# 49. Sarcoidosis

**168**

**Methotrexate vs azathioprine in chronic sarcoidosis**

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**Background:** Although steroids remain the mainstay of therapy in sarcoidosis, chronic use is associated with toxicity. Evidence is missing on which second line therapy is most appropriate.
**Aim:** To compare the effect of methotrexate (MTX) and azathriopine (AZA) in second line treatment of chronic sarcoidosis patients regarding steroid use, lung function and side effects.
**Methods:** This is a retrospective cohort study, reviewing all patients who started MTX or AZA in two Dutch/Belgian tertiary referral centres. Demographic data, steroid use, lung function tests and side effects were noted from initiation until 2 years after or discontinuation. Treatment effect was calculated with a linear mixed model with FEV1, VC, DLCO and prednisone dose changes over time as endpoints. Differences in side effects were calculated with $\chi$2-tests.
**Results:** 200 patients were included, 145 received MTX and 55 received AZA. Prednisone daily dose decreased with 6.32 mg/year ($p<0.0001$) while on therapy, with no difference between MTX and AZA. FEV1 showed a mean increase of 52 ml/year ($p=0.006$) and VC of 95 ml/year ($p=0.001$), with no difference between drugs for both. DLCO (% predicted) increased with a mean of 1.23%/year ($p=0.018$). Mean DLCO was 5.12% lower in the AZA group ($p=0.05$), but this difference was constant over time. There were significantly more patients with infections in the AZA group (26.5 vs 16.0% $p=0.01$). No significant differences were found regarding other side effects.
**Conclusions:** This is the first study comparing the effect of MTX and AZA in sarcoidosis treatment. Although more infections occurred in the AZA group, this study shows both drugs were equally effective in terms of lung function improvement and had a significant steroid sparing effect.

**169**

**Concentration of sFas in bronchoalveolar lavage fluid of smoking patients with sarcoidosis**

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Sarcoidosis (SA) is a multiorgan granulomatous disease of unknown etiology. SA is more prevalent among non-smokers. The postulated protective role of smoking on inflammation in SA remains enigmatic. Fas/FasL system is assumed to participate in the regulation of immune response and granuloma formation in SA. Soluble Fas (sFas) is known to inhibit Fas-induced apoptosis. In previous study we reported an elevated number of Fas positive cells in the bronchoalveolar lavage fluid (BALf) of smoking sarcoidosis patients.
The aim of this study was to find out whether sFas concentration in BALf differs between ever smoking (S) and never smoking (NS) patients with sarcoidosis.
We investigated 57 patients with confirmed SA: 36 NS and 21 S. Total and differential cell count in the BALf samples were performed according to standard methods. The sFas concentration was measured by ELISA.
There were significant differences in BALf composition between S and NS. The total cell count and the percentage of macrophages were significantly higher among S than among NS (11.3 vs. 6.5 x$10^6$, 62 vs. 50%, respectively), while the percentage of lymphocytes was significantly lower among S than among NS (29 vs. 41%). The sFas concentration was lower in the BALf of S compared with NS (median values 68.3 vs 95.5 pg/mL, $p=0.09$). Furthermore, the sFas concentration among active smokers was significantly lower than among NS (65.6 vs 95.5 pg/mL, $p=0.01$).
We found out that sFas concentration is reduced in the BALf of smoking sarcoidosis patient. Lower sFas concentration may result in higher apoptosis rate of inflammatory cells, thereby promoting granulomatous inflammation resolution.

