximab.
Case presentation:
We describe a case of refractory sarcoidosis with severe multi-system involvement and marked osseous lesions successfully treated with infliximab. Intravenous Infliximab infusions at 5mg/kg were instituted at weeks 0, 2 and 8, thereafter eight-weekly with a profound improvement of the skin lesions, the small joint arthritis of the hands and lung function. The radiological appearances improved markedly following 30 months infliximab therapy.
Conclusion:
This case extends our success in managing refractory osseous sarcoid lesions. Previous reports of response of osseous sarcoid to anti-TNF therapy are limited. Currently, infliximab, along with other anti-TNF agents, is approved for the treatment of rheumatoid arthritis and crohns disease. Our patient was unique in the extensive healing of bone fracture and destructive distal phalangeal lesions.

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D10: The correlation between the duration of sarcoidosis, certain clinical features of sarcoidosis and pulmonary function parameters

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Background: The purpose of this study was to determine the correlation between the duration of the disease and the stage of the disease, extrapulmonary localization (EPS) of the disease, diffusion coefficient (DLco), vital capacity (VC) and forced expired volume per second (FEV1).
Methods: The experiment involved 68 patients with a histologically proven sarcoidosis, 45 of which were women (66.18%) and 23 men (33.82%), average age 50.63+11.21 (23 to 73 years old). The average duration of the disease was 18.69+19.39 months (from 3 to 120). The duration of the disease was statistically longer in patients with EPS (32.08+33.08) than in those without it (15.53+13.03), $p<0.05$ (Mann-Whitney U test).
Results: The average value of DLco was 83.85+16.28 (min 26, max 116). Spearman linear correlation coefficient was used to determine a very small, negative and statistically irrelevant correlation between DLco and the duration of treatment ($p=-0.16$). The average value of FEV1 was 90.02+17.15% (min 51, max 139). Moreover, Spearman linear correlation coefficient was used to determine a very small, negative and equally statistically irrelevant correlation between FEV1 and the duration of treatment ($p= -0.14$). The average value of VC was 96.10+15.60% (min 60.50, max 138%). Spearman linear correlation coefficient of -0.08 shows that the negative correlation VC and the duration of treatment is minimal.
Conclusion: Based on the aforementioned data, it can be concluded that the duration of treatment minimally and negatively correlates with DLco, FEV1 and VC, with little statistically relevant connection.

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E: **Impact of Sarcoidosis in patients' lives**

E1: Measurement of fatigue in sarcoidosis; defining Minimal Clinically Important Differences

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Background:
The Minimal Clinically Important Difference (MCID) represents the smallest change score which is considered clinically worthwhile. Knowledge of the MCID is essential for the measurement of fatigue in clinical practice. Currently, this knowledge is lacking in sarcoidosis. Therefore, the aim of this study was to estimate the MCID for the Fatigue Assessment Scale in sarcoidosis.
Methods:
Outpatients (n = 321) of the sarcoidosis management center of the Maastricht University Medical Centre, participated in this prospective follow-up study. Anchor-based and distribution-based methods were used to estimate the MCID for the Fatigue Assessment Scale. Based on the anchor Physical Quality of Life, a Receiver Operating Characteristic was obtained. The distribution-based methods consisted of the Effect Size and Standard Error Measurement.
Results:
The anchor-based MCID found with Receiver Operating Characteristic was 3.5. The distribution-based methods showed that a small Effect Size corresponded with a MCID of 4.2. Furthermore, the Standard Error Measurement criterion identified a MCID of 3.6.
Conclusions:
The MCID was estimated on a change of 4 points on the Fatigue Assessment Scale, based on the anchor- and distribution-based methods. This MCID of the Fatigue Assessment Scale may be used in the follow-up of fatigue in clinical trials and in the management of fatigue in individual sarcoidosis patients.

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E2: Three types of fatigue in sarcoidosis patients

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Background: Fatigue is frequently reported in sarcoidosis and appears to differ between patients. Intermittent fatigue, Early Morning Fatigue, and Afternoon Fatigue have been described in sarcoidosis, but these types of fatigue are not yet validated. Therefore, the aim of this study was to examine whether these types of fatigue can be identified in sarcoidosis, and to describe the characteristics of those types.
Methods: Sarcoidosis outpatients (n = 434) of the Maastricht University Medical Centre, participated in this study. They completed questionnaires regarding depressive symptoms, fatigue, quality of life, restless legs, dyspnea, depressive symptoms, anxiety, sleeping problems, and symptoms indicative for small fiber neuropathy. Medical data en demographics were taken from the records.
Results: Latent Cluster Analysis revealed three clusters: 1) Intermittent Fatigue: patients with complaints of fatigue that varied during the day, 2) Not Tired: patients with mild or no complaints of fatigue, and 3) All Day Fatigue: patients who felt tired the whole day. The three patient clusters differed regarding several demographics, clinical and psychological characteristics. All Day Fatigue patients reported the most complaints and they were most often declared to be unfit to work.
Conclusions: Intermittent fatigue was validated and two other types were found. This clustering provides a useful typology of individual patients that may be applied in clinical settings. Especially for the All Day Fatigue type psychological counselling is recommended, in order to improve the wellbeing of the patients.

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E3: Chronic fatigue in sarcoidosis-in-clinical-remission: psychological and physical characteristics

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Background: When sarcoidosis is in clinical remission, complaints of chronic fatigue often persist. So far, the exact features of this post-inflammatory fatigue are unknown. The present study assesses the severity of fatigue and the presence of fatigue-related symptoms in sarcoidosis-in-clinical-remission. Furthermore, we evaluate whether fatigue is associated with psychological distress, pain, and patient-reported sleep quality, and record physical activity levels and muscle strength as objective assessments of fatigue. Lastly, we assess the severity of fatigue at a follow-up one year later.
Methods: Seventy-five patients with sarcoidosis-in-clinical-remission were evaluated with the Checklist Individual Strength (fatigue), the Symptom Checklist-90 (psychological distress), the McGill Pain Questionnaire (pain), standardized interview (fatigue-related symptoms), sleep diary, accelerometer and muscle strength tests.
Results: Fatigue severity mean score in sarcoidosis patients in-clinical-remission was high (fatigue-severity score: 30.5±15.5), and fatigue-related symptoms were significantly more present in the fatigued patients. Median time since diagnosis was 9 years. Fatigue was significantly associated with increased psychological distress, higher pain severity scores and more pain points, reduced physical activity levels and reduced muscle strength. Scores on sleep quality were normal. Response at follow-up was 87% (92% of previous non-fatigued patients, 81% of previous fatigued patients). Fatigue severity scores of the responding group were significantly increased compared to a year before.
Conclusions: Fatigue in sarcoidosis patients in clinical remission is a long-lasting and severe problem that deteriorates over time. This post-inflammatory chronic fatigue is associated with a constellation of psychological and physical symptoms.

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E4: Fatigue in sarcoidosis

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Background:
Fatigue in common symptom in sarcoidosis. The aim of this study was to establish the prevalence of fatigue in our patients and to determine weather fatigue was related with duration of therapy, clinical and radiological presentation.

Methods:
Study was performed on clinic for lung disease, Clinical Center Nis, and 69 patients (mean age 50.63±11.21) were included. Fatigue was measured on the fatigue Assessment Scale.

Results:
Fatigue was presented in 23 (33.82%) patients. Duration of therapy was statistically significant longer in that group, compare with group without this symptom (21.78±16.35 vs 17.11±20.76). Fatigue was most common in patients with radiological stage III of the disease (62.22%), and stage II of the disease (35.56%) Fatigue was more frequently present in patients with extrapulmonary (34.78%) sarcoidosis.

Conclusions:
Fatigue was common symptom in our patients with sarcoidosis. Fatigue was related with longer duration of therapy, radiological stage III of the disease, and extra pulmonary sarcoidosis.

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E: **Impact of Sarcoidosis in patients' lives**

E5: “Pride and prejudice” in sarcoidosis. Does a prescribed treatment match a patient’s priorities?

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Background: Sometimes sarcoidosis patients need a life time treatment. In this study we wanted to elucidate the patients perception about medications used to treat sarcoidosis. We also want to test the influence of gender, education and sarcoidosis duration using Beliefs about Medications Questionnaire (BMQ).
Methods: 92 biopsy positive sarcoidosis patients were enrolled in this study. (70 female/22 male) mean age 50±12yrs. Education: 30 patients -elementary school, 45pts- high school and 17pts- university. Duration of therapy: 26pts were treated for sarcoidosis from 1-5 years, 15 for 6-10 years and 10 years or longer-13pts. Patients were given self-administered BMQ, an 18 item questionnaire assessing beliefs about specific medications and beliefs about medications in general. There are 4 subscales including: specific necessity, specific concern, harmful effects of sarcoidosis medicines, general overuse, and general harm. Scoring: 5 point Likert scale-a higher score indicates a stronger belief. The reliability was assessed using intraclass correlation coefficient (ICC) and Cronbach alpha; the differences in BMQ scores and the education level one way MANOVA, while the differences in BMQ scores between gender and therapy duration for sarcoidosis were analysed using Mann Whitney test.
Results: High reliability was found for specific necessity (Cronbah α=0.83; ICC 95% Confidence Interval [CI]: 0.77-0.88), harmful effects (Cronbah α=0.67; ICC 95% CI: 0.54-0.76) and general harm (Cronbah α=0.78; ICC 95% CI: 0.71-0.85).Significant but low reliability was found between the items of overuse (Cronbah α=0.43; ICC 95% CI: 0.20-0.61). Males (mean 3.34) perceived medications in general to be more harmful than females (mean 2.86) and also strongly believe that drugs are overused. P=0.001. Considering the general harm and duration of sarcoidosis least prejudice had patients with the longest treatment - more that 10 yrs. (2.49). The difference was statistically significant between the groups. P=0.008. Analyzing the level of education the highest prejudice for harmful effects had patients with the highest university degree. (2.74) P=0.000.The age significantly correlated with all BMQ scores.
Conclusion: Patients’ priorities may be very different from prescribers’ priorities, or indeed from the priorities prescribers assume their patients to have.

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E6: The coexistence between fatigue and oxidative stress – total antioxidant status (TAS) in sarcoidosis

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Background: The aim of this study is to elucidate a link, if any, between total antioxidant status (TAS) and fatigue in sarcoidosis.
Methods: 69 sarcoidosis patients were analyzed (53female/16male). 39 patients had chronic form of sarcoidosis. Fatigue was measured using Fatigue Assessment Scale (FAS) and total antioxidant status (TAS) level as a measure of total non-enzymatic power, was measured by Erel method. (In one of our previous studies in 213 sarcoidosis patients and 187 healthy adults we found the TAS level was significantly higher in sarcoidosis patients [1.46 (1.39-1.56) vs. 0.84 (0.73-0.9) mmol/L,P<0,001]. To assess the link between total FAS score and total TAS, the curve estimation procedure was used. In order to find the discrimination, sensitivity and specificity between the groups ((FAS≥22) and TAS values the ROC curve procedure was used. For making the decision on coexistence between high total FAS score and TAS we used the commutation rule and the opposite probabilities. Significance level 0.05. Statistical analyses were done using SPSS version 18, Chicago, Illinois.
Results: Mean total FAS score in the analyzed group was 27.06±9.67 and mean TAS was 1.45±0.20 mmol/L. The curve estimation procedure resulted in exponential curve (R square=0.96) with the link between total FAS score and TAS (FAS=e2.177xTAS).Using the logarithm transformation the equivalent value for FAS=22 is TAS=1.40 mmol/L. 33 patients in the analyzed group had (FAS≥22) and (TAS≥1.40mmol/L). The sensitivity for FAS cut off 22.5 was 97% and specificity 65% while the sensitivity for TAS cut point 1.40 mmol/l was 94% and specificity 64%. Applying the opposite probability rule and the commutation we concluded that the coexistence of high values of FAS score and high values of TAS score among patients with sarcoidosis had sensitivity over 99% and specificity of 87.4%.
Conclusion: High total FAS score (FAS≥22) in sarcoidosis patients in more than 99% coexists with high total antioxidant status (TAS≥1.40) suggesting the fact that better control of total antioxidant status (TAS) level, as a measure of total non-enzymatic power, may result in better control of fatigue.

