POS0390

SIMULTANEOUS ASSESSMENT OF JOINT AND VASCULAR INFLAMMATION BY PET-CT IN TOFACITINIB-TREATED PATIENTS WITH RHEUMATOID ARTHRITIS: A PROSPECTIVE STUDY

A. Hamar¹, Z. Hascsi², A. Pusztai¹, M. Czókolyová¹, E. Végh¹, Z. Pethő¹, K. Gulyás¹, B. Soós¹, G. Kerekes³, É. Szekanecz⁴, K. Hodosi¹, S. Szántó¹, G. Szücs¹, T. Seres⁵, Z. Szekanecz¹, S. Szamosi¹. ¹University of Debrecen, Rheumatology, Debrecen, Hungary; ²Scanomed Ltd., PET/CT, Debrecen, Hungary; ³University of Debrecen, Medicine, Debrecen, Hungary; ⁴University of Debrecen, Oncology, Debrecen, Hungary; ⁵University of Colorado Anschutz Medical Campus, Anesthesiology, Aurora, Co, United States of America

Background: Rheumatoid arthritis (RA) has been associated with atherosclerosis and cardiovascular (CV) disease. 18F-fluorodeoxyglucose positron emission tomography/computed tomography (¹⁸FDG-PET/CT) is suitable to detect synovial and vascular inflammation. Tofacitinib has been used to effectively treat RA. **Objectives:** We wished to assess the effects of tofacitinib treatment on synovitis and vascular inflammation simultaneously by ¹⁸FDG-PET/CT.

Methods: Thirty RA patients with active disease were treated with either 5 mg bid or 10 mg bid tofacitinib and evaluated at baseline and after 6 and 12 months. We determined DAS28, CRP, IgM rheumatoid factor (RF) and anti-cyclic citrullinated peptide (aCCP) levels. All patients underwent 18F-fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) in order to determine vascular and synovial inflammation in five aortic segments and five articular regions, respectively. In the joints, mean (SUV_{mean}) and maximum standard uptake values (SUV_{max}), while in the aorta, mean (TBR_{mean}) and maximum target-to-background ratios (TBR_{max}) were determined. Carotid intima-media thickness (IMT), arterial stiffness (PWV) and endothelial dysfunction (FMD) were determined by ultrasound.

Results: One-year tofacitinib treatment significantly attenuated vascular and synovial inflammation as visualized by PET/CT. Articular SUV_{mean} (p=0.010), SUV_{max} (p=0.001), as well as aorta TBR_{max} (p<0.001) significantly decreased over time. Synovial inflammation as determined by PET/CT variably and positively associated with aCCP, RF, CRP, ApoB, lipoprotein A (LpA), IMT and PWV. Vascular inflammation (TBR_{max}) inversely correlated with HAQ and positively with ESR, ApoA, and PWV. Uni- and multivariable analyses suggested that articular SUV values were independently associated with CRP, ApoB, LpA, IMT and PWV, while aortic TBR_{max} was determined by HAQ and PWV.

Conclusion: ¹⁸F-PET/CT is suitable to simultaneously assess synovial and vascular inflammation in RA. One-year tofacitinib treatment dampened inflammation. PET/CT changes were associated with markers of systemic inflammation, atherogenic lipids, carotid atherosclerosis and arterial stiffness.

REFERENCES:

- [1] Gotthardt M, Bleeker-Rovers CP, Boerman OC, Oyen WJ. Imaging of inflammation by PET, conventional scintigraphy, and other imaging techniques. *J Nucl Med.* 2010;51(12):1937-49.
- [2] Bucerius J, Hyafil F, Verberne HJ, Slart RH, Lindner O, Sciagra R, et al. Position paper of the Cardiovascular Committee of the European Association of Nuclear Medicine (EANM) on PET imaging of atherosclerosis. *Eur J Nucl Med Mol Imaging.* 2016;43(4):780-92.

Acknowledgements: This research was supported by the European Union and the State of Hungary and co-financed by the European Social Fund in the framework of TAMOP-4.2.4.A/2-11/1-2012-0001 'National Excellence Program' (Z.S.); by the European Union grant GINOP-2.3.2-15-2016-00015 (G.P., G.T. and Z.S.) and by the Pfizer Investigator Initiated Research Grant no. WI188341 (Z.S.).