**170**

**Association study of *ANXA11*-R230C with sarcoidosis**

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**Introduction:** Recently, a genome wide association study demonstrates that the polymorphism *ANXA11*-R230C (rs 1049550) is strongly associated with sarcoidosis, a chronic granulomatous disease (GD) of unknown etiology.
**Aim:** Considering that also other diseases are accompanied by granulomas formation, as observed in 10% of patients with common variable immunodeficiency (CVID), the purpose of this study was to confirm the association of *ANXA11*-R230C with sarcoidosis and to examine its possible association with GD in CVID.
**Methods:** DNA was extracted from peripheral blood of 71 patients with sarcoidosis (M/F:24/47, mean age: 52.5 years, range:20-75), 101 normal individuals (M/F:35/66, mean age: 54.1 years, range:19-76), and 19 CVID patients (M/F:7/12, mean age: 28.1 years, range: 2-60), two of which had granulomatous disease (one with familial CVID history including a sister without GD). A PCR-RFLP protocol was designed to detect *ANXA11*-R230C and statistical analysis was performed using the SPSS software (ver.-10.0).
**Results:** The difference in the allele frequency of *ANXA11*-R230C between patients with sarcoidosis and healthy individuals was not marginally reached to be significant ($p=0.073$). The allele frequency of *ANXA11*-R230C in CVID patients was 50.0% and no significant difference between patients with and without GD was observed. However, in the family with CVID, the member with GD carried only the R alleles, associated with granulomas formation, while her sister was heterozygous.
**Conclusions:** The association of *ANXA11*-R230C with sarcoidosis was not confirmed, while the emerged contribution of this polymorphism in the granoulomas formation in CVID needs to be further clarified.

**171**

**HLA-DRB1 allele distributions in Danish sarcoidosis patients. Increased prevalence of HLA-DRB1*15**

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**Background:** The MHC class II region appears to play an important role in the development and clinical picture of sarcoidosis, probably due to the influence on the immune response against yet unknown antigen(s). A previous HLA-class II study in Danes has shown that HLA-DR3 antigen is associated with a favorable prognosis and HLA-DRw6 with susceptibility to sarcoidosis (Ødum et al, Exp Clin Immunogenet 1991;8:227-321).
**Subjects and methods:** HLA-DRB1 allele classification was performed by PCR in ethnic Danish patients with biopsy-verified sarcoidosis (n=94). Danish blood donors (n=10146) served as controls.
**Results:** The prevalence of DRB1*01 was lower and the prevalence of DRB1*15 was higher in sarcoidosis patients compared to controls.

HLA-DRB1 allele frequencies

| DRB1*-allele | Sarcoidosis (n=94) | | Controls (n=10146) | | Chi-square test |
|---|---|---|---|---|---|
| | n | frequency (%) | n | frequency (%) | p-value |
| 01 | 7 | 7.4 | 2048 | 20.2 | 0.0033 |
| 03 | 23 | 24.5 | 2563 | 25.3 | 0.95 |
| 04 | 29 | 30.9 | 3276 | 32.3 | 0.85 |
| 07 | 20 | 21.3 | 2111 | 20.8 | 0.91 |
| 08 | 7 | 7.4 | 705 | 6.9 | 0.85 |
| 09 | 1 | 1.1 | 215 | 2.1 | 0.73 |
| 10 | 1 | 1.1 | 155 | 1.5 | 0.71 |
| 11 | 14 | 14.9 | 1373 | 13.5 | 0.82 |
| 12 | 8 | 8.5 | 475 | 4.7 | 0.13 |
| 13 | 19 | 20.2 | 2522 | 24.9 | 0.36 |
| 14 | 3 | 3.2 | 441 | 4.3 | 0.77 |
| 15 | 42 | 44.7 | 2945 | 29 | 0.0013 |
| 16 | 1 | 1.1 | 148 | 1.5 | 0.75 |

**Conclusions:** There exist no specific biochemical markers of sarcoidosis. Diagnosis relies on histological examination of tissue biopsy specimens in combination with the exclusion of infection with mycobacteria and other causes of the granulomatous response. Due to the paucity of prognostic markers, we suggest that analysis of HLA-DRB1 (and BTNL2 genotype (Milman et al, Clin Respir J 2011;5:105-11)) should be incorporated in the evaluation of sarcoidosis patients.