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E7: Trascranial ultrasound (TCS) in sarcoidosis- relation to fatigue, depression and anxiety

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Background: Up to date neuroimaging is known to complement clinical findings in the diagnostic work up mostly of parkinsonian syndromes. No investigation was performed to evaluate the trans cranial ultrasound findings (TCS) in sarcoidosis. It is already recognized that impaired ehogenicity of the mesencephalon structures can correlate with the impaired cognitive functions. In this study we wanted to elucidate the TCS findings and the relation with the sensation of fatigue, depressive feelings and anxiety in sarcoidosis patients.
Methods: 40 biopsy positive sarcoidosis patients were enrolled in this study (28female/12male); mean age 48.5±12.15yrs. TCS was performed in the Neurology Clinic, Belgrade, Serbia, by an experienced neurologist. Prior to TCS patients were evaluated for fatigue, depression and anxiety using: Fatigue Assessment Scale, (FAS - mean total score 25.55±7.54), Beck Depression inventory (BDI- mean 18.25±12.52), Hamilton Anxiety Scale (HAMA mean - 16.15±7.79) and Hamilton depression Scale (HAMD - mean 13.75±7.79). Statistical analyses were done using Fisher's Exact Test and Discriminant Analysis with CCD ≥0.51.
Results: 7/40pts had hyperehogenicity of substantia nigra (SN); and 15/40 had hyperechogenicity of nucleus rubber (NR). Fisher's Exact Test revealed significant hyperechogenicity of NR in patients with FAS≥22. (Fisher's value =0.042). Significant hyperechogenicity of NR was found in patients with BDI >10, (Fisher's value=0.017). HAMA anxiety score >17 significantly coexisted with hyperechogenicity of SN and NR (Fisher's value=0.041). Discriminant Analysis revealed significant discrimination of depressive pts. Patients with BDI≥10 (28/40pts) had chronic sarcoidosis, steroid therapy ≤10mg and FAS total score ≥22, and NR hyperechogenicity. (Lambda=0.643, Chi ² =15.702, CCD=0.598, df=5, p<0.01). Reliability of discrimination 85%. Discriminant Analysis of HAMA showed that patients with HAMA score ≥17 (20/40 pts) were older, had FAS total score ≥22 and hyperechogenicity of NR or SN (Lambda=0.738, Chi² =10.916, CCD=0.511, df=4, p<0.05) Reliability of discrimination 75%. Hypoechogenicity of SN was found in 33/40pts suggesting the restless leg syndrome in these patients.
Conclusion: Further analyses are necessary in this field, possibly to reveal the role of other findings and other possible causes of fatigue in sarcoidosis.

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E8: The assessment of three health-related quality of life measurements assessed in an ambrisentan trial for sarcoidosis associated pulmonary hypertension

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Background: Sarcoidosis associated pulmonary hypertension (SAPH) is a medical condition that adversely affects health-related quality of life (HRQOL) and is potentially life threatening. We examined the impact of ambrisentan upon health related quality of life as assessed by the sarcoidosis health questionnaire (SHQ), short form 36 (SF-36), and St. George Respiratory Questionnaire (SGRQ).
Methods: This was a substudy as part of a proof of concept pilot study to assess ambrisentan 5 mg/day for 1 month followed by 10 mg/day for 5 additional months for SAPH. The primary outcome measure was change in 6 minute walk distance (6MWD) between 0 and 24 weeks. HRQOL was measured by SF-36, SGRQ, and SHQ.
Results: 21 subjects were enrolled. 10/21 (48%) completed this trial. 11/21 dropped out: 8/21 (38%) for medical reasons and 3/21 (14%) for social reasons. There were no significant changes in 6MWD, any gas exchange or hemodynamic variable. No significant change in SF-36 total score was noted over the 24 weeks (Δ = 5.8 +/- 6.1, p=0.63). The change in SGRQ was (Δ = -15.3 +/- 25.0, p=0.12), far above the minimal importance difference of -4.0. However, this change failed to reach statistical significance. The mean change in SHQ over the 24 weeks did change significantly (mean week 0 = 3.71 +/- 0.64, mean week 24 = 4.4 +/- 1.0, p=0.03). Interestingly, the NYHA class of the subjects as assessed by the investigators improved.
Conclusion: In this 6-month trial of ambrisentan for SAPH, physician assessment in level of dyspnea as assessed by NYHA improved as did SHQ assessed by the subjects. SGRQ trended toward improvement and there was no appreciable change noted in SF-36. These data suggest despite the absence of documented physiologic improvement, ambrisentan did improve sarcoidosis-specific and respiratory-specific HRQL measures suggesting that ambrisentan may have some benefit in patients with SAPH.
This study was funded by Gilead Sciences, Inc.

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E: **Impact of Sarcoidosis in patients' lives**

E9: Clinical characteristics of Czech patients with sarcoidosis: A case review of 124 patients

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Background: It is known that the presentation of sarcoidosis can demonstrate race and ethnical heterogenity. Our aim was to find out extent of thoracic and extrathoracic involvement of sarcoidosis at our own population.
Methods: Retrospectively, 124 (59% female; mean age 44 yrs) Caucasian patients with sarcoidosis which were followed up at out patient department of Thomayerś University Hospital in Prague during years 2001-2010 were enrolled. Data at the time of diagnosis were gathered.
Results: Chest X-ray stages were scored at time of diagnosis (stage 0: 3.2%; I: 8%; II: 53.2%; III: 11.3%; IV: 2.4%). Solely intrathoracic involvement was found in 21.9% of patients. Forced vital capacity (FVC) was not impaired (FVC >80% predicted) in 75%, mild (FVC 70-79% predicted) in 15.3%, moderate (FVC 50-69% predicted) in 9.7%, and severe impaired (FVC <50% predicted) in 0% of patients. Carbon monoxide lung diffusion capacity (DLCO) was not impaired (DLCO >80% predicted) in 51.6%, mild impaired (DLCO 70-79% predicted) in 19.4 %, moderate impaired (DLCO 50-69% predicted) in 23.4%, and severe impaired (DLCO <50% predicted) in 3.2% of the patients at time of diagnosis.
Conclusion: Sarcoidosis at our study population, in comparison with ACCESS study has lesser sex related heterogenity, approximately the same age distribution as American study with relatively frequent onset at older age. Our study group has tendency to lesser occurrence of irreversible stadium IV and to more frequent absence of FVC impairment. However, DLCO impairment was found at nearly half of patients.

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E10: Cutaneous lesions of sarcoidosis in Japan

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Background: The cutaneous manifestations of sarcoidosis often enable the dermatologist to be the first physician to make the diagnosis. The predominance of any particular type of cutaneous lesions of sarcoidosis is known to be influenced by race.
Methods: We conducted a retrospective chart review in our institution. Dermatological data were obtained by using a standardized protocol, including the medical history and type of cutaneous manifestation that was evaluated by clinical manifestations and histopathological examinations.
Results: Papular and nodular lesions are universally the most common specific cutaneous manifestations of sarcoidosis. Plaque type is secondly frequently seen in Japan but the frequency varies every race. Lupus pernio is extremely rare in Japan but common in European countries. Subcutaneous lesions tend to be more frequently seen in Japan than other European countries and the United States. Infiltrated scar is the most common skin lesion of sarcoidosis in Japan. The frequency however varies among previous reports, because of overlooking of asymptomatic tiny lesions on the knees and elbows. Erythema nodosum, a nonspecific lesion without granulomas, is frequently (40%~65%) seen in Europe and the United States. On the other hands, the incidence is low (less than 5%) in Japan.
Conclusions: The recognition and adequate examinations of skin lesions are important in diagnosis of sarcoidosis. Therefore, clinical manifestations characteristic for cutaneous lesions of sarcoidosis in each country should be understood.

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E11: Sarcoidosis in Southern East Serbia

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The aim of this work was to investigate clinical and demographic characteristics of sarcoid patients in region of Southern east Serbia.

Methods:
We investigate ten years period, from 2000 to 2010. Sixty eight patients with histologically confirmed sarcoidosis were included.

Results:
There were 45 (66.18%) women and 23 (33.82%) men, middle age 50.63+11.21 (23 to 73 year). The most common symptoms were dry cough (52.17%), dyspnea (40.58%) and join pain (37.68%). Erythema nodosum was initial presentation in 42.02% of patients. Using Scadding classification system, patients were classified in four groups. Thirty eight (55.88%) of patients were in stage one, twenty tree (33.82%) stage two, five patients (7.35%) stage three and two patients were in stage fourth. Extra pulmonary involvement was seen in 13 (19.12%) of patients, most commonly was affected eyes (4.34%) and skin (erythema nodosum) (4.34%). Nineteen patient (27.5%) have chronic disease, with one of more exacerbation.

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E12: Spatial QRS-T angle is significantly increased in asymptomatic sarcoid patients with ventricular arrhythmias

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Background: Aim of the present study was the evaluation of the QRS-T-a in asymptomatic sarcoidosis patients without known heart disease and to investigate the possible relationship between QRS-T-a and occurrence of potentially serious ventricular arrhythmias.
Methods: The ECG derived QRS-T angle of 112 sarcoidosis patients was calculated from the surface ECG while cardiac involvement was assessed based on the evaluation of the surface electrocardiogram, the echocardiogram, the 24-hour ambulatory ECG (Holter) and the cardiac Magnetic Resonance Imaging (MRI). Assessment of the ventricular arrhythmias was based at the Lown classification criteria from holter. Four subgroups were formed according to the combination of the arrhythmic risk (Lown >3 was high and Lown ≤3 was low) and the detection of cardiac involvement). Patients were compared to 65 age and sex adjusted healthy controls.
Results: Of the sarcoidosis patients 32% fulfilled the criteria of cardiac involvement while 12 patients were classified as Lown 4A (Couplets of Premature Ventricular Beats) and 3 as 4B (Non sustained Ventricular Tachycardia). Systolic blood pressure, disease duration, QRS and T amplitude weren’t significantly different between groups. The spatial QRS-T angle of the sarcoidosis patients were significantly increased compared to the controls, while the subgroup analysis showed that patients with cardiac involvement and Lown >3 had significantly elevated angle compared to the other disease groups. Spatial QRS-T angle is associated with the age and the Lown classification
Conclusion: Sarcoidosis patients, especially these with severe arrhythmias and cardiac involvement, have increased spatial QRS-T angle compared to healthy subjects.

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F: **Pulmonary fibrosis and other ild**

F1: Diffuse alveolar hemorrhage associated with variant alleles increases the risk of pulmonary fibrosis in patients using oral anticoagulants

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Background: Diffuse alveolar damage (DAH) causes oxidative stress and inflammation in the lung. It has been suggested that oxidative damage plays a role in the pathophysiology of pulmonary fibrosis. Recently, we found that DAH was associated with vitamin K epoxide reductase (VKORC1) and cytochrome P450 CYP2C9 and CYP2C19 variant alleles in patients using oral anticoagulation therapy. We hypothesized that the presence of one or more of these variant alleles in patients with DAH caused by oral anticoagulants also increases the risk of developing pulmonary fibrosis.
Methods: During a 9-year period (7-year inclusion period and at least 2-year follow-up), data on patients using coumarins with confirmed DAH were gathered. Of 63 confirmed DAH cases receiving oral anticoagulants DNA was available. CYP2C9*2/*3 (C430T/A1075C), CYP2C19*2/*3 (G681A/G636A), and VKORC1 (G-1639A/C1173T) single nucleotide polymorphisms were genotyped, using real-time PCR.
Results: In 62 (98%) of 63 patients (age 62.9±15.8) with DAH, variant alleles were found. Out of these 63 DAH cases 34 (54%) subsequently developed pulmonary fibrosis. A total of 37 (59%) patients died within a 2±2 (range 0-10) year period after the DAH diagnosis was confirmed and 18 (49%) of the deceased were patients with pulmonary fibrosis.
Conclusions: Genotyping of four SNPs for VKORC1 and CYP2C9 polymorphisms is useful in predicting the risk of DAH in patients receiving oral anticoagulants. Early and timely use of genotyping is recommended to prevent an unstable anticoagulation and also to reduce the risk of bleeding complications including DAH and subsequent pulmonary fibrosis.

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F2: KL-6, a Human MUC1 Mucin, as a prognostic factor for diffuse alveolar hemorrhage syndrome

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Background: Diffuse alveolar hemorrhage (DAH) syndrome is a group of life-threatening diseases following diverse pathogenesis. To date, prognostic factors for DAH have not been well investigated. KL-6, a complex sialo-carbohydrate glycoprotein present in the human MUC1 mucin, is a sensitive serum marker for various interstitial lung diseases (ILD). The purpose of our study was to investigate a utility of KL-6 as a prognostic factor for DAH.
Methods: We retrospectively collected the consecutive patients with DAH that were admitted to our institute between 2004 and 2011. Correlations between outcome and age, sex, laboratory/ radiological findings or therapeutic regimens were evaluated.
Results: A total of 39 patients were included in this study (male/female, 23/16; median age, 69 (16-83)). The etiologies of DAH were infection (n=21), excessive anticoagulation (n=10), vasculitis (n=4), interstitial pneumonia (n=3), and idiopathic (n=1). Thirteen patients survived, and 26 died. There were no significant differences in age, sex, smoking status and radiological findings between the groups. Initial and peak serum KL-6 levels were significantly higher in non-survivors compared with survivors (p=0.01, p=0.0002, respectively). Patients with deterioration in PaO2/FIO2 (P/F) ratio or oxygenation index during initial one week showed a significant increase in serum KL-6 level (p=0.002, p=0.003, respectively). In univariate analysis, initial P/F ratio <200, P/F ratio 48 hours after admission <200, initial serum KL-6 level ≥240 U/mL and peak serum KL-6 level ≥700 U/mL were correlated with survival (p<0.05, p=0.04, p=0.009, p= 0.001, respectively). In multivariate analysis, only peak serum KL-6 level ≥700 U/ml was an independent poor prognostic factor for DAH (p=0.03; Hazard ratio, 55.6; 95%CI, 1.56-1000).
Conclusions: Peak serum KL-6 level ≥700 U/ml appears to be of clinical utility as poor prognostic factor for DAH.

