463. Biomarkers in sarcoidosis

The role of vitamin D3 in the local inflammatory process in sarcoidosis (SA)
Anna Kowalska1, Elzbieta Pascinska1, Anna Gollan-Guremek 1,
Agnieszka Skoczylas1, Justyna Czerniawska1, Adriana Roz1, Paulina Jagus1,
Joanna Chorostowska-Wynimko2, Dorota Górecka 1.
12nd Department of Respiratory Medicine, Institute of TB & Lung Diseases, Warsaw, Poland;
2Laboratory of Molecular Diagnostics and Immunology, Institute of TB & Lung Diseases, Warsaw, Poland

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-V we measured 1.25(OH)2D (pg/ml), 25(OH)D (ng/ml) in serum, BALF and EBC.

Results: Results are shown below:

Table 1

<table>
<thead>
<tr>
<th>Concentration of vitamin D3</th>
<th>Mean value ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>25D serum</td>
<td>15.5±8.1</td>
</tr>
<tr>
<td>1.25D serum</td>
<td>54.2±22.8</td>
</tr>
<tr>
<td>25D BALF</td>
<td>7.4±1.4</td>
</tr>
<tr>
<td>1.25D BALF</td>
<td>8.9±3.1</td>
</tr>
<tr>
<td>25D EBC</td>
<td>7.7±3.8</td>
</tr>
<tr>
<td>1.25D EBC</td>
<td>8.5±3.1</td>
</tr>
<tr>
<td>LD serum</td>
<td>4.5±2.1</td>
</tr>
</tbody>
</table>

LD = 1.25(OH)2D/25OHD

Table 2

<table>
<thead>
<tr>
<th>Correlations of vitamin D3 concentration</th>
<th>R</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>25D serum/macrophage rate in BALF</td>
<td>0.35</td>
<td>0.001</td>
</tr>
<tr>
<td>1.25D serum/macrophage rate in BALF</td>
<td>-0.33</td>
<td>0.002</td>
</tr>
<tr>
<td>1.25D serum/macrophage rate in BALF</td>
<td>-0.21</td>
<td>0.047</td>
</tr>
<tr>
<td>1.25D serum/macrophage rate in BALF</td>
<td>-0.22</td>
<td>0.039</td>
</tr>
<tr>
<td>25D serum/CD4 lymphocyte count in BALF</td>
<td>-0.28</td>
<td>0.013</td>
</tr>
<tr>
<td>LD serum/cell count in BALF</td>
<td>0.24</td>
<td>0.025</td>
</tr>
</tbody>
</table>

LD = 1.25(OH)2D/25OHD

4515

4516

Vitamin D3 and markers of systemic inflammation in sarcoidosis (SA)

Anna Kowalska1, Elzbieta Pascinska1, Anna Gollan-Guremek 1,
Agnieszka Skoczylas1, Justyna Czerniawska1, Adriana Roz1, Paulina Jagus1,
Joanna Chorostowska-Wynimko2, Dorota Górecka 1.
12nd Department of Respiratory Medicine, Institute of TB & Lung Diseases, Warsaw, Poland;
2Laboratory of Molecular Diagnostics and Immunology, Institute of TB & Lung Diseases, Warsaw, Poland

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-V we measured 1.25(OH)2D (pg/ml), 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, phosphate 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/ALP -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 25OH D or 1.25OH2D and other evaluated inflammatory markers.

Conclusions: Significant correlations between some inflammatory markers and 25OH D or 1.25OH2D concentration in serum may suggest a role of vitamin D3 as an indicators of inflammatory process in SA.

Supported by: Polish Ministry of Science Grant No 1594/P01/2007/32

4517

Polymorphisms in CCR5 confer susceptibility to Löfgren’s syndrome and may regulate the immune response

B. Karakaya1, C.H.M. van Moorsel 1, G.T. Rijkers2, A.H.M. van der Helm 3,
T.W.J. Huizinga1, J.M.M. van den Bosch1, J.C. Grutters1.
1Department of Pulmonology, Center for Interstitial Lung Disease, St Antonius Hospital, Nieuwegein, Netherlands; 2Department of Medical Microbiology and Immunology, St Antonius Hospital, Nieuwegein, Netherlands; 3Department of Rheumatology, Leiden University Medical Center, Leiden, Netherlands

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. 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.