**172**

**Whole-body magnetic resonance imaging in sarcoidosis to assess extrapulmonary organ involvement**

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**Introduction:** Sarcoidosis is a systemic inflammatory disorder that may affect any organ of the body. There is no valid tool to assess the extent of extrapulmonary organ involvement. Whole-body magnetic resonance imaging (MRI) might be a promising modality to detect extrapulmonary disease activity.
**Aim:** To assess the validity of whole-body MRI with regard to extrapulmonary disease activity in patients with sarcoidosis.
**Methods:** 24 consecutive patients at the Clinic of Respiratory Medicine, University Hospital Basel, Switzerland, with histologically-confirmed sarcoidosis were prospectively included. All patients underwent whole-body MRI. Pulmonary function tests and the *extrapulmonary physician organ severity tool* (ePOST) were assessed for each patient.
**Results:** In total 9/24 (38%) patients showed findings of probable or possible sarcoidal origin. 5/24 (21%) showed skeletal lesions, 3/24 (13%) had muscular findings and 1/24 (4%) had enhancement of the cauda equina. ePOST score was significantly higher in those 9 patients with abnormal whole-body MRI-findings (17.3) than in those with normal images (10.6). FVC percentage predicted (%P), TLC,%P, and DLCO,%P were significantly lower in those patients with abnormal skeletal enhancement compared to those without skeletal abnormalities.
**Conclusions:** Whole-body MRI depicted manifestations of extrapulmonary sarcoidosis in 38% of cases in an unselected patient sample. Abnormal whole-body MRI findings correlated with high ePOST scores, and might thus be a valid tool to assess extrapulmonary disease activity. Abnormal skeletal findings correlated with decreased lung volumes, and might therefore be a marker of total disease activity.

**173**

**HLA-DPB1 and chronic sarcoidosis**

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**Background:** Beryllium disease and sarcoidosis have clinical similarities. Beryllium exposure together with the HLA-DPB1 alleles containing a glutamic acid at amino acid position 69 ($Glu^{69}$) is associated with beryllium sensitization and chronic beryllium disease (CBD).
**Aim:** To determinate whether the same genetic variations of HLA-DPB1 that are found in CBD are associated with chronic sarcoidosis.
**Methods:** HLA-DPB1 was determined in 98 Finnish patients with chronic sarcoidosis not resolved within 2 years and in 150 control subjects. The DPB1 alleles were genotyped with sequence specific primers (Olerup SSP™) and haplotypes were formed with MHC class II and class III markers.
**Results:** 17 different DPB1 alleles were observed. The DPB1*04:02 allele was less frequent among the patients (10.2% vs.20.3%; p=0.003, OR=0.45) than controls. A haplotype with DPB1*04:01, one C4A gene and one C4B gene was increased among sarcoidosis patients (30.9% vs. 22.5%; p=0.036, OR=1.5). By studying polymorphic amino acid residues of DPB1, we did not detect an association of $Glu^{69}$, but a DPB1*04:02 specific amino acid variant was detected at the position 178 (Met) suggesting a protective role for chronic sarcoidosis (p=0.004, OR=0.42). Furthermore, preliminary SNP analyses of HLA class II and III region showed that the SNP associations are independent from DPB1.
**Conclusion:** We confirm and further describe the contributory role of DPB1 with the risk associated for chronic sarcoidosis. Preliminary results suggest that both DRB1 and certain independent markers in the HLA class II and III region increase the risk for chronic sarcoidosis. However, HLA association analyses are complicated by the extensive linkage disequilibrium (LD) across the HLA region.