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F3: Involvement of the immune system in chronic Beryllium disease model. A role for DCs

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Background:
Beryllium (Be), a widely used metal in industries, can cause chronic beryllium disease (CBD). CBD has similarities with sarcoidosis, a noncaseating interstitial lung disease characterized by granulomatous inflammation. The cause of sarcoidosis is unknown.

Methods:
Here we are studying the airway pathophysiology and the role of DCs in a mouse model for CBD. CBD was induced in C3H mice by 9 repeated intratracheal injection of 100 µg Be-BeO mixture during 3 weeks.

Results:
Eight weeks after the first exposure animals manifested abnormalities, characterized by focal infiltrates containing CD4+, CD8+ T-cells, CD11c+ DC, MHC-classII+ cells, B-cells and BALT-formation and collagen deposition. In the alveolar space aggregates of macrophages (granulomata) were commonly observed. Analyses of bronchoalveolar lavage (BAL) fluid established the presence of T-cells, DCs and macrophages that were localized around the Be and BeO particles as nibbling cells. To address the functional role of DCs in maintaining inflammation, CD11c+DCs were conditionally depleted in chronic phase of CBD by local intratracheal administration of diphtheria toxin to CD11c-DTR-Tg mice. In the absence of CD11c+ DCs, granuloma structures disappear.

Conclusion:
We observed the involvement of the adaptive immune system, including the accumulation of T cells in BALT-like structures, suggesting a cell-mediated immunity against Be and BeO.

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F4: Mortality in idiopathic pulmonary fibrosis (IPF) on the waiting list for lung transplantation in the Netherlands

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Background: Idiopathic Pulmonary Fibrosis (IPF) is a progressive and lethal disease, with a prognosis of 2.5 to 5 years. IPF generally does not respond to medicinal treatment, therefore lung transplantation is the only therapeutic option to prolong life and improve quality of life. Because of its progressive nature and relative shortage of donor lungs in the Netherlands, mortality of IPF on the waiting list appears high. The aim of this study is to evaluate waiting list mortality of this disease in the Netherlands.
Methods: Data were retrospectively collected during the period from September 1989 till June 2010 of all IPF patients registered for lung transplantation in the Netherlands. Patients were included after revision of the diagnosis based on the IPF criteria set by the ATS/ERS. Clinical data and lung function measurements were collected at the time of screening.
Results: 167 IPF patients were referred for lung transplantation and after initial evaluation for contraindications, 122 patients were accepted for further screening. After screening, 90 patients were listed for lung transplantation. During the waiting list period 30 patients with IPF (33.3%) died, compared to mortality rate in Cystic Fibrosis (CF) and Chronic Obstructive Pulmonary Disease (COPD) subjects of 13.8% and 16.3%, respectively. Analysis of lung function showed a severe restrictive lung function pattern with mean FVC %-predicted of 51.1% (SD 19.0) and mean DLCO %-predicted of 27.1% (SD 9.4) at time of screening.
Conclusions: The study revealed a 2 to 3-fold higher waiting list mortality rate for IPF compared to COPD and CF in the Netherlands. Analysis of lung function at time of screening showed a mean DLCO %-predicted of 27.1 %. International guidelines of lung transplantation in IPF advise referral to a transplant center in case of histological or radiographic evidence of UIP, and screening for transplantation in case of DLCO < 39% of predicted. Considering these guidelines, this study indicates that referral of IPF to one of the transplant centers in the Netherlands might be improved. This might lead to a lower waiting list mortality.

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F5: Outcome of patients with interstitial lung disease admitted into ICU

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Background: Limited data are available on the clinical course of patients with Interstitial Lung Disease (ILD) and acute respiratory failure requiring admission to ICU.

Objectives: To investigate the outcome of patients with ILD and acute respiratory failure with special attention to Idiopathic Pulmonary Fibrosis (IPF) or Drug-induced ILD (DI-ILD).

Methods: Retrospective identification of patients with ILD admitted into ICU between 1993 and 2009. Primary endpoint was in-hospital mortality.
Results: 72 subjects could be included, divided into 3 groups: IPF, n=28; DI-ILD, n=20 and Miscellaneous, n=24. The in-hospital mortality rates were 68, 40 and 25% for IPF, DI-ILD and Miscellaneous, respectively, (p=0.006) and reached 100, 64 and 60%, respectively, in those receiving mechanical ventilation (p=0.007)
On multivariate analysis, the need for invasive or non invasive ventilation (OR= 35; [95% IC, 5-255]), the type of ILD (IPF vs Miscellaneous) (OR=22; [95% IC, 3-147]), and high-dose steroids during ICU stay (OR=0.19; [95% IC, 0.04-0.99]) were found to be independent determinants of in-hospital mortality.

Conclusion: This study highlights the poor prognosis of IPF in ICU particularly if mechanical ventilation is required. DI-ILD and Miscellaneous with comparable severity criteria have a better prognosis than IPF. High-dose steroids appear as a protective factor whatever the type of ILD.

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F6: Long-term follow-up of the patient with pulmonary Langerhans'-cell histiocytosis

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Background: Langerhans' Cell Histiocytosis (LCH) is a rare disease involving clonal proliferation of dendritic mononuclear cells (Langerhans cells). Clinically, its manifestations range from isolated bone lesions to multisystem disease with infiltration into organs locally or diffusely. Pulmonary Langerhans' Cell Histiocytosis (PLCH) is a unique form of LCH in that it occurs almost exclusively in cigarette smokers. It is now considered a form of smoking-related interstitial lung disease.
Case report:
A 29-year-old chimney-sweep and smoker presented with left sided rib/chest pain for several months before hospital admission. In this period, he had no history of febrility or skin lesions and he didn't have any other problems other than the above. The stool and urine were normal. Physical examination showed very painful chest motility without any signs of lymph adenopathy, organomegaly or other abnormalities. All laboratory studies including pulmonary function tests were within normal limits. Chest radiography as well as Tc-99m bone scintigraphy revealed multiple osteolytic lesions which predominantly affected the ribs. A high resolution computed tomography (HRCT) of the chest revealed bilateral, nodular, and cystic formations within lung parenchyma. A flexible fiberoptic bronchoscopy including analysis of bronchoalveolar lavage and transbronchial lung biopsy specimens, were normal. The patient underwent peripheral osteotomy of the sixth rib with biopsy of the left lower lung lobe. Histopathologic analysis of biopsy specimens showed infiltration of the ribs and lung tissue with Langerhans' cells. Electron microscopy examination demonstrated the presence of Birbeck's granules within of the histiocytes. Immunohistochemically, tumour cells give a positive reaction to anti-CD1a and anti-S100 consistent with the diagnosis of PLCH. In this case, we have decided to start with immunosuppressive therapy (prednison) with good clinical outcome.
Conclusion: The cornerstone of treatment for PLHC is smoking cessation. Nine years later, the patient is considered to be in complete remission. These observation suggests that at least some forms of LHC may have prevailing immunological features where an immunosuppressive effect with smoking cessation could be beneficial.

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F7: BAL examination in patients with cryptogenic organising pneumonia. Is it possible to predict relapse?

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Background: Cryptogenic organising pneumonia (COP) is a rare form of idiopathic interstitial pneumonia and the significance of BAL findings according clinical course of the disease have not been presented.
Material and methods: The BAL examination was performed in 28 patients COP. During the time of observation 7 of them were relapsed. All of patients were nonsmokers, however 6 of them were ex-smokers. They were in mean age of 57.3 years (range 41-76 years). Open lung biopsy was performed in 16 patients and in 12 patients diagnosis was established on the basis of transbronchial lung biopsy. Dyspnoea (80%), cough (84%), weakness (60%), fever (64%), lose of weight (64%), sweats (30%) chest pain (18%) were the most frequently noticed symptoms. Radiographically, bilateral consolidations with areas of ground glass attenuations were shown in 27 patients and 1 patient had localized infiltration in the upper part of right lung. Reticular pattern of lung lesions was observed in 4 cases. In 1patient nodular changes and in 5 patients enlarged lymph nodes were shown. A migratory pattern of pulmonary lesions was observed in 20(71%) patients. Twenty patients were treated with Clarithromycin (CLA) and 7 with prednisone(P). In one patient spontaneous regression was noticed. Two patients in CLA treated group and 5 patients treated with P were relapsed.
Results: Only in one patient BAL examination was within the normal limits. Decreased number of macrophages and increased number of lymphocytes was revealed in 6 (96%) patients in relapsed group and 16 (76%)patients who experienced only one episode of COP. Eosynophils in number between 3% to 20% in BAL was shown in 4 (57%) relapsed and 18(86%) non relapsed patients. Eight patients had increased number of neutrophils- 3 (41%) in relapsed group and 5 (24%) in non-relapsed group. Decreased CD4/CD8 ratio below 0.9 was reveled in 1 (14%) relapsed and 5 (24%) non-relapsed patients.
Conclusions: Relapses were more frequently observed in P treated group. In patients with relapsed COP more frequently lymphocytosis and higher percentage of neutrophils in BAL were noticed.

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F8: Association between polymorphisms in the P53 and P21 genes and IPF

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Background: Idiopathic pulmonary fibrosis (IPF) is devastating and progressive lung disease. Its aetiology remains unclear but is thought to involve damage to the epithelium and abnormal repair. Alveolar epithelial cells near areas of remodelling show an increased expression of proapoptotic molecules.[1,2] The purpose of this study was to investigate the role of genes involved in cell cycle control in IPF.

Methods: Genotypes for five polymorphisms in the P53 gene and four polymorphisms in CDKN1A, the gene encoding p21, were determined in 77 IPF patients and 353 controls. In PBMC from 16 healthy controls mRNA expression of p53 and p21 was determined. Genotypes were tested for correlations with patient characteristics and expression levels.

Results: The rs12951053 and rs12602273 polymorphisms in the p53 gene were significantly associated with survival in IPF patients. Carriers of the minor allele had a 4-year survival of only 22% versus 57% in the non-carrier group (p=0.006). All four polymorphisms in CDKN1A significantly predisposed to IPF. Rs2395655 and rs733590 were most associated with increased risk of developing IPF. In addition, the rs2395655G allele was associated with a rapid decline in lung function. The rs733590 polymorphism was significantly associated with p21 mRNA expression levels.

Conclusions: This study reports the novel finding that polymorphisms in the p53 gene are associated with survival in IPF and polymorphisms in the p21 gene predispose to IPF. This suggests cell cycle defects are involved in the pathology of IPF.

[1] K. Kuwano et al. Am J Respir. Crit Care Med., 1996, 154, 477-483.
[2] M. Plataki et al. Chest, 2005, 127, 266-274.

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F9: Idiopathic pulmonary fibrosis – Characterization of a population and assessment of prognostic markers

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Background: Idiopathic Pulmonary Fibrosis (IPF) is the idiopathic interstitial pneumonia with worst prognosis; to date this entity it is not epidemiologically characterized in Portugal.
Aim: Characterize IPF patients attending our Interstitial Lung Diseases specific consultation, according to clinical and functional parameters, bronchoalveolar lavage (BAL) characteristics and 6-MWT at presentation. Assessment of survival predictors.
Methods: Retrospective analysis of patients diagnosed IPF, followed in our hospital from January 2006 to December 2010.
Results: We included 33 patients, aged 67.97±13.4 years, 24 male. Fifty-three percent were non-smokers. We estimated an annual incidence of 1.88 new cases per 100.000 inhabitants and a prevalence of 4.28 cases per 100.000 inhabitants. Average time from onset of symptoms to first consultation: 20.4 months. Functional parameters at first evaluation: TLC: 75.5±15.5%, FVC: 72.5±16.9%, DLCO: 46.3±17.3%. Average walked distance in 6-MWT: 406m; desaturation in 76.0%. Echocardiographic signs of pulmonary hypertension (EcoPH) were observed in 54,5%. BAL showed on average 15.0% neutrophils, 6.2% eosinophils and 17,2% lymphocytes. Fifteen patients died (45.5%), most frequently due to respiratory infection (n=5), acute exacerbation of IPF (n=3), and lung surgery complications (n=2). Median survival: 43.6±8.3 months. A positive correlation between survival and %TLC (p=0.041), and a trend towards statistical significance for %FVC (p=0.071; positive correlation) and BAL % neutrophils (p=0.072; negative correlation) were observed. Male gender (p=0.018) and EcoPH (p=0.022) were related to worse prognosis.
Discussion: Male gender predominated, as observed in other studies. Contrary to expectations, we found a higher proportion of non-smokers. Infectious complications, in contrast to other series, were the leading cause of death, followed by acute exacerbation of IPF. As expected %TLC, male gender and EcoPH were associated with a poor prognosis; %FVC and %DLCO did not correlate to survival, probably due to reduced sample.