Disclosure of Interests: Attila Hamar: None declared, Zolt Hascsi: None declared, Anita Pusztai: None declared, Monika Czókolyová: None declared, Edit Végh: None declared, Zsófia Pethő: None declared, Katalin Gulyás: None declared, Boglárka Soós: None declared, György Kerekes: None declared, Éva Szekanecz: None declared, Katalin Hodosi: None declared, Sándor Szántó Speakers bureau: Abbvie, MSD, Novartis, Consultant of: Abbvie, MSD, Novartis, Gabriella Szücs Speakers bureau: Boehringer, Actelion, Roche, Consultant of: Boehringer, Actelion, Roche, Tamas Seres: None declared, Zoltán Szekanecz Speakers bureau: Abbvie, Pfizer, Roche, Novartis, Lilly, Sager, Janssen, Consultant of: Pfizer, Abbvie, Roche, Novartis, Grant/research support from: Pfizer, Szilvia Szamosi Speakers bureau: Roche

DOI: 10.1136/annrheumdis-2021-eular.2473

POS0391

ASSESSING SENESCENT HUMAN LYMPH NODE STROMAL CELLS DURING RHEUMATOID ARTHRITIS DEVELOPMENT

T. de Jong^{1,2}, J. W. Bolt^{1,2}, J. F. Semmelink^{1,2}, L. van Baarsen^{1,2}. ¹Amsterdam UMC, University of Amsterdam, Department of Rheumatology & Clinical Immunology and Department of Experimental Immunology, Amsterdam, Netherlands; ²Amsterdam UMC, Amsterdam Rheumatology & Immunology Center (ARC), Amsterdam, Netherlands

Background: Cellular senescence is a state of proliferation arrest of cells. The persistence and accumulation of senescent cells has been implicated in the pathogenesis of age-related diseases like rheumatoid arthritis (RA). Clinical disease is preceded by loss of immune tolerance and autoimmunity. Lymph node stromal cells (LNSC) are important regulators of this tolerance. Therefore, senescent LNSC may affect tolerance and the development of systemic autoimmunity. **Objectives:** To determine the extent of cellular senescence of LNSC during early phases of systemic autoimmunity.

Methods: We included individuals with arthralgia without any evidence of arthritis who were positive for IgM rheumatoid factor (IgM-RF) and/or anti-citrullinated protein antibodies (ACPA; RA-risk group), early arthritis patients (ACR/EULAR 2010 criteria; disease duration < 1 year) and seronegative healthy controls. All study subjects underwent ultrasound-guided inguinal lymph node biopsy. LNSC were isolated and cultured from freshly collected lymph node needle biopsies and passages 0-9 were used for experiments. Cellular senescence was assessed by measuring cell size, granularity, autofluorescence, senescence-associated gene expression levels, DNA damage and senescence-associated β -galactosidase (SA β -gal) activity.

Results: Preliminary flow cytometry data shows that the cell size and autofluorescence of LNSC from RA patients (n=11) and RA-risk individuals (n=7) is increased compared with healthy LNSC (n=7), while granularity was specifically increased in LNSC from RA patients (n=9). Initial stainings indicate higher SA β -gal activity and more DNA damage in LNSC from RA-risk (n=3) and RA patients (n=4) compared with healthy controls (n=3). Expression levels of senescence-associated genes such as p21 and p53 were significantly higher in LNSC from RA patients compared with healthy controls (n=6 per group).

Conclusion: These preliminary findings suggest senescence already in early RA and provide a rationale for investigating the consequence of senescent LNSC on immune cell responses

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2021-eular.2501

POS0392

PRESENCE OF FOUR SERUM AUTOANTIBODIES ASSOCIATES WITH THE ACPA STATUS IN EARLY RHEUMATOID ARTHRITIS

L. Lourido¹, C. Ruiz-Romero^{1,2}, L. Collado¹, M. Hansson³, L. Klareskog³, R. Sjöberg⁴, E. Pin⁴, P. Nilsson⁴, F. J. Blanco^{1,5}. ¹Unidad de Proteómica, Grupo de Investigación de Reumatología (GIR), Instituto de Investigación Biomédica de A Coruña (INIBIC), Complejo Hospitalario Universitario de A Coruña (CHUAC), Sergas, Universidade da Coruña (UDC), A Coruña, Spain; ²Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina, (CIBER-BBN), Madrid, Spain; ³Karolinska Institutet, Karolinska University Hospital, Division of Rheumatology, Department of Medicine Solna, Stockholm, Sweden; ⁴KTH Royal Institute of Technology & SciLifeLab, Division of Affinity Proteomics, Department of Protein Science, Stockholm, Sweden; ⁵Unidad de Investigación de Reumatología y Salud (GIR-S), Departamento de Fisioterapia, Medicina y Ciencias Biomédicas, Facultad de Fisioterapia, A Coruña, Spain

