463. Biomarkers in sarcoidosis

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The role of vitamin D3 in the local inflammatory process in sarcoidosis (SA)

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Background: SA is an inflammatory disease characterized by granulomas that can produce 1.25(OH)2D hormone. No reports are available in the literature on the role of vitamin D3 derivatives in the local inflammation.
Aims: To assess the role of vitamin D3 derivatives in the local inflammatory process in SA based on the determination of their concentration in the bronchoalveolar lavage fluid (BALF) as well as in exhaled breath condensate (EBC).
Material and Methods: In 108 pts (48 women, 60 men; (mean±SD) age 44.3±9.5 yrs, BMI=27.5±4.8) with SA stage I-IV we measured 1.25(OH)2D (pg/m), 25(OH)D (ng/ml) in serum, EBC and BALF.
Results: Results are shown below:

Table 1

Concentration of vitamin D3	Mean value ± SD
25D serum	15.5±8.1
1.25D serum	54.2±22.8
25D BALF	7.4±1.4
1.25D BALF	8.9±3.1
25D EBC	7.7±3.8
1.25D EBC	8.5±3.1
LD serum	4.5±3.1

LD = 1.25(OH)2D/25OHD.

Table 2

Correlations of vitamin D3 concentration to	R	p
1.25D serum/macrophage rate in BALF	0.35	0.001
1.25D serum/lymphocyte rate in BALF	–0.33	0.002
1.25D BALF/neutrophil rate in BALF	–0.21	0.047
1.25D EBC/neutrophil rate in BALF	–0.22	0.039
25D serum/CD4 lymphocyte count in BALF	–0.28	0.013
LD serum/cell count in BALF	0.24	0.025

LD = 1.25(OH)2D/25OHD.

No correlation was found between 25OHD or 1.25(OH)2D and CD8 lymphocyte count or BALF CD4/CD8.
Conclusions: 1. It is possible to measure vitamin D3 derivates concentration in BALF and EBC. 2. Many correlations found between macrophage, lymphocyte and neutrophil counts in BALF and concentration of 1.25D and 25D in serum, BALF and EBC suggest an important role of vitamin D derivates in local and systemic inflammatory process in SA.
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Vitamin D3 and markers of systemic inflammation in sarcoidosis (SA)

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Background: SA is an inflammatory disease characterized by granulomas that can produce 1.25(OH)2D hormone.
Aims: To assess the relationship between vitamin D3 derivatives concentration in serum and inflammatory markers in SA.
Material and Methods: In 108 pts (48 women, 60 men; (mean±SD) age 44.3±9.5 yrs, BMI=27.5±4.8) with SA stage I-IV we measured 1.25(OH)2D (pg/m), 25(OH)D (ng/ml) in serum and BALF as well as leukocytes, lymphocyte phenotypes CD4/CD8, alkaline phosphatase (ALP), ACE, D-dimer, fibrinogen, albumin and gammaglobulin, phosphorate in serum and 24h calcium urine excretion.
Results: Results are shown below:

Correlations of vitamin D3 concentration to	R	p
25D serum/ALP	–0.22	0.049
1.25D serum/phosphorate	–0.26	0.02
25D serum/albumin	0.30	0.005
LD serum/albumin	–0.21	0.055
LD serum/leukocytes	0.24	0.031
LD serum/ACE	0.20	0.085
25D serum/D-dimer	–0.33	0.002
25D serum/CRP	–0.21	0.051
25D serum/CD4 lymphocyte count in BALF	–0.28	0.013
1.25D serum/24h calcium urine excretion	0.26	0.023

LD = 1.25(OH)2D/25OHD.

There was no other significant correlation between 25OHD or 1.25(OH)2D and other evaluated inflammatory markers.
Conclusions: Significant correlations between some inflammatory markers and 25OHD or 1.25(OH)2D concentration in serum may suggests a role of vitamin D3 as an indicators of inflammatory process in SA.
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Polymorphisms in CCR5 confer susceptibility to Löfgren's syndrome and may regulate the immune response

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Rationale: 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: Hunderd 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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Chitotriosidase: A sensitive biomarker of sarcoidosis

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Chitotriosidase is a member of family of glycosylhydrolases, enzymes involved

in the degradation of chitin and chitin-like substrate, identified in a wide variety of organisms. Increased concentrations of chitotriosidase have been reported in several lysosomal storage diseases and more recently also in sarcoidosis.
In this study chitotriosidase concentrations were evaluated in a population of 233 sarcoidosis patients and 70 controls in order to verify enzyme specificity and sensibility and to evaluate chitotriosidase prognostic meaning.
Chitotriosidase has been found significantly increased in serum of patients with sarcoidosis than in controls ($p < 0.0001$). ROC curve analysis revealed: cut-off value of 39.50 nmol/h/ml, sensitivity 89,70% and specifity 90%. The analysis of chitotriosidase in different phenotypic subgroups of patients revealed very high serum enzyme levels in symptomatic patients requiring systemic steroid therapy at onset and after disease relapses.
In conclusion as a new potential biomarker of sarcoidosis severity, chitotriosidase resulted sensitive, reproducible and easily detectable in serum.