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F10: Cryptogenic organising pneumonia (COP) – Experience at a Portuguese hospital centre

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Background: COP is an unusual pulmonary condition but it frequently mimics other common diseases such as infective pneumonia, tuberculosis and primary bronchogenic neoplasm making it difficult to diagnose. Lung biopsy is the preferred method for establishing the diagnosis and clinicians must pursue it in order to institute early steroid therapy (ST).
Methods: Retrospective study of all histopathological proven cases of COP over a 16-yr period (1995-2010). The purpose was to describe the clinical, laboratory, functional and imaging features at onset, the diagnostic approach and the outcome.
Results: Total of 15 patients: 8 females and 7 males, mean age of 61±11.8 yrs (range 39-79). All had cough and 10 (66.7%) had fever and asthenia. Other common complaints: anorexia, weight loss, dyspnea and night sweats. Inspiratory crackles were the most typical finding. Relevant laboratory findings: leukocytosis (50.0%), anemia (71.4%) and elevated levels of C-reactive protein (100.0%). Most frequent patterns on X-ray: patchy bilateral (46.7%) and unilateral (26.7%) consolidation. HRCT findings showed a bilateral airspace consolidation (in 86.7%) predominantly at the bases. COP also presented as nodular and as isolated ground-glass opacities (in 13.3% and 6.7%). Lung function tests: normal in 6 (40.0%) patients, 5 (33.3%) had a restrictive and 3 (20.0%) an obstructive pattern. Two (13.3%) had a decreased DLCO/VA and 8 (53.3%) hypoxemia. Cytologic BAL profile was available in 6 cases, showing a mixed pattern in 5 (83.3%) with an increase of lymphocytes (range 26.0-57.0%) and neutrophils (4.0-17.6%). Open lung biopsy was diagnostic in 6 (only 1 was performed in 2010), transbronchial biopsy (TBB) in 5 and transthoracic core-biopsy in 4. Most patients showed a rapid response to ST, one required mechanical ventilation but none died. Seven (46.7%) received ST for 6-24 months and of this group, 5 (71.4%) had relapse of symptoms and X-ray abnormalities on reducing the dose or after stopping ST. Seven (46.7%) are still on ST.
Conclusions: This series suggests that TBB (coupled with BAL) is a very important diagnostic tool and should be considered the first diagnostic step. Although COP has a good prognosis and patients respond well to ST, relapses are very frequent, meaning that duration of therapy after initial recovery is very difficult to assess and that close follow-up is required.

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F11: Bronchogenic carcinoma in idiopathic pulmonary fibrosis patients after lung transplantation

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Background: The incidence of bronchogenic carcinoma after lung transplantation is increased. In transplanted pulmonary fibrosis patients this is 2-4%, in the majority of cases in the native lung and sometimes unexpected in the explanted lung. Diagnosis is often hard because of the diffuse lung abnormalities due to the underlying fibrosis. Moreover, it may mimic a pulmonary infection. We describe three transplanted idiopathic pulmonary fibrosis (IPF) patients.
Cases: Patient A, a 48-year old male with IPF presented 7 years after a successful single lung transplantation with dyspnoea, weight loss, cough and malaise. A high resolution computed tomography of the chest showed an increasing opacity in the native lung replacing the fibrotic lesions. Biopsies showed a large cell carcinoma of the lung with lymphangitis carcinomatosa, also in the transplant lung. Due to the rapid progression no oncological treatment was started and he died shortly after. Patient B, a 58-year old male with IPF, underwent a bilateral lung transplantation. In the explanted lung a squamous cell carcinoma was found with mediastinal lymph metastases. Chemo-radiotherapy was started resulting in a partial remission. Two years later progression appeared and second line chemotherapy was started but shortly after he died. Patient C, a 53-year old female with IPF was diagnosed with an adenocarcinoma in the explanted lung at the time of transplantation. At that time she already had skeletal metastases. Chemotherapy was not succesful and she died three months after her bilateral lung transplantation.
Conclusion: The symptoms of bronchogenic carcinoma after lung transplantation are aspecific, diagnosis is often difficult and prognosis is poor. These cases stress the importance of actively searching for bronchogenic carcinoma before as well as after lung transplantation in patients with IPF.

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F12: Is the clinical outcome in pulmonary Langerhans' cell histiocytosis associated with polymorphisms in the ITPA gene?

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Background: The enzyme ITPase is involved in the intracellular inosine-nucleotide homeostasis. The clinical consequences of a mutation or a polymorphism in the ITPA gene, encoding ITPase, are still unclear, although there is some evidence that adverse drug reactions on thiopurine therapy are associated with ITPA polymorphisms. An unexpected association was found between the outcome in Langerhans'cell histiocytosis (PLCH) and the presence of ITPA polymorphisms
Methods: In 150 patients with various ILD TPMT and ITPase was measured before starting Azathioprine therapy, from this group 8 patients suffered from PLCH.
Erythrocyte ITPase activity measurement and ITPA polymorphisms detection were established using described methods. Clinical outcome in PLCH was defined by improvement of HCRT and increase in diffusing capacity for CO.
Results: In general cessation of smoking improves the clinical outcome in PLCH substantially, as can be detected by HCRT and CO diffusion capacity of the lung. In a 4 out 8 patients, suffering from PLCH the clinical outcome was negatively associated with a decreased ITPase activity in erytrocytes and the presence of one or two ITPA polymorphisms, despite changes in lifestyle.
Conclusion: The nature of the negative association between ITPA polymorphisms and the clinical outcome in PLCH is still under investigation. We hypothesize that the housekeeping function of ITPA is essential for an adequate response to cellular stress. In patients with PLCH, carrying ITPA polymorphisms, this function is compromised, resulting in an unfavorable clinical outcome. We propose that ITPA and it protein products posses an immunomodulating function and hereby attenuates disease outcome.

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G: **Inflammation: Serum, BAL, and induced sputum in sarcoidosis**

G1: Different T-cell phenotypes in bronchoalveolar lavage fluid, blood and enlarged lymph nodes in patients with sarcoidosis

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Background: Sarcoidosis is characterized by the formation of non-necrotizing granulomas in affected organs and usually an increased ratio of CD4+/CD8+ T-lymphocytes in bronchoalveolar lavage (BAL) fluid. The main purpose of this study was to compare the T-cell phenotype in paired samples from BAL fluid, blood and enlarged mediastinal lymph nodes in sarcoidosis.
Methods: Patients underwent a clinical investigation including bronchoscopy with BAL and blood samples were drawn. Endoscopic ultrasound-guided fine needle aspiration of enlarged mediastinal lymph nodes via esophagus (EUS-FNA) was also performed. Cells from all three compartments were analyzed regarding the CD4+/CD8+ ratio, presence of T-regulatory cells and expression of activity markers on T-cells.
Results: We found that the CD4/CD8-ratio differed significantly between BAL, blood and lymph nodes [7.8, 5.5 - 23.0 (median, p25 - p75)], (1.5, 1.0 - 3.1), (2.8, 2.4 - 5.6), ($p < 0.05$ for all). The frequency of CD4+ T-cells expressing CD27 was significantly reduced i.e. indicating a later stage of differentiation, in BAL versus blood and lymph nodes (23.0, 13.1 - 30.1), (87.7, 84.0 - 94.0), (91.5, 86.1 - 94.0), ($p<0.0001$), but did not differ between blood and lymph nodes. The percentage of CD4+ T-cells expressing the activation marker CD69 differed significantly between BAL, blood and lymph nodes (63.4, 61.3 - 96.0), (1.6, 1.2 - 4.5), (34.6 17.9 - 45.6) ($p < 0.0001$ for all).
Conclusions: The select accumulation of CD4+ T-cells that are expressing markers of activation and differentiation in BAL fluid of patients with sarcoidosis, indicates an active ongoing immune response localized to the alveolar space.

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G2: CD8+ T cell exhaustion in sarcoidosis disease

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Background: Sarcoidosis is a unique disease which is at the crossroads of microbial infection and host immune response. Specifically, sarcoidosis is a multisystem granulomatous disease of unknown etiology, characterized by a Th1 immunophenoytpe. Recent published work from our lab demonstrates antigen-specific recognition of mycobacterial proteins in CD4+ T cells derived from 72% of sarcoidosis subjects at diagnostic bronchoscopy. Furthermore, 60% of sarcoidosis subjects exhibited antigen-specific recognition by CD8+ T cells. The unexpected finding of antigen-specific CD8+ T cells suggests that CD8+ T cells may play an important role in sarcoidosis pathogenesis. Accordingly, CD8+ T cells have been well studied in chronic infection. These studies demonstrate that persistent antigen stimulation leads to antigen-specific CD8+ T cell exhaustion, characterized by upregulation of inhibitory receptors such as PD-1 that negatively regulate antigen-specific cells. Therefore we hypothesized that T cell exhaustion contributes to sarcoidosis disease pathogenesis. Methods: Ten sarcoidosis and five healthy control subjects were enrolled in this study. Subject PBMC were stimulated with ESAT-6 peptide or polyclonal T cell stimulation using plate bound anti-CD3 and soluble anti-CD28 antibodies. PD-1 expression was then measured on CD8+ T cells by multiparameter flow cytometry. Concurrently, intracellular IFN-γ and IL-2 production was assessed. Results: PD-1 expression was upregulated on CD8+ T cells in sarcoidosis subjects with active disease. Furthermore, PD-1 expression was highest on monofunctional IFN-γ+ T cells compared to polyfunctional (IFN-γ+IL-2+) T cells. In five of the sarcoidosis subjects, we assessed PD-1 expression during active disease and upon disease improvement. Improvement of disease correlated with reduction in PD-1 expression. Conclusions: These results indicate that PD-1 expression may correlate with sarcoidosis disease severity. Furthermore, these data suggest that the manifestations of chronic infection may contribute to sarcoidosis disease pathogenesis.

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G3: Peripheral blood responses to specific antigens in sarcoidosis and the role of CD28

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Background: Potential antigens inducing granulomatous inflammation in sarcoidosis include certain mycobacterial and auto-antigens associated with production of interferon-γ. Paradoxically, peripheral anergy to common recall antigens is prevalent, which may be explained by the presence of regulatory T-cells or impaired dendritic cell responses. This phenomenon may also reflect impaired T-cell co-stimulation. The aim of this study was to examine peripheral blood responses of patients with sarcoidosis to potential antigens and CD28 T-cell co-stimulation.
Methods: Peripheral blood mononuclear cell (PBMC) responses from patients with sarcoidosis (n=16) and purified protein derivative (PPD)-negative and positive healthy controls (n=22) were measured using interferon-γ ELISPOT with the following stimuli: anti-CD3/CD28 coated beads, M. tuberculosis ESAT-6 and KatG peptides, vimentin and lysyl tRNA peptides, and common recall antigens including cytomegalovirus (CMV) lysate and also combined CMV, Epstein-Barr virus and influenza virus (CEF) 9-mer peptides. Additionally, using a multiplex cytokine assay, IL-2, IL-4, IL-6, IL-10 and TNF-α were measured in PBMC culture supernatants following stimulation with ESAT-6/KatG, CEF peptides and CD3/CD28 beads.
Results: Following stimulation with ESAT-6 and KatG peptides, patients with sarcoidosis exhibited higher numbers of IFN-γ producing T-cells ($p = 0.02$), and elevated production of IL-2 ($p = 0.02$), IL-6 ($p = 0.04$) and TNF-α ($p = 0.02$) compared to PPD-negative healthy controls. In contrast, PBMCs from patients with sarcoidosis showed reduced IFN-γ producing T-cells following stimulation with CMV lysate ($p = 0.01$), CEF peptides ($p = 0.04$) and CD3/CD28 beads ($p < 0.001$); and reduced production of IL-2 ($p = 0.05$), IL-4 ($p = 0.04$), IL-6 ($p = 0.05$) and TNF-α ($p = 0.05$) following CD3/CD28 bead activation.
Conclusions: PBMCs from patients with sarcoidosis exhibit greater responses to M. tuberculosis antigens compared to PPD negative healthy controls. Additionally, patients with sarcoidosis have reduced CD4 and CD8 immune responses to common recall antigens. One mechanism contributing to the peripheral anergy in sarcoidosis may be impairment of the T-cell CD28 co-stimulatory receptor pathway.

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G4: Measurement of neopterin, transforming growth factor-β1 (TGF-β1) and angiotensin converting enzyme (ACE) in the exhaled breath condensate of patients with sarcoidosis

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Background: Exhaled breath condensate (EBC) is a simple, non-invasive method of sampling airway lining fluids in a variety of respiratory diseases. Since sarcoidosis affects the lungs in approximately 90% of cases, we sought markers of granulomatous airway inflammation and disease activity including neopterin, TGF-β1 and ACE in EBC. This may be a useful approach in identifying novel exhaled biomarkers to determine which sarcoidosis patients are at risk of developing pulmonary fibrosis.

Methods: Exhaled breath condensate was collected from patients with sarcoidosis (n=16) and healthy control subjects (n=22). EBC neopterin, and active-TGF-β1 were measured by ELISA. EBC ACE activity was measured using a colorimetric assay. To adjust for differences in total protein between subjects, EBC total protein concentration was measured using a bicinchoninic assay (BCA).