Background: The presence of anti-citrullinated protein antibodies (ACPAs) is a hallmark of rheumatoid arthritis (RA) that precedes the development of the disease by years and is used for its clinical diagnosis. However, there are RA subjects that test negative for ACPA and thus the early diagnosis on these patients may be delayed. Furthermore, the presence or absence of ACPA in RA supports the hypothesis that on these two subsets of patients underlie different pathogenesis and clinical outcomes.

Objectives: In this work, we searched for serum autoantibodies useful to assist the early diagnosis of ACPA-seronegative RA and its management.

Methods: We profiled the serum autoantibody repertoire of 80 ACPA-seronegative and 80 ACPA-seropositive RA subjects from the Swedish population-based Epidemiological Investigation of RA (EIRA) cohort. A suspension bead array platform built on protein fragments within Human Protein Atlas and selected from an initial untargeted screening using arrays containing 2660 total antigens was employed to identify IgG and IgA serum autoantibodies. A validation phase on antigen suspension bead arrays was carried out on another set of samples from EIRA containing 386 ACPA-seropositive, 358 ACPA-seronegative and 372 randomly selected control subjects of the same age and sex. A sample-specific threshold based on 20 times the median absolute deviation plus the median of all signals was selected to determine the reactivity of samples. The Wilcoxon rank sum test and Fisher's test were applied for the comparison of autoantibody levels and reactivity frequencies between the groups.

Results: Our data revealed four antigens associated with the ACPA status (Table 1). Testis-specific Y-encoded-like protein 4 (TSPYL4) showed significantly higher IgG reactivity frequency in ACPA-seronegative subjects compared to ACPA-seropositive (8% vs. 3%; P<0.05). Significant differences at IgG autoantibody levels (P<0.05) were also observed between ACPA-seronegative subjects and controls for this specific antigen. Significantly higher

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.

Characters from table content including title and footnotes:

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2021-eular.2514

POS0393

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

T. Hügler¹, S. Nasi¹, D. Ehrichou¹, P. Omoumi², A. So¹, N. Busso¹. ¹Lausanne University Hospital, CHUV, Department of Rheumatology, Lausanne, Switzerland; ²Lausanne University Hospital, CHUV, Radiology, Lausanne, Switzerland

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

DOI: 10.1136/annrheumdis-2021-eular.2521

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-Aguilera¹, N. Barbarroja Puerto¹, M. Luque-Tévar¹, A. M. Patiño-Trives¹, I. Arias de la Rosa¹, J. A. Moreno², M. I. Burón², J. A. González-Reyes², E. Collantes Estevez¹, A. Escudero Contreras³, C. Lopez-Pedraza¹, J. M. Villalba². ¹IMIBIC, Rheumatology Service, IMIBIC/Reina Sofia Hospital/University of Cordoba, Cordoba, Spain, Córdoba, Spain; ²IMIBIC, Department of Cell Biology, Physiology and Immunology, University of Cordoba, Campus de Excelencia Internacional Agroalimentario, ceiA3, Córdoba, Spain, Córdoba, Spain; ³IMIBIC, Rheumatology Service, IMIBIC/Reina Sofia Hospital/University of Cordoba, Cordoba, Spain, Cordoba, 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 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.

Acknowledgements: Supported by PI18/00837, RIER RD16/0012/0015, RTI2018-100695-B-I00, P18-RT-4264 and CVI276, co-funded with FEDER.

Disclosure of Interests: None declared

DOI: 10.1136/annrheumdis-2021-eular.2580

POS0395

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

T. J. van Wesemael¹, A. L. Dorjée¹, T. Huizinga¹, A. van der Helm - van Mil¹, R. Toes¹, D. van der Woude¹. ¹Leiden University Medical Center, Rheumatology, Leiden, Netherlands

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