Results: Carriage of the G-allele was significantly higher in patients with Löfgren’s syndrome than in healthy controls (p=0.0057, 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 were 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.

4518

Chitotriosidase: A sensitive biomarker of sarcoidosis

Elena Bargagli, David Bennett, Claudia Maggiorelli, Maria Grazia Pieroni, Rosa Metella Refini, Maria Grazia Perari, Paola Rottoli.
Department of Respiratory Diseases, Section of Pulmonology, Center for Interstitial Lung Disease, St Antonius Hospital, Nieuwegein, Nieuwegein, Netherlands; 2Department of Medical Microbiology and Immunology, St Antonius Hospital, Nieuwegein, Netherlands; 3Department of Rheumatology, Leiden University Medical Center, Leiden, Netherlands

Chitotriosidase is a member of a family of glycosylhydrolases, enzymes involved
in the degradation of chitin and chitin-like substrate, identified in a wide variety of organisms. Increased concentrations of chitinase have been reported in several lysosomal storage diseases and more recently also in sarcoidosis. In this study chitinase concentrations were evaluated in a population of 233 sarcoidosis patients and 70 controls in order to verify enzyme specificity and sensitivity and to evaluate chitinase prognostic meaning. Chitinase 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 mmol/1/h/ml, sensitivity 89.70% and specificity 90%. The analysis of chitinase 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, chitinase resulted sensitive, reproducible and easily detectable in serum.

4519 Mycobacterial heat shock protein 16 kDa, marker of dormant stage of mycobacteria, in precipitated circulating immune complexes in sarcoidosis

Anna Dabanievic1, Adam Holowna2, Mahavir Singh3, 1Department of Pulmonology, Medical University of Gdańsk, Gdańsk, Poland; 2Department of Clinical Pharmacology, Medical University of Białystok, Białystok, Poland; 3Department of Genome Analysis, Helmholtz Center for Infection Research and LEIENEX Diagnostics & Therapeutics GmbH, Braunschweig, Germany

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, Mtb-hsp16 in sampled 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 in autoimmune response in SA related to stationary-phase of M. tuberculosis.

4520 Use of discriminant analysis to assess pulmonary functional worsening in patients with sarcoidosis by means of a panel of inflammatory markers

Gregoriana Fauci1, Maria Rutili1, Alessandro Belli1, Armanda Propoli1, Angelo Strocchia2, Salvatore D’Antonio2, Alfredo Sebastiani3, Giovanni Puglisi3, Giovanni Galluccio1, Sandro Batella1, 1Department of Cardiovascular, Respiratory and Morphologic Sciences, University “La Sapienza”, S. Camillo-Forlanini Hospital, Rome, Italy; 2RCS Fondazione Don Carlo Gnocchi - Onlus, Don Gnocchi, Milan, Italy; 3Department of Respiratory Diseases, S. Camillo-Forlanini Hospital, Rome, Italy

Background: Sarcoidosis’ protein clinical course has prompted many studies to discover biomarkers which could help to track 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 functional 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, PIIP and SII 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: C= PMNLx 0.18 +ECPBAL x 1.20-MP0BAL x 0.03 +TryptaseBAL x 1.21-PIIPBAL x 0.20-SII2RBAL x 0.01-1.183, 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.

4521 Chronic fatigue in sarcoidosis-in-clinical-remission: Psychological and physical characteristics

Ingrid Korenromp1, 2, Cobi Heijnen2, Oscar Vogels1, Jan Grutters1, 1Department of Pulmonology, St. Antonius Hospital, Nieuwegein, Netherlands; 2Laboratory for Neuroruninmunology and Developmental Origins of Disease, University Medical Center, Utrecht, Netherlands; 3Department of Neurorunlogy, St. Antonius Hospital, Nieuwegein, Netherlands; 4Division Heart & Lungs, University Medical Center, Utrecht, Netherlands

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 fatigue 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.

825s