Results: EBC neopterin was detectable in 3/20 controls and 7/16 patients with sarcoidosis. Patients with sarcoidosis had greater mean levels of neopterin compared to control subjects (0.57 ± 0.45nmol/l versus 0.41 ± 0.22nmol/l, $p = 0.04$), although there was no difference when neopterin levels were normalized to EBC total protein ($p = 0.13$). TGF-β1 was detectable in the EBC of all subjects and concentrations were higher in patients with sarcoidosis compared with control subjects (115.5 ± 79.6 pg/mol versus 82.3 ± 16.2 pg/mol, $p = 0.048$) but the difference was not significant when comparing the ratios of TGF-β1 to total protein ($p = 0.60$). There was no difference in EBC ACE activity, which was only detectable in 3/20 healthy controls and 2/16 patients ($p = 0.91$).

Conclusions: EBC markers of granulomatous inflammation are detectable at greater levels in patients with sarcoidosis compared to healthy controls subjects. Larger studies are warranted to examine the disease correlates and predictive utility of these markers.

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G: **Inflammation: Serum, BAL, and induced sputum in sarcoidosis**

G5: Circulating cytokines in sarcoidosis

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Background: Sarcoidosis is a systemic, granulomatous condition of unknown etiology that predominantly affects the lungs but also commonly involves various extra-pulmonary organs. The mechanisms of heterogeneity of organ involvement are unknown. The immunopathogenesis of sarcoidosis is also largely unknown; immune cells are recruited and activated via poorly understood cell signaling mechanisms. In the lung, levels of cytokine cell signals are frequently altered. There may be important alterations in circulating cytokines as well. In addition, alterations may correlate with organ involvement. To explore these hypotheses, we measured circulating cytokines in patients with sarcoidosis and in controls, and evaluated cytokine levels according to organ phenotype.
Methods: We used a bead-based multiplex cytokine assay to measure levels of 17 cytokines in 56 patients with biopsy-established, chronic sarcoidosis, and in 18 controls. Organ phenotypes for subgroup analysis included all pulmonary, fibrotic pulmonary, non-fibrotic pulmonary and neurosarcoid. IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, G-CSF, GM-CSF, IFN-γ, MCP-1, MIP-1β, and TNF-α were measured simultaneously in serum samples. Non-parametric t-tests were performed to compare cytokine levels in controls to levels in sarcoidosis, and to levels in organ phenotypes.
Results: In patients with sarcoidosis compared to controls, IL-5 was decreased ($p=0.002$), and IL-7 ($p=0.033$) and GM-CSF ($p=0.044$) were increased. By organ phenotype, IL-5 was decreased in all pulmonary ($p=0.010$) and in non-fibrotic pulmonary ($p=0.040$) compared to controls. In addition, IL-5 was decreased in neurosarcoid compared to controls ($p=0.036$), to non-fibrotic pulmonary ($p=0.048$) and to fibrotic pulmonary ($p=0.040$). IL-7 was increased in fibrotic pulmonary ($p=0.041$) compared to controls. GM-CSF was increased in neurosarcoid ($p=0.007$), and trended towards an increase in all pulmonary ($p=0.051$) and non-fibrotic pulmonary ($p=0.054$) compared to control.
Conclusions: In a comprehensive evaluation of serum cytokines in sarcoidosis, we found serum IL-5, IL-7 and GM-CSF to be significantly altered, with an affect of organ phenotype on cytokine patterns. Our findings are supportive of important, detectable alterations in systemic immunity in sarcoidosis, and of a relationship between cytokine alterations and organ phenotype. Cytokine alterations provide a window into the immunopathogenesis of sarcoidosis, and provide attractive targets for follow-up studies.

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G6: Differences in BAL macrophages cytology determined by morphometric analysis in patients with sarcoidosis

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Background:
Morphometric and image cytometric DNA analysis of the macrophages nuclei in BAL fluid of patients with sarcoidosis have been investigated in order to establish their diagnostic and clinical significance.

Methods:
Investigated morphometric parameters were: area, outline, convex area, length, breadth, maximal radius, minimal radius, form factor (FF), elongation factor (EF), area/convex area (ACA), DNA index (DI), percentage of cells before histogram peak, percentage of cells in peak, percentage of cells after histogram peak, percentage of cells in S-phase and G2M phase. Seventy-three patients were included in the investigation, separated in four groups: sarcoidosis with acute onset (n=16), sarcoidosis with chronic onset (n=17), interstitial lung diseases other then sarcoidosis (n=30) and controls (n=10).

Results:
Statistical significant differences were observed between groups in all statistical descriptions of morphometric parameters area, outline, convex area, length and maximal radius of the macrophages nuclei in BAL fluid ($p<0,05$) and in percentage of the cells in S-phase between patients with acute and chronic sarcoidosis determined with DNA cytometric analysis of BAL fluid. Multivariate discriminant forward step-wise analysis resulted in classification functions for investigated groups allowing 100% separation of sarcoidosis patients with acute and chronic sarcoidosis. In all, 43 variables of morphometric and DNA cytometric parameters of macrophages nuclei in BAL fluid have been separated.

Conclusion: Investigated morphometric parameters allow correct classification of BAL fluid macrophages nuclei in the groups of acute or chronic sarcoidosis.

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G7: Mycobacterial heat shock proteins 16 kDa, marker of stationary-phase of M. tuberculosis, in precipitated circulating immune complexes in sarcoidosis

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Mycobacterial heat shock proteins (Mtb-hsp), genetic factors and autoimmunity have been considered as potential causes of sarcoidosis (SA). Mtb-hsp may provide a link between infection and autoimmunity by cross-reactivity between the mycobacterial and human hsp. Mtb-hsp16, similar to small hsp human α-crystallin, is implicated in forming of circulating immune complexes (CIs) in tuberculosis (TB). Mtb-hsp16 is also necessary for the survival of M. tuberculosis in the stationary-phase, especially in an anaerobic condition in a granuloma.We have recently demonstrated opposite presence of Mtb-hsp70, Mtb-hsp65 and Mtb-hsp16 in sarcoid tissues. In lymph nodes, Mtb-hsp70 and Mtb-hsp16 were more expressed than Mtb-hsp65, whereas in sera, Mtb-hsp70 was more frequent than Mtb-hsp65 or Mtb-hsp16. Higher occurrence of Mtb-hsp70 than Mtb-hsp65 and Mtb-hsp16 in sarcoid tissues could be caused by sequestration of Mtb-hsp65 and Mtb-hsp16 in CIs. Therefore, we have evaluated and quantified Mtb-hsp70, Mtb-hsp65 and Mtb-hsp16 in precipitated CIs from blood of the same individuals: 20 patients with SA, 19 patients with TB and 21 healthy volunteers using the precipitation with 3,5% PEG and Western Blot. Our results showed significantly increased CIs levels in SA vs TB and healthy individuals, whereas there was no difference between TB and Control. The Mtb-hsp16, Mtb-hsp65, and Mtb-hsp70 concentrations in precipitated CIs were significantly higher in SA than in TB and Control. There were no differences between TB and Control. Mtb-hsp16 concentration in CIs was significantly higher than Mtb-hsp65 and Mtb-hsp70 in all tested groups. Mtb-hsp16 and Mtb-hsp70 presence in CIs was comparable in both Stage I and Stage II of SA, whereas Mtb-hsp65 occurrence was significantly more increased in Stage II than in Stage I. In summary, our results show increased concentrations of Mtb-hsp16, Mtb-hsp65 and Mtb-hsp70 in precipitated CIs in sarcoidosis comparing to corresponding levels in TB and healthy individuals. It seems that Mtb-hsp16 may be more important than Mtb-hsp70 and Mtb-hsp65 in circulating immune complexes formation and possibly the protein may be implicated (auto)immune response in SA related to stationary-phase of M. tuberculosis.

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G8: Is there any association between presence of Mycobacterium avium subsp. paratuberculosis and SLC11A1 in sarcoidosis?

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Infectious agents, genetics and autoimmunity have been explored as potential causes of sarcoidosis (SA). Mycobacterium tuberculosis (MTB) and Mycobacterium avium ss. paratuberculosis (MAP) are suspected as causal agents for sarcoidosis; MAP exposure probably being more common in countries where MTB infections are well controlled. In genetically predisposed host, especially those with a polymorphism of allele 3 in the (GT)n promoter of SLC11A1 (formerly natural resistance associated macrophage protein 1, NRAMP1), host response to infection or mycobacterial antigen(s) may provide a link between infection and autoimmunity through cross-reactivity between human heat shock proteins (hsp) and those of MTB or MAP (hsp cross reactivity ~90%, ~65%, respectively). SLC11A1 plays a key role the host response to intracellular pathogens through its involvement in the acidification of the phagosomes and regulation of nitric oxide, IL-10 and intra-phagosomal iron concentrations. We hypothesized that SA patients with a polymorphism at allele 3 in SLC11A1 and serum antibodies to MTB-hsp are MAP-infected more often than controls. To test this hypothesis, 18 SA patients and 24 healthy controls who were positive for both the polymorphic allele 3/3 at SLC11A1 and MTB-hsp antibodies were tested for MAP by two methods. MAP organisms were detected in patient peripheral blood leukocytes (PBL) by culture of in MGIT liquid media followed by nested IS900 PCR as described by Naser et al. Antibodies to MAP early secreted antigens (culture filtrate) were measured by ELISA. We found MAP in the PBL of 1 of 18 (5%) SA patients and 6 of 24 (25%) healthy controls ($p=0.0001$). The level of anti-MAP antibodies was not different between groups; 27% and 17% for SA and controls, respectively. These data suggest that MAP infections are not associated with SA. It is possible that since both groups had the polymorphism of allele 3 in the (GT)n promoter of SLC11A1, both are more susceptible to MAP infection. To explore this idea, humans without this genetic defect must be tested for MAP.

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G: **Inflammation: Serum, BAL, and induced sputum in sarcoidosis**

G9: Lung and blood Th1 and Th17 responses against mycobacterial antigens in patients with pulmonary sarcoidosis

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Background: Sarcoidosis is an inflammatory disorder characterized by granulomas most commonly affecting the lungs. The presence of mycobacterial antigens, and recently a specific protein, M. tuberculosis catalase-peroxidase (mKatG), in sarcoidosis tissue has been reported. Th1 cell responses against mKatG have been observed in sarcoidosis lung and peripheral blood cells.

Methods: In the present study, IL-17 and IFNγ production were evaluated by ELISPOT after stimulation of bronchoalveolar lavage (BAL) fluid and peripheral blood cells with mKatG and M. tuberculosis PPD proteins. Two groups of sarcoidosis patients were compared: HLA-DR3 positive (good prognosis) versus HLA-DR3 negative (bad prognosis). We also did immunohistochemistry on bronchial biopsies for further characterization of cytokine expression.

Results: Both mKatG and PPD stimulation of BAL cells resulted in higher frequencies of cells producing IFNγ compared to IL-17. We did not detect any significant difference between BAL and blood regarding IL-17 secretion after stimulation with both mKatG and PPD, while significantly more BAL cells produced IFNγ in comparison to blood after stimulation with mKatG and PPD ($p<0.05$). The existence of IL-17+ cells in the granulomas also supports the role of IL-17 in sarcoidosis.

Conclusions: The observed Th17 responses against mycobacterial antigens could contribute to the inflammation in sarcoidosis, although they generally occur at lower frequencies than corresponding Th1 responses.

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G10: Higher frequencies of perforin- and granzyme B-expressing CD8+ T cells in peripheral blood of sarcoidosis patients

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Background: Sarcoidosis is a granulomatous disease of unknown etiology, mainly affecting the lungs. Previous studies have extensively studied the CD4+ T helper cells and established their role in the pathological process. However, CD8+ T cells can sometimes make up the majority of cells recovered by bronchoalveolar lavage (BAL). Previous studies have demonstrated that both CD4+ and CD8+ T cells produce Th1 cytokines in the lungs of sarcoidosis patients, but little is known about the cytotoxic capacity of CD8+ cells in this disease. Our aim was to characterize CD8+ T cells as well as NK cells in sarcoidosis with regard to cytotoxic intracellular mediators.
Methods: Peripheral blood (from 17 sarcoidosis patients and 11 healthy controls) and BAL fluid (from 19 patients and 4 controls included so far) was obtained. Flow cytometric analysis of cell subsets was performed after staining with antibodies against the surface molecules CD3, CD4, CD8 and CD56 as well as antibodies against the cytotoxic intracellular mediators perforin, granzyme B and granulysine.
Results: Sarcoidosis patients had a significantly higher percentage of both perforin positive (49% in sarcoidosis patients vs. 18% in healthy controls, p-value < 0.001) as well as granzyme B positive (52% in sarcoidosis patients vs. 24% in healthy controls, p-value < 0.05) CD8+ T cells in blood. In BAL fluid however, the tendency seems to be the opposite, with sarcoidosis patients having a lower percentage of CD8+ T cells positive for intracellular cytotoxic mediators, although recruitment of more healthy controls to be included in the study is ongoing.
Conclusion: Cytotoxic T cells may be involved in the propagation or regulation of the inflammatory process in sarcoidosis, but functional studies are needed to delineate their role.