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Mycobacterial heat shock protein 16 kDa, marker of dormant stage of mycobacteria, in precipitated circulating immune complexes in sarcoidosis

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M. tuberculosis antigens, e.g., heat shock proteins (Mtb-hsp), genetic factor and autoimmunity have been explored as potential causes of sarcoidosis (SA). Mtb-hsp inducing both cellular and humoral immune response may provide a link between infection and autoimmunity. We have recently demonstrated the presence of Mtb-hsp70, Mtb-hsp65 and Mtb-hsp16 in sarcoid tissue. Higher occurrence of serum Mtb-hsp70 than Mtb-hsp65 and Mtb-hsp16 in SA patients could be caused by sequestration of Mtb-hsp65 and Mtb-hsp16 in circulating immune complexes (CIs). To test this hypothesis, we have evaluated and quantified Mtb-hsp70, Mtb-hsp65 and Mtb-hsp16 in precipitated CIs from blood of 20 patients with SA, 19 patients with active tuberculosis (TB) and 21 healthy volunteers using PEG precipitation and Western Blot. The results showed significantly increased CIs levels in SA vs TB and Control, whereas there was no difference between TB and healthy individuals. The Mtb-hsp16, Mtb-hsp65 and Mtb-hsp70 concentrations in precipitated CIs were significantly higher in SA than in TB and Control, but there was no difference between TB and Control. In all tested groups, the Mtb-hsp16 concentration was significantly increased than Mtb-hsp70 and Mtb-hsp65. In summary, our results show increased presence 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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Use of discriminant analysis to assess pulmonary functional worsening in patients with sarcoidosis by means of a panel of inflammatory markers

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Background: Sarcoidosis' protean clinical course has prompted many studies to discover biomarkers which could help to trace disease progression and response to therapy.
Aims: In our study we performed discriminant analysis, to investigate whether a panel of selected markers measured in BALF and serum from patients with sarcoidosis would help to predict pulmonary functional worsening.
Methods: We enrolled in the study 30 consecutive individuals with sarcoidosis. At enrolment participants underwent pulmonary function tests, fiber-optic bronchoscopy and radiological evaluation. PFTs were also performed at follow-up visits during a 2 year period. Pulmonary function worsening was defined as a decline of TLC, FVC, FEV1> 15% and DLCO > 10%. BALF differential cell counts were performed in all participants and BAL and serum ECP, MPO, Tryptase, PIIIP and SIL2r concentrations were quantified by RIA and ELISA tests. Discriminant analysis was performed to optimize the accuracy of selected variables in predicting functional worsening.
Results: Pulmonary function worsening was observed in 24% of participants. Applying discriminant analysis function a high classification rate was obtained.The following formula: CV= PMNBAL x 0.18 +ECPBAL x 1.20 -MPOBAL x 0.03 +TryptaseBAL x 1.21- PIIIPBAL x0.1 -sIL2RBAL x0.01-1.183, allowed the correct allocation of 100% of participants. The positive likelihood ratio was >20 and the negative likelihood ratio was 0.
Conclusions: Our results show that a panel of BAL markers may be used to distinguish patients with stable disease from individuals with pulmonary function worsening and may help to decide therapeutic strategies.

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Chronic fatigue in sarcoidosis-in-clinical-remission: Psychological and physical characteristics

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When sarcoidosis is in clinical remission, complaints of chronic fatigue often persist. The exact features of this post-inflammatory fatigue are unknown. This study assesses the severity of fatigue and the presence of fatigue-related symptoms in sarcoidosis-in-clinical-remission. Furthermore, we evaluate 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.
Methods: Seventy-five patients with sarcoidosis-in-clinical-remission were evaluated with the Checklist Individual Strength (fatigue), the SymptomChecklist-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 and reduced muscle strength. Scores on sleep quality were normal. Response at follow-up was 87%. 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.