IgG autoantibody levels (P<0.05) towards another antigen, dual specificity mitogen-activated protein kinase kinase 6 (MAP2K6), were also observed in ACPA-seronegative subjects compared to ACPA-seropositive and controls. In contrast, we found significantly higher IgG autoantibody levels (P<0.05) in ACPA-seropositive individuals compared to ACPA-seronegative and controls towards two antigens, anosmin-1 (ANOS-1) and muscle related coiled-coil protein (MURC). ANOS-1 shows also significantly higher IgG reactivity frequency in ACPA-seropositive individuals compared to ACPA-seronegative and controls (22%, 9% and 6% respectively; P<0.05). Interestingly, three out of the four antigens discovered to be associated with the ACPA status in early RA are highly expressed in lungs and heart, two of the main extraarticular sites affected in RA. No significant differences were observed at IgA levels for any of the antigens analyzed.

Table 1. Scheme of the different phases of the study, the features within each phase and the results. The reactivity to four antigens allows to distinguish ACPA-seronegative (ACPA−), ACPA seropositive (ACPA+) and controls.

<table>
<thead>
<tr>
<th>Phases</th>
<th>Untargeted discovery</th>
<th>Targeted discovery</th>
<th>Validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
<td>80 ACPA−</td>
<td>80 ACPA−</td>
<td>358 ACPA-372 Controls</td>
</tr>
<tr>
<td>Antigen array</td>
<td>Planar</td>
<td>Suspension</td>
<td>Suspension</td>
</tr>
<tr>
<td>platform</td>
<td>arrays</td>
<td>bead array 1</td>
<td>bead array 2</td>
</tr>
<tr>
<td>Number of antigens</td>
<td>2660</td>
<td>62</td>
<td>27</td>
</tr>
<tr>
<td>Number of candidate</td>
<td>62</td>
<td>27</td>
<td>4 (TSPYL4,MAP2K6, ANOS1,MURC)</td>
</tr>
</tbody>
</table>

Conclusion: Upon further validation in other early RA sample cohorts, our data suggest the measurement of these four autoantibodies may be useful for the early diagnosis of ACPA-seronegative RA and give insight into the pathogenesis of the different RA subsets.

Characteristics from table content including title and footnotes:

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**POS0394**

**NAD+ boosters reestablish the altered NAD+ metabolism of leukocytes from rheumatoid arthritis patients improving their oxidative, apoptotic and inflammatory status**

C. Perez-Sanchez1, L. M. Sánchez-Mendoza2, M. D. C. Abalos-Aguilera3, N. Barbarroja Puerto4, M. Luque-Tevec5, A. M. Patric-Hivres1, I. Arias de la Rosa1, J. A. Moreno1, M. I. Burton6, J. A. Gonzalez-Reyes7, E. Collantes Estevez8, A. Escudero Contreras9, C. Lopez-Pedrera10, J. M. Villalba3, IMIBIC, Rheumatology Service, IMIBIC/Reina Sofia Hospital/University of Cordoba, Cordoba, Spain, Cádiz, Spain; IMIBIC, Department of Cell Biology, Physiology and Immunology, University of Cordoba, Campus de Excelencia Internacional Agroalimentario, ceiA3, Córdoba, Spain, Cádiz, Spain; IMIBIC, Rheumatology Service, IMIBIC/Reina Sofia Hospital/University of Cordoba, Cordoba, Spain, Cádiz, Spain

**Background:** NAD+ is an important cofactor and second messenger for multiple cellular processes that exhibits antioxidant, anti-apoptotic and anti-inflammatory properties. Pre-clinical studies in animal models of Rheumatoid Arthritis (RA) have demonstrated the therapeutic potential of NAD+ boosters in the control of the disease activity. However, to date no studies have been set up to evaluate the NAD+ metabolism and the therapeutic effects of NAD+ boosters in RA patients.

**Objectives:**

1. To study the NAD+ metabolism in RA patients and their association with key clinical features.
2. To evaluate the effect of anti-TNF therapy in the NAD+ metabolism.
3. To analyze the beneficial effects of NAD+ boosters in leukocytes from active RA patients.

**Methods:** Plasma and PBMCs were purified from 100 RA patients and 50 healthy donors (HDs). Moreover, an additional cohort of 50 RA patients treated with Anti-TNF therapy was analyzed before and after 6 months of treatment. NAD+ levels were determined by using the NAD+/NADH-Glo Assay. NAD+-consuming genes expression were analyzed by RT-PCR. In parallel, PBMCs from eight HDs and eight active RA patients were treated ex vivo with 1mM of NAD+ boosters including nicotinamide (NAM), nicotinamide riboside (NIR), and nicotinamide mononucleotide (NMN). After 24 hours, intracellular reactive oxygen species (ROS) levels (DCFHDA) and the percentage of apoptotic PBMCs (annexin V/PI) were assessed by flow cytometry. Lastly, a panel of key pro-inflammatory genes were evaluated by reverse RT-PCR.

**Results:** NAD+ and NADH levels were significantly reduced in plasma and PBMCs of RA patients compared with HDs and directly related to disease activity (DAS28, CDAI, SDAI). Accordingly, the expression levels of genes involved in the consumption of NAD+ such as SIRT1, CD38 and PARP-1 were found up-regulated in PBMCs from RA patients. Anti-TNF therapy for 6 months restored the altered NAD+ levels towards those showed by HDs. Furthermore, the clinical response promoted by Anti-TNF therapy (changes in DAS28) correlated with changes in NAD+ levels. The in vitro treatments of PBMCs isolated from active RA patients with NAD+ boosters significantly increased the NAD+ levels and promoted a deep reduction of intracellular ROS levels, the percentage of apoptotic cells and the expression levels of key inflammatory mediators, such as IL-6, IL-8, IL-18, TNF-α, CCL2, IL-23, and STAT-3.

**Conclusion:** 1. NAD+ metabolism is altered and associated with the disease activity of RA patients, involving both, reduced NAD+ levels and increased expression of NAD+-consuming genes. 2. Anti-TNF therapy restored NAD+ levels, which were directly linked to the clinical effectiveness. 3. NAD+ boosters reduced the oxidative, apoptotic and inflammatory profile of RA leukocytes through the parallel increase of intracellular NAD+ levels. Thus, NAD+ boosters might be considered novel therapeutic tools for RA patients.

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