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G11: Influence of smoking on sFas concentration in the bronchoalveolar lavage fluid of patients with sarcoidosis

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Background: Sarcoidosis (SA) is a multisystem granulomatous disease with frequent localization in the lungs. The incidence of SA in smokers is reported to be low but the role of cigarette smoking on inflammation in SA remains unclear. Some studies postulated the participation of Fas/FasL system in the regulation of immune reaction and granuloma formation. Soluble Fas (sFas) is known to inhibit apoptosis. Previously we reported an elevated number of Fas positive cells in the BALF of smokers with SA. The aim of this study was to compare sFas concentration in bronchoalveolar lavage fluid (BALf) of ever smokers (S) with never smokers (NS) with SA.

Methods: We investigated 57 patients with confirmed SA: 36 NS and 21 S (mean pack/years = 8.2, 7 current smokers, 14- ex smokers). Patients in the S group were significantly younger than in the NS (mean 34 vs. 42 years) with predominance of men (70%). Total and differential cell count in the BALF was performed with standard methods. The concentration of sFas was measured using ELISA method.

Results: Composition of BAL differed significantly between the S and NS group with significantly higher macrophage percentage and count in the S group when compared with the NS group (62 vs 50%, 11.3 vs 6.5 x106, respectively), while the lymphocyte percentage was significantly lower (in S when compared with NS (29 vs 41% in the S group and NS group, respectively). The total granulocyte count was higher in the S group than in the NS group, however the difference was not significant. The BALF concentration of sFas was lower in the S group (median values 68.3 vs 95.5 pg/mL, p=0.09). The sFas concentration in active smokers was significantly lower than in NS (65.6 vs 95.5 pg/mL, p=0.01). There was a significant correlation of the sFas concentration with the proportion of lymphocytes.
Conclusions: Thus, our findings showed reduced sFas concentration in the BALF of smokers with SA. The low concentration of sFas may cause a higher apoptosis rate of inflammatory cells and facilitate resolving of granulomas.

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G12: Tobacco consumption and impaired immune surveillance in lower airways. Data from bronchoalveolar lavage (BAL) harvested from patients with sarcoidosis (PS), idiopathic pulmonary fibrosis (IPF) and from controls

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Background: Tobacco consumption is established as a cause of lung tumors, due to its mutagenic potential. Less is known about another possible mechanism, i.e. suppressed local immune surveillance, impaired function of alveolar lymphocytes (AL) and immune polarization towards Th2-dependent immunity.
Methods: BAL was harvested from patients with PS (n=53), IPF (n=25) and control subjects (n=13), subdivided according to their smoking status. Selected cytokines with potential impact on antitumor immunity (IFNγ, TNFα, FasL, TRAIL) were tested in BAL supernatants by ELISA. AL were phenotyped for cytokine receptors (CD119, CD120a, CD120b, Fas). AL apoptosis was examined with 1) TUNEL (both in BAL cytospins and flow cytometric samples) 2) Annexin V/PI staining 3) cell cycle analysis (evaluation of sub-G1 late apoptosis peak).
Results: Average 1% of AL enters apoptosis in cell cycle analysis of nonsmoking controls. 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. Tobacco consumption in all groups resulted in significant increase in AL apoptosis rate (e.g. 11±7.5% in IPF smokers, $p<0.05$ as compared to nonsmokers). Corresponding results were found in TUNEL. IFNγ, FasL and TRAIL levels were lower in all smoking subgroups, as compared to their nonsmoking counterparts (e.g. 6.7±1.6pg/ml in PS nonsmokers vs 2.6±1.9 pg/ml in smokers for IFNγ; 49.8±12.7 pg/ml in IPF nonsmokers vs 10.6±4.1 pg/ml in smokers for TRAIL, $p<0.05$ for both). No changes were found TNFα levels, however TNF type 1 (CD120a) receptor expression was significantly risen in all smoker subgroups.
Conclusions: Increased AL apoptosis rate found in smokers could be explained in part by enhanced AL susceptibility to proapoptotic TNFα function. BAL supernatant levels of factors with potential antitumor activity were decreased in smokers as compared to respective nonsmoking subgroups. Our collective data indicate decreased immune surveillance in smoker lower airways. It may be responsible for severe course of IPF, blunted symptoms in PS and, in general, increased risk of lung cancer.

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H: **Inflammation: Serum, BAL, and induced sputum in ild**

H1: Improved outcome in ANCA associated renal vasculitis over the last 30 years

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Background: Renal small-vessel vasculitis is associated with anti-neutrophil cytoplasmic antibodies (ANCA) directed against myeloperoxidase (MPO) and proteinase 3 (PR3). Recently, it was demonstrated that mortality in patients with ANCA associated vasculitis (AAV), not involving the kidneys, declined over the last decades. In the current study, we assessed whether prognosis of patients with ANCA-associated renal vasculitis also improved over the last three decades.
Methods: Patients with necrotizing crescentic glomerulonephritis due to AAV were included between January 1979 and December 2009. Inclusion criteria were the presence of PR3- or MPO-ANCA and the availability of a kidney biopsy. Biopsies were classified according to the recently developed EUVAS classification. End points for survival analysis were renal replacement therapy, death or closure of study. To assess renal and patient survival, patients were divided in three groups through time: 1979-1989, 1990-2000 and 2001-2009.
Results: 181 patients were included. One, five and ten year survival were 77%, 66% and 49%. Age, serum creatinine, ANCA subtype and time groups were predictors of patient survival. Survival within the time groups was significantly different, yielding a hazard ratio for death of 3.5 for 1990-2000 and of 5 for 1979-1989 compared to 2001-2009 ($p<0.01$). Serum creatinine and active lesions as found in the kidney biopsy significantly decreased through the three decades.
Conclusion: Both patient and renal survival in patients with ANCA associated renal vasculitis have improved over the last three decades. We postulate that both earlier diagnosis and better therapeutic management of patients are responsible for this effect.

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H2: Extracellular concentration of proteasome in serum and BAL of patients with interstitial lung diseases

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Background: Interstitial lung diseases (ILD) are a heterogeneous group of diffuse parenchymal disease. Proteasomes are responsible for nonlysosomal protein degradation. They are involved in apoptosis, stress response and inflammation. ARDS and sarcoidosis show elevated levels of extracellular proteasome. The aim of this study was to detect proteasome concentration and enzyme activity in BALF of ILD patients.
Patients and methods: 240 patients with ILD (94 IPF, 52 HP, 47 NSIP, 33 BOOP, 8 RB-ILD, 6 DIP) and 18 healthy controls (HC) were studied. The 20S proteasome subunit (murine monoclonal antibody by Biomol Int. L.P., Exeter, UK) was measured by ELISA in BALF and serum.
Results: BOOP patients showed significantly higher levels of extracellular proteasome than healthy controls and other ILDs in BALF (BOOP 118, HC 60, and HP 85, DIP 84, IPF 59, NSIP 45, RB-ILD 28 ng/mL, overall p=0,047). The same tendency was also found in serum, although not statistically significant (BOOP 521, HC 405, and IPF 369, NSIP 369, HP 319, RB-ILD 275 ng/mL, overall $p = 0.175$). Age, BMI, smoking habits or steroid treatment did not influence BALF or serum proteasome levels. A positive correlation was found between proteasome BALF and serum levels ($r=0.3$, $p=0.041$) and with BAL neutrophilia ($r=0.3$, $p=0.006$). Serum but not BALF proteasome levels correlated negatively with DLCO ($r=-0.4$, $p=0.04$).
Conclusion: Proteasome extracellular concentration seems to be higher in BOOP, a disease with a marked inflammatory component, than in other ILDs.

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H3: Presence of mimivirus in bronchoalveolar lavage fluid of critically ill patients suspected of ventilator-associated pneumonia

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Background: Acanthamoeba polypaga mimivirus belongs to the amoebae-associated micro-organisms (AAMO). Antibodies to mimivirus have been found in patients presenting with pneumonia suggesting a potential role for this virus as a respiratory pathogen. In addition, positive serology for the mimivirus was associated with an increased duration of mechanical ventilation and intensive care unit (ICU) stay in patients with ventilator-associated pneumonia (VAP). The aim of the present study was to evaluate the occurrence of mimivirus in bronchoalveolar lavage fluid (BALF) samples of critically ill patients suspected of VAP by means of real time polymerase chain reaction (RT-PCR).
Material and Methods: This study was conducted at the intensive care unit (ICU) of the Maastricht University Medical Centre+, a 750-bed hospital. All consecutive BALF samples obtained between January 2005 and October 2009 from patients suspected of VAP were eligible for inclusion. Data on BALF total cell count, differential cell count, quantitative bacterial culture and detection of viruses, mycobacteriae and fungi were noted. BALF samples were retrieved from -80°C storage. All samples were analyzed by an RT-PCR targeting the mimivirus.
Results: A total of 260 BALF samples from 214 patients (139 male, 75 female) were included in the study. The mean age in the study group was 63 (19-84) years. A total of 105 out of 260 (40%) suspected episodes of VAP (86 patients), were microbiologically confirmed bacterial VAP. Of the patients without a microbiologically confirmed VAP, seven were diagnosed with Pneumocystis pneumonia, three with a herpes simplex virus type 1 pneumonia, one with human metapneumovirus infection and one with tuberculosis. Unfortunately, neither in the bacterial VAP positive nor in the bacterial VAP negative BALF samples the presence of mimivirus DNA could be demonstrated.
Conclusion: Although mimivirus was suggested as a potential respiratory pathogen in patients with pneumonia, its presence could not be confirmed in this study-population of critically ill patients suspected of VAP.

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H4: Alternative diagnosis in the ventilator-associated pneumonia suspected bronchoalveolar lavage negative patient

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Background: Other infectious and non-infectious diseases have proven responsible for mimicking the clinical picture of ventilator-associated pneumonia (VAP). Aim of this study was to determine potential alternative diagnosis in patients suspected of VAP with negative bronchoalveolar-lavage (BAL) results.
Methods: All adult intensive care patients with a clinical suspicion of VAP and negative BAL results were included. The clinical suspicion of VAP was based on the combination of clinical, radiological, and microbiological criteria. BAL was considered positive if cell differentiation revealed $\geq 2\%$ cells with intracellular organisms and/or quantitative culture results of ≥ 104 cfu/ml. Retrospectively, the most likely alternative diagnosis of fever, pulmonary densities and both combined were determined.
Results: 110 patients with suspected VAP and negative BAL results were included. Regarding fever, bacteremia was considered in 9 (13.2%) patients, caused by central venous line infection (n=2), infected ascitis (n=1), urinary tract infection (n=3), infected hematoma (n=1). In two cases its origin remained obscure. Resorption fever was considered in 8 (11.8%) patients originating from neurotrauma (n=3), multitrauma (n=2), lung bleeding (n=1), brainstem hemorrhage (n=1) and a postoperative bleeding after thoracic-abdominal aortic aneurysm repair (n=1). Ischemia was found to be the alternative cause of fever in 6 (8.8%) patients, 5 due to intestinal ischemia and 1 due to a large ischemic cerebrovascular, accident. In 54% of patients an alternative diagnosis of fever and pulmonary densities combined was found. Non-bacterial infectious pneumonia was diagnosed in 12 patients. Herpes simplex virus-1 was the causative pathogen in 7 cases, followed by Cytomegalovirus in 2, Pneumocystis jiroveci, Proteus mirabilis and Candida albicans each 1 case. In 8 patients non-infectious pneumonia was diagnosed. BOOP (n=3) and drug-induced pneumonia (n=3) were the leading causes, followed by eosinophilic pneumonia (n=1), and Wegener's granulomatosis (n=1).
Conclusions: There is a wide spectrum of alternative diagnosis in patients suspected of VAP with negative BAL results. Viral pneumonia and non-infectious pneumonitis are found frequently. Early identification of the exact cause may be vital for adequate treatment and outcome.

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H: **Inflammation: Serum, BAL, and induced sputum in ild**

H5: Diagnostic value of pulmonary NKT CD3+CD16/56+ cells

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Background: Natural killer T (NKT) cells, a unique subgroup of T cells, may be implicated in the pathogenesis of interstitial lung diseases (ILDs).

Methods: We used multi-parameter flow cytometry with antibodies to CD3, CD4, CD8, CD14, CD45 and CD16/56 in BAL fluid (BALF) to examine the diagnostic relevance of pulmonary CD3+CD16/56+ NKT cells and to compare them with NK cell frequencies and CD4/CD8 index. We selected and analysed only BALF of patients with initial increased (> 8%) frequency of CD3+CD16/56+ NKT cells.

Results: Of 88 selected patients with increased frequencies, 25 patients were diagnosed as hypersensitivity pneumonitis (HP) and 35 as sarcoidosis (SA), 14 patients demonstrated diffuse ILDs, 7 had malignant diseases and 7 patients had bacterial infection. The uppermost frequencies of BALF CD3+CD16/56+ NKT cells were demonstrated in patients with HP (median of 16%, range 8% to 52%), and those values were significantly higher as CD3+CD16/56+ frequencies observed in patients with SA (10%, range 8% to 19%) or diffuse ILDs (10.5%, range 8% to 17%; P<0.0001). In contrast, there was no difference in the proportion of CD3-CD16/56+ natural killer (NK) cells between all study groups (median value from 3 up to 4.5% with the range from 0% up 18%; P=0.17) Patients with sarcoidosis have also significantly higher CD4/CD8 ratio (median 2.4, range 0.26 to 12.14) in comparison to patients with HP (median 0.46, range 0.04 to 10.4; P<0.0001).

