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.

Phases	Untargeted discovery	Targeted discovery	Targeted validation
Number of samples	80 ACPA- 80 ACPA+	80 ACPA- 80 ACPA+	358 ACPA-372 Controls 386 ACPA+
Antigen array platform	Planar arrays	Suspension bead array 1	Suspension bead array 2
Number of antigens	2660	62	27
Number of candidate biomarkers	62	27	4 (TSPYL4, MAP2K6, ANOS1, MURC)

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.

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POS0393

FIBRIN DEPOSITION IS AN ACTIVE TRIGGER OF CARTILAGE DEGENERATION IN RHEUMATOID ARTHRITIS

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Background: Fibrin(ogen) maintains inflammation in various disorders but has never been linked to cartilage damage in rheumatoid arthritis (RA) or other forms of inflammatory arthritis.

Objectives: To investigate the role of fibrin deposition on cartilage integrity in arthritis.

Methods: Fibrin deposition on knee cartilage was analyzed by immunohistochemistry in RA patients and in murine adjuvant-induced arthritis (AIA). In chondrocytes, fibrinogen expression ($Fg\alpha$, $Fg\beta$, $Fg\gamma$) and procoagulant activity were evaluated by qRT-PCR and turbidimetry respectively. Fibrin-induced catabolic genes were assessed by qRT-PCR in chondrocytes. Fibrin-mediated chondro-synovial adhesion (CSA) with subsequent cartilage tears was studied in co-cultures of human RA cartilage with autologous synovial cells, in the AIA model, and by MRI. The link between fibrin and calcification was examined in human RA cartilage stained for calcific deposits and *in vitro* in fibrinogen-stimulated chondrocytes.

Results: Fibrin deposition on cartilage correlated with the severity of cartilage damage in human RA explants and in AIA wildtype (WT) mice, while fibrinogen deficient ($Fg^{-/-}$) mice were protected. Accordingly, fibrin upregulated catabolic enzymes (*Adamts5* and *Mmp13*) in chondrocytes. Secondly, CSA was present in fibrin-rich and damaged cartilage in AIA WT but not in $Fg^{-/-}$ mice. In line, autologous human synovial cells, cultured on RA cartilage explants, adhered exclusively to fibrin-positive degraded areas. Gadolinium-enhanced MRI of human joints showed contrast-enhancement along cartilage surface in RA patients but not in controls. Finally, fibrin co-localized with calcification in human RA cartilage and triggered chondrocyte mineralization inducing pro-calcification genes (*Anx5*, *Pit1*, *Pc1*) and cytokine (IL-6). Although at a much lesser extent, we observed similar fibrin-mediated mechanisms in osteoarthritis (OA).

Conclusion: Fibrin deposition directly impacts on cartilage integrity via induction of catabolism, mechanical stress, and calcification. Potentially, fibrin is a key factor of cartilage damage occurring in RA as a secondary consequence of inflammation.

Disclosure of Interests: None declared

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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

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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 its 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 1 mM of NAD⁺ boosters including nicotinamide (NAM), nicotinamide riboside (NR), and nicotinamide mononucleotide (NMN). After 24 hours, intracellular reactive oxygen species (ROS) levels (DFCHDA) 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 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 SIRT-1, 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-1 β , 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 profiles 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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POS0395

ANTI-ACETYLATED PROTEIN ANTIBODIES IN RHEUMATOID ARTHRITIS (RA): CLUES FOR THE STARTING POINT OF AUTOANTIBODY RESPONSES IN RA

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Background: Rheumatoid arthritis (RA) is characterized by autoantibodies such as rheumatoid factor (RF) and anti-modified protein autoantibodies (AMPAs) like anti-citrullinated protein antibodies (ACPA) and anti-carbamylated protein