**174**

**Evaluation of ANXA11 rs1049550 SNP linkage with sarcoidosis susceptibility**

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**Introduction:** A recent genome-wide association detected a strong association between Annexin A11 (*ANXA11*) polymorphisms and sarcoidosis susceptibility. *ANXA11* take part in several biological pathways, namely apoptosis and proliferation.
**Aim:** Evaluation of *ANXA11* rs1049550 SNP association with sarcoidosis susceptibility in a Portuguese population and after stratification of clinically distinct disease presentations.
**Methods:** A case-control study included 136 unrelated patients (37.7±12.6 years, 58.8% women) and 92 healthy controls (32.3±7.0 years, 62% women). Allele frequencies were compared with Chi-square (or Fisher exact test when appropriate) and genotype frequencies with Chi-square for trend test. Odds ratios (OR) and 95% CIs were calculated as association measures. Samples were genotyped for *ANXA11* rs1049550 C/T (R230C) polymorphism using real time PCR with TaqMan SNP genotyping assay.
**Results:** The frequency of *ANXA11* rs1049550 (R230C)*T allele was significantly lower in sarcoidosis patients (33.1%) compared with controls (46.2%, p<0.01, OR=0.58, 95%CIs=]0.38;0.86[). An OR=0.44 and OR=0.4 for sarcoidosis was obtained respectively, in the carriers of one (genotype *ANXA11* CT) and two (genotype *ANXA11* TT) copies of *ANXA11* rs1049550*T allele normalised to the CC wild type genotype (p<0.01). When patients with erythema nodosum (EN) were removed, this association persists only in the group without EN. There was no significant difference among radiological Scadding stages.
**Conclusions:** In this population an association between *ANXA11* rs1049550*T SNP and protection to sarcoidosis was observed, confirming previous data in populations from different geographic regions, but only in those patients without EN.

**175**

**Bosentan for sarcoidosis associated pulmonary arterial hypertension (BoSAPH) was effective in advanced parenchymal lung disease**

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**Introduction:** We reported significant improvement in pulmonary artery mean (PAM) and pulmonary vascular resistance (PVR) for those treated with 16 weeks (wks) of bosentan (BOS) versus no change in those treated with placebo (PLA) for sarcoidosis associated pulmonary arterial hypertension (SAPH).
**Purpose of the study:** To determine whether patients with advanced parenchymal disease were less likely to respond to BOS.
**Methods:** Patients with SAPH confirmed by right heart catheterization (RHC) were randomized 2:1 to receive either BOS or PLA. After 16 wks of therapy, patients underwent repeat RHC. Patients had forced vital capacity (FVC) and Scadding chest x-ray (CXR) stage.
**Results:** Of the 25 patients treated with BOS, 2 stopped study drug before wk 8. At 16 wks, 21had repeated RHC. Patients were subgrouped into those with FVC% ≥70% predicted (FVC ≥70%) or below 70% (FVC ≤70%) and those with CXR stage 0-3 (CXR<4) versus stage 4 (CXR=4). The table demonstrates the changes in PAM and PVR. There was significant improvement in PAM and PVR for those with FVC <70% but was not for those with FVC ≥70%. No changes were seen for the PLA group.

PAM and PVR

| | Number | Wk 0 PAM | Wk 16 PAM | Wk 0 PVR | Wk 16 PVR |
|---|---|---|---|---|---|
| FVC>70% | 7 | 35+7.9 | 36+6.7 | 6.7+2.81 | 4.9+1.95 |
| FVC<70% | 14 | 36+6.6 | 32+10.0 * | 5.5+3.01 | 4.1+2.08 * |
| CXR Stage 1-3 | 9 | 37+6.0 | 35+6.5 | 6.8+4.10 | 4.4+2.00 * |
| CXR Stage 4 | 12 | 35+7.6 | 30+10.1 * | 5.1+1.34 | 4.4+2.14 |

Mean+S.D., *P<0.05.

**Conclusion:** After 16 wks of treatment, bosentan therapy was associated with a significant improvement in PAM and PVR. These changes remained significant for patients with an FVC<70% predicted. SAPH patients with advanced parenchymal lung disease may respond to vasodilatory therapy.