Conclusion: This study showed that high frequencies of pulmonary NKT CD3+CD16/56+ cells, but not NK cells, might have important role for diagnostic evaluation of HP.

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H6: Induced sputum analyses in Beryllium-exposed dental technicians reflect hygiene and oxidative stress

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Background: Chronic beryllium disease and beryllium sensitization are caused by occupational exposure to beryllium. The incidence of the disease continues to increase despite environmental controls.
Objective: To assess whether size and shape of induced sputum particulate matter and oxidative stress parameters can biologically monitor beryllium-exposed workers.
Methods: This cross-sectional study included 83 dental technicians. Induced sputum and beryllium lymphocyte proliferation tests were done by conventional methods. Particle size distribution and shape image analysis were done by laser and video technologies. Shape analysis was by aspect ratio, convexity, circularity, average concavity, and gray level. Heme oxigenase-1 gene expression was evaluated in induced sputum samples by quantitative PCR.
Results: A cut-off of 92% of particles that were <5 μ in induced sputum samples was correlated to the presence of a risk for a positive beryllium lymphocyte proliferation test (odds ratio of 3.4 [0.9-13]). Particle opacity (gray level) in induced sputum was associated with beryllium exposure (odds ratio 0.95 [0.91-0.98]) and it was higher in non-exposed compared to exposed workers. Exposure to fumes vs dust and the use of hood/personal masks vs no protection yielded differences in opacity as well. HO1 gene expression was associated with opacity (r=0.25, P=0.04) with high values in fume- vs dust-exposed workers.
Conclusions: We describe novel biological markers for screening workers exposed to hazardous dust. The opaqueness of particles in induced sputum is sensitive to the hygienic condition in the workplace and affects the oxidative stress molecular pathway.

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H7: Levels of autoantibodies against granulocyte-macrophage colony-stimulating factor (GM-CSF) are elevated in the sera of pneumoconiosis patients

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Background:
Pulmonary alveolar proteinosis (PAP) is a rare diffuse lung disease characterized by excessive accumulation of surfactant proteins in the alveoli and terminal bronchioles. Recent progress in the pathogenesis of PAP is the discovery of elevated levels of autoantibodies against GM-CSF in idiopathic PAP patients. Epidemiological studies have shown that about a quarter of PAP patients had the history of dust exposure.

Objective:
To examine the levels of anti-GM-CSF antibodies in the sera of pneumoconiosis patients.
Methods:
We obtained sera from 150 pneumoconiosis patients and 58 healthy controls, and measured the levels of anti-GM-CSF antibodies by enzyme-linked immunosorbent assay (ELISA).

Results:
The levels of GM-CSF antibodies were significantly elevated in the sera of pneumoconiosis patients compared with healthy controls (p<0.0001).
Conclusion: Dust exposure may play roles in the generation of anti-GM-CSF autoantibody.

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H8: Endotoxin markers assessed in BAL of patients with lung fibrosis and sarcoidosis

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Background: Endotoxins (lipopolysaccharide, LPS), components of Gram-negative bacteria cell wall, are ubiquitous in the indoor environment; inhalation of LPS is associated with e.g. airway inflammation and asthma exacerbation. Analytical measurement of the chemical markers, 3-hydroxy fatty acids (3-OHFAs, exclusively present in lipid A, the LPS most conservative part) was previously applied for endotoxin assessment in serum, saliva and newborn feces*. The aim of this pilot study was an evaluation of endotoxin in BAL from patients with different interstitial lung diseases prior to explore LPS impact in disease progress and aggravation.
Methods: BAL was obtained from patients with lung fibrosis (n=20), sarcoidosis (n=12), chronic eosinophilic pneumonia (n=3), and undefined diseases (n=14). Samples of BAL supernatants were lyophilized, weighted and subjected to hydrolysis to release 3-OHFAs, followed by derivatisation and analysis by gas chromatography-tandem mass spectrometry. Internal standard was added to quantify the data.
Results: Among selected diseases the highest level of endotoxin has been detected in BAL of lung fibrosis patients (median 7.7 pmol LPS/mg; SD 4.0). It was significantly higher when compared to sarcoidosis (median 4.1 pmol LPS/mg, SD 2.3). Significant correlation between endotoxin level and proportion of neutrophils and eosinophils (p<0.05, R>0.04) has been revealed.
Conclusions: Determination of chemical markers of endotoxin, previously used in different biological specimen, has been proven to accurately assess its level in complicated biological matrices, like BAL fluid. Presuming the role that LPS holds as potent stimulator of inflammatory response and one of PAMPs in innate immunity, this analytical tool for lung disorder studies will be of a remarkable practical value.
* Szponar B. (2011) Endotoxins in environmental and clinical samples assessed by GC-tandem MS. Detection of Biological Agents for the Prevention of Bioterrorism. Ed. J. Banoub. NATO Science for Peace and Security Series A: Chemistry and Biology, Springer, pp. 245-266

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H9: Impact of adenosine A2A receptor activation on calcium transients in alveolar macrophages

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Background: The nucleoside adenosine, acting through different cell membrane receptors, is an important modulator of cell-mediated inflammation. Previous studies in animal models indicate an anti-inflammatory role for adenosine A2A receptors in peripheral inflammation. However, the specific receptors present in different human leukocytes and their actions are still unclear. We investigated the actions of the adenosine A2A receptor in alveolar macrophages, a key player in the pathogenesis of different respiratory diseases, including COPD and interstitial lung disease.
Methods: Bronchoalveolar lavage was collected from patients with different interstitial lung diseases and cells were plated in gridded coverslips. Adherent cells were incubated with a fluorescent calcium-sensitive indicator FURA-2, and single-cell intracellular free calcium transients were recorded in response to a chemotactic peptide, Formyl-Methionyl-Leucyl-Phenylalanine (FMLP) in the presence and absence of a selective adenosine A2A receptor agonist, CGS21680. At the end of the experiment the cells were stained using May-Grünwald Giemsa and the macrophages were identified using the grid.
Results: Three patients were included. FMLP 100 nM caused a significant increase in intracellular free calcium in single macrophages (mean: 97,8% above baseline), although a high variability between different cells was observed (standard deviation: 46.1%). The presence of 100 nM CGS21680 failed to significantly modify the FMPL-induced calcium transients (mean increase: 128.1 ±60.89%). Different concentrations of this drug (10-300 nM) were also tested, with the same results.
Conclusions: These preliminary results suggest that A2A receptors are either not present or present but inactive in this experimental model of alveolar macrophage activation. Further studies will evaluate the presence and subcellular distribution of this receptor in human alveolar macrophages. (Supported by FCT)

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H10: Evaluation of tricolor flow cytometry in CD4+/CD8+ subtyping of BAL fluid from patients with interstitial lung diseases (ILD)

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Background: Flow cytometry (FCM), though offering major advantages over cytospin techniques, is still unappreciated by clinical laboratories analyzing BAL specimens and favouring conventional immunocytochemical (IC) staining to determine CD4/CD8 cell ratio in ILD. We validated FCM utility in CD4+/CD8+ subtyping of BAL fluid from ILD patients with respect to standard IC staining.

Methods: Diagnostic BAL specimens from 50 patients with suspected sarcoidosis, idiopathic pulmonary fibrosis (IPF) and hypersensitivity pneumonitis (HP) were evaluated by both IC (LSAB+System-AP, Dako) and FCM (Tritest, Becton-Dickinson). In FCM CD4+/CD8+ cells were identified by light scattering with CD3 selection.

Results: Relative amounts of CD4, CD8 T cells and CD4/CD8 ratio demonstrated by the FCM showed excellent, significant Spearman's correlation with IC results ($R=0.90$; $p<0.0000$). Kruskal-Wallis ANOVA showed that FCM values did not differed significantly from IC results ($p>0.5$). IC failed to assess CD4/CD8 in 10 (20%) cases due to low lymphocyte count or artifactual staining.

Conclusions: Our results validate the use of blood-standardized, commercially available antibody cocktails for BAL lymphocyte typing by FCM. FCM allowed reliable, precise and rapid T cell subset measurement in all samples with excellent data repeatability. Apparently validated FCM protocol allows diagnostically relevant CD4/CD8 ratio determination by simple gating strategy. Therefore, FCM should be method of choice for clinical laboratories with access to a three-color flow cytometer.

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H11: Alveolar lymphocytes (AL) subsets in flow cytometry (FCM) examination - proposal of normal value range in both smokers and nonsmokers. May detailed phenotyping help to understand the role of AL in health and disease?

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Background: FCM is a common tool applied to examine AL, for lower airways diseases research and diagnostics. However, there is no consensus on the processing of BAL material for FCM. Moreover, the nature of AL in healthy subjects (in steady state conditions) has not been precisely established.
Methods: AL were obtained from BAL in 58 individuals free of lung pathology (37 nonsmokers, NS, and 21 smokers, S). Three-color typing of AL major subsets was competed with detailed staining of a) cytotoxic function, b) apoptosis susceptibility and c) antigen presenting cell (APC) markers.
Results: We proposed: 1) normal value range of AL subsets (CD4/CD8: 0.9-4.0 in NS, 0.5-1.6 in S); 2) precise criteria for BAL material recruitment, acquisition and FCM analyses. AL were almost exclusively T cells (90±1.0% in NS and 89±1.6% in S, respectively, median±SEM) with only a few B and NK cells (2±0.4 and 3±0.6% in NS, 1±0.7 and 4±1.2% in S, respectively), as compared to PBL parallel typing ($p<0.0001$ for all). BAL CD8+ cells presented characteristic T cytotoxic cell phenotype (CD8+CD28+CD11b–). Since relatively high percentage of Th cells expressed CD28 and granzyme B (>50% in NS), we hypothesize that Th in healthy controls are locally active as cytotoxic cells. BAL fluid recovery in NS (but not in S) was positively correlated with percentage of AL CD4+ and CD4/CD8 ratio (Rs +0.39, $p=0.03$). Despite high CD95 expression (up to 100% cells), average 1% only of AL entered apoptosis. Other death receptors, as TNF-R1, DR3 and DR4 were found exceptionally on AL both in NS and S. Interestingly, remarkable percentage of AL presented HLA-DR+CD80+86+ phenotype suggesting possible APC function. CD3 and CD4 antigen mean fluorescence intensity was significantly lower on AL than on PBL of the same subjects ($p<0.001$).
Conclusions: 1. The results support the view on AL as effector/memory T cell population. 2. Even upon physiological (steady state) conditions, T cells in lower airways seem to be antigen-specifically restimulated. 3. The cytotoxic function is probably manifested in a similar extent, by both Th and Tc alveolar cells.

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H12: Different susceptibility of BAL immune cells to transforming growth factor β (TGFβ) in Th1 interstitial lung diseases (ILDs) and idiopathic pulmonary fibrosis (IPF)

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Background: TGFβ, as the most potent activator of lung fibrosis, stimulating fibroblast proliferation and inducing secretion of collagen as well as other extracellular matrix proteins. It reveals its profibrotic effect by receptor complex, including CD105 co-receptor 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), IPF (n=9), non-specific interstitial pneumonitis (NSIP, n=6) and extrinsic allergic alveolitis (EAA, n=7). CD105 appearance was also assessed in lung model 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 experiments) 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 co-receptor, CD105, is commonly present in lower airways. Higher BAL CD105+ lymphocyte percentage in IPF, and lower one in PS and EAA, may reflect different Th1/Th2 polarization pattern. Summarizing, the clue role of diverse lower airway cell reactivity to TGFβ in ILDs should be considered.

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O: Oral presentations

O1: GWAS in Japanese patients with sarcoidosis by the use of pooled samples

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Background: Sarcoidosis is a polygenic granulomatous disorder with predominant manifestation in the lung. Genome-wide association study (GWAS) has been receiving attention as a new master card for a searching method of disease susceptibility genes. The first GWAS study for sarcoidosis has suggested the localization of the susceptibility genes on several chromosomes. Though replication study is needed for Japanese patients, high economic burden is a great concern. To circumvent this problem, we conducted GWAS using pooled samples.
Methods: The study subjects were 128 control subjects and 163 patients with sarcoidosis. Pooled DNA samples for each group were prepared by adding equal amount of DNA from individual subjects. Quadruplicated and triplicate samples were prepared for controls and patients, respectively. We used Human610-Quad v1.0 DNA Analysis BeadChip (Illumina) as a SNP array. After adjusting fluorescence signals for each allele, we selected SNPs with significant difference in the simulated allele frequencies between controls and patients. Some of those SNPs were genotyped for individual subjects using TaqMan probes.
Results: From array information, we found a number of SNPs whose genotype distribution in patients is potentially different from that for controls. Among such SNPs, individual genotyping revealed that rs7755224 (p=0.002) and rs11749676 (p=0.009) were significantly associated with the development of sarcoidosis.
Conclusion: Our results indicated that GWAS by the use of pooled samples is feasible and potentially useful for the first stage screening method for susceptibility genes of sarcoidosis.

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O2: Two novel ways for distinguishing sarcoidosis from sputum negative tuberculosis

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Background: To differentiate sarcoidosis (SA) from sputum negative tuberculosis (TB) is a challenge for clinical doctors, especially in a high TB prevalence country like China. We established two novel ways for sarcoidosis and sputum negative tuberculosis differentiation.
Methods: 1. Biopsy samples were retrospectively collected from 104 patients with sarcoidosis, 31 patients with tuberculosis, and 55 controls. The samples were collected to amplify Mycobacterium tuberculosis (MTB) genome by real-time quantitative PCR. Cut-off values of real-time PCR quantification for differential diagnosis were selected by receiver-operating characteristic (ROC) curves. 2. Data of 181 TB and 117 SA were entered, Univariate comparisons between SA and TB were performed to screen the candidate variables that could be applied in the scoring system. A logistic regression analysis with backward conditional method was used to choose the final variables and to develop the scoring system.
Results: 1. Cut-off values (1.14×103copies/ml) of real-time PCR quantification for differential diagnosis were selected by receiver-operating characteristic (ROC) curves. The sensitivity for the differential diagnosis of sarcoidosis and tuberculosis was 96.8% and specificity was 98.1%. We applied this method to 49 new cases whose diagnosis were uncertain of sarcoidosis or tuberculosis, 33 of the patients' diagnosis was confirmed as sarcoidosis and 2 was confirmed as tuberculosis, 72% (35/49) of the patients got affirmative diagnosis. 2. Four scoring systems were established: clinical-radiographic (CR), clinical-radiographic-pathological (CRP), clinical-radiographic-radionuclide (that is, emission computed tomography, ECT) (CRE) and clinical-radiographic-radionuclide-pathological (CREP). We prospectively applied the scoring system to evaluate 130 new cases who were suspected sarcoidosis, the sensitivity and specificity of the four scoring system to distinguish the two diseases respectively were 91.78%, 97.26%, 94.52%, 98.63% and 87.72%, 98.25%, 96.49%, 98.25%.
Conclusions: These two novel methods can help doctors to differentiate sarcoidosis and sputum negative tuberculosis very effectively. Combination of the two methods can help doctors to get much more correct diagnosis.

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O3: Reduced expression of PPAR-alpha in bronchoalveolar lavage CD4+ T cells of sarcoidosis patients

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Background: Sarcoidosis is a systemic inflammatory disease characterized by noncaseating granulomas affecting many organs, especially the lungs and intrathoracic lymph nodes. Activated CD4+ T cells with a type 1 cytokine profile are considered to be of central importance for the pathogenesis. The peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that play important regulatory roles in numerous cellular processes, including inflammation. Three PPARs have been described, namely PPAR-alpha, -beta/delta and –gamma. They are expressed in many cell types, including macrophages and T cells. A reduced activity of PPAR-gamma in sarcoidosis patients´ alveolar macrophages has previously been reported. The purpose of this study was to investigate on the mRNA and protein level the expression of PPARs in lung cells of sarcoidosis patients.
Methods: Seventeen sarcoidosis patients and nine healthy controls underwent bronchoscopy with broncoalveolar lavage (BAL) thereafter CD4+ T cells and alveolar macrophages (AMs) were sorted by flow cytometry and subjected to real-time PCR analysis for mRNA expression of PPARs. Immunofluorescence staining of BAL cytospin slides with anti-PPAR antibodies was also performed.
Results: PPAR-alpha relative gene expression was significantly downregulated in CD4+ T cells of sarcoidosis patients, but no differences were observed with regard to T cell expression of the other PPARs. PPAR expression in AMs did not differ between patients and controls. No differences were observed between patients with or without Löfgren´s syndrome.
Conclusion: The observed downregulation of PPAR-alpha in T helper cells may contribute to ongoing inflammation in pulmonary sarcoidosis via failure to repress proinflammatory genes.

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O4: Increased local and circulating T helper 17 cells in pulmonary sarcoidosis

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Background: Sarcoidosis is a systemic inflammatory disorder characterized by granulomas in the lungs and other organs. Although the etiology is unknown, sarcoidosis is thought to emerge from an exaggerated T-helper (Th)1 response upon presentation of an unidentified antigen by dendritic cells (DCs).Myeloid DC in bronchoalveolar lavage (BAL) from sarcoidosis patients were increased in number and maturation status, providing evidence for local DC activation in pulmonary sarcoidosis. This was supported by the finding of increased numbers of mature DCs in granuloma containing airway mucosal biopsies. Because IL-17A has recently been implicated in granuloma formation in various diseases, including tuberculosis, we hypothesized that besides Th1 cells, also Th17 cells may have a role in sarcoidosis.
Methods: We used intracellular flow cytometry for blood and BAL samples and immunohistochemistry for bronchial biopsies to identify Th17 cells.
Results: We found that memory CD4+ T cell populations in peripheral blood of sarcoidosis patients contained significantly increased proportions of IL17A+ cells, when compared with healthy controls. Interestingly, proportions of IL17A/IFNγ+ and IL17A/IL-4+ double-producing T cells, which are normally very rare, were significantly increased in the circulation of sarcoidosis patients and were present in substantial numbers in the BAL. In this context, it is of note that in autoimmune conditions IL-17A/IFNγ+ CD4+ T cells have been shown to be more pathogenic than IL-17A single producers. In addition, we found increased regulatory T cell numbers , but with impaired survival in vitro, suggesting diminished immune suppression. Immunohistochemical analyses revealed a significant increase in the numbers of IL17A+ cells, located in and around granuloma throughout the lamina propria. These cells were not detected in non-granulomatous sarcoidosis biopsies. IL-22+ cells were increased in the subepithelial layer.
Conclusions: The enhanced IL-17A expression in granulomas and the presence of IL-17A+, IL-17A/IFNγ+ and IL-17A/IL-4+ memory T cells in the circulation and the BAL provides evidence for Th17 cell involvement in granuloma induction or maintenance.

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O: Oral presentations

O5: Impaired gas exchange, exercise capacity, and muscle strength in sarcoidosis patients

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Background: Recently we found that beside fatigue reduced exercise intolerance (reduced six-minute walk test) and muscle weakness are frequent problems in sarcoidosis. The aim of this study was to evaluate the additional value of cardio pulmonary exercise testing to depict impaired gas exchange and exercise limitation.
Methods: The clinical records of 160 (age: 41.3±10.0; female 63) sarcoidosis patients referred to the ild care center of the MUMC during a 3 year period were reviewed. All performed a symptom limited incremental exercise test (10W/min) on an electronically braked cycle ergometer from which peak work rate and maximal oxygen uptake (VO2 max) were determined. Patients also performed hand grip and inspiratory muscle strength tests.
Results: The diffusing capacity for carbon monoxide (DLCO; mean of 83.2±18.0% of predicted) was decreased (<80%) in 61 cases (38.1%). In the cases with a normal DLCO ≥ 80% (n=99; 61.9%), a PA-a,O2 at maximal exercise was moderate increased (>2.5 kPa) in 69 cases (69.7%), and obviously impaired (>4.7 kPa) in 18 cases (18.2%). The VO2 max was reduced (<80%) in 44 cases (44.4%), Watt% (<80%) in 52 cases (52.5%). In this group the hand grip force and inspiratory muscle strength were both reduced in 43 cases (43.4%).
Conclusion: A normal DLCO does not exclude impairment of gas exchange and exercise limitation. Moreover, the DLCO cannot reliably predict exercise performance in an individual person with sarcoidosis. Testing the response of sarcoidosis patients to exercise can enhance the objective basis of the severity of respiratory functional impairment and impairment of gas exchange.

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O6: The clinical course of sarcoidosis: Description and treatment response in a large American cohort

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Background: There is relatively minimal clinical information concerning the clinical course of sarcoidosis in Americans. We review the characteristics of a large cohort of sarcoidosis patients cared for at major USA sarcoidosis center over a 12 year period. Methods: Clinical information was obtained from an institution-approved clinical database. This study was approved by the MUSC institutional review board. Definitions of organ involvement were based on the ACCESS organ assessment instrument (SVDLD 1999; 16:75-86). Results: This analysis covered 12 years from 1999 through 2010. Over this period, 1774 unique sarcoidosis patients were identified in the database. Not all patients had complete data entered into the database, therefore several categories do not reach the sum of 1774. 91% (1176/1298) were biopsy-confirmed. 66% (818/1247) of the patients were black and 34% (429/1247) were white. The average length of follow-up was 42.2 months (SD=39.3). 61% (1083/1774) were on some anti-sarcoidosis medication at some time. 53% (981/1774) were on corticosteroids, 12% (213/1774) were on methotrexate, and 11% (192/1774) were on an antimalarial at some time. The mean daily prednisone-equivalent dose lowered from 14 mg to 9 mg over the first two years of follow-up. The lung was the most common organ involved (89%, 1403/1582) followed by skin (26%, 522/1582), eye (23%, 363/1582), and liver (20%, 323/1582). For specific organs, cardiac sarcoidosis required treatment in the highest percentage of cases (62%, 46/74) followed by muscle (58%, 11/18), neurosarcoidosis (56%, 78/139), and lung (51%, 712/1403). Blacks required anti-sarcoidosis treatment at some time more frequently than whites (73%, 594/819 versus 52%, 225/429). The average number of organs involved was 2.48 for blacks (N=819) and 2.06 for whites (N=429). Over the first five years of follow-up, the number of organs involved per patient increased from 2.19 to 2.88.
Conclusions: This analysis of the characteristics of patients seen in a large USA referral sarcoidosis clinic may give some insights into the phenotypic expression, diagnostic features, therapeutic approach, and outcome of this disease.

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O7: Imbalance between circulating endothelial cells and endothelial progenitors in idiopathic pulmonary fibrosis

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Background: Fibrogenesis during idiopathic pulmonary fibrosis (IPF) is associated with abnormal vascular remodeling. Respective abundance of circulating endothelial cells (CEC) and endothelial progenitor cells (EPC) might reflect the balance between vascular injury and repair and potentially serve as a biomarker of the disease.
Objectives: We postulated that CEC and all EPC subtypes might be differently modulated in IPF. We aimed at 1) assessing them in early stages of IPF and 2) searching for correlations with disease severity.
Methods: 64 consecutive patients with newly diagnosed IPF and 10 healthy age-matched volunteers were studied. CEC were isolated with CD146-coated beads. CD34, CD133 and KDR antigens, characterizing EPC, were assessed through flow cytometry. EPC (early CFU-Hill and late endothelial cells forming colonies (ECFC)) were also counted using cell culture.
Results: CEC numbers were significantly increased in IPF (p=0.004) whereas EPC assessed using both flow cytometry (CD34+KDR+) and cell culture were decreased vs controls (p<0.05). CEC did not differ according to disease severity (DLCO > or < 40%) nor did CD34+KDR+ cells. In contrast, progenitors obtained in culture were markedly increased in the most severe vs the least severe IPF subgroup (p=0.04 and p=0.01 for CFU-Hill and ECFC, respectively, for DLCO<40% vs >40%). ECFC was the only cell type found to be correlated to DLCO (Spearman correlation test, p=0.04).
Conclusion: IPF is associated with markers of vascular injury and with a global decrease in EPC. Disease severity is associated with an EPC mobilization whose mechanisms and clinical impact need to be explored.

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O8: Mutations in SFTPC, SFTPA2 and TERT explain 60% of Familial Pulmonary Fibrosis and correlate to specific disease phenotypes

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Background: Idiopathic Pulmonary Fibrosis (IPF) is a fatal lung disease, histologically characterized by diffuse interstitial remodelling and patchy inflammation. A significant percentage of IPF patients have a familial form of the disease. Separate reports have identified mutations in Surfactant Protein-C (SFTPC), Surfactant Protein-A2 (SFTPA2), Telomerase Reverse Transcriptase (TERT) or Telomerase RNA component (TERC) in these families.
Methods: We determined the frequency of mutations in SFTPC, SFTPA2, TERT and TERC in 20 families with Familial Pulmonary Fibrosis (FPF).
Results: Heterozygous non-tolerated sequence changes were detected in 12 out of 20 patients, consisting of 5 SFTPC, 2 SFTPA2 and 5 TERT mutations. Mutations segregated with disease in each family and haplotype analysis showed that identical mutations had arisen independently. Families with SFTPC and SFTPA2 mutations always had evidence of parent-offspring disease transmission, while in families with TERT mutation sibs were affected. Pediatric pulmonary disease occurred only in families with SFTPC mutations. Carriers of an SFTPA2 mutation also suffered from lung cancer. Families with a TERT mutation usually presented as typical IPF and did not show clear symptoms associated with other known syndromes of telomere shortening.
Conclusions: This is the first report of a cohort of IPF families that is completely sequenced for candidate genes. We could identify a mutation in 60% of patients with FPF. These mutations correlated with a specific disease phenotype. The function of each of the mutated genes is very different, but all indicate towards a central role for the alveolar type II cell in disease pathogenesis.

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