(SNP) was studied on endothelium-denuded aortic rings. The effect of MTX on hind paw radiographic score, serum lipids and plasma pro-inflammatory cytokines (TNF α and IL-1 β) levels was measured.

Results: As compared to Vehicle rats, MTX significantly reduced arthritis score ($p < 0.01$) but did not change radiographic score. It reduced plasma cytokines levels ($p = 0.02$) but not total cholesterol and triglycerides levels. MTX did not change Ach-induced relaxation as compared to Vehicle. As regards endothelial pathways, MTX increased vascular NOS activity ($p < 0.0001$) and decreased superoxide anions production but did change neither COX-2 and arginase activities nor EDHF production. Vascular smooth muscle reactivity to NE and SNP was unchanged by the treatment.

Conclusions: Despite a reduction of clinical and biological inflammation, MTX did not improve endothelial function in AIA rats. The study of endothelial mechanisms highlights the role of COX and arginase as seminal targets for reducing ED in RA. This study suggests that other mechanisms than improvement of endothelial function are involved in the CV benefits of MTX in RA that remain to be elucidated. Our data also suggest that the adjunction of drugs targeting endothelial function to MTX in RA patients might improve their CV prognostic.

References:

- [1] Agca R, Heslinga SC, Rollefstad S, et al. EULAR recommendations for cardiovascular disease risk management in patients with rheumatoid arthritis and other forms of inflammatory joint disorders: 2015/2016 update. *Ann Rheum Dis* 2016;annrheumdis-2016-209775. doi:10.1136/annrheumdis-2016-209775.
- [2] Prati C, Demougeot C, Guillot X, et al. Endothelial dysfunction in joint disease. *Joint Bone Spine Rev Rheum* 2014;81:386–91. doi: 10.1016/j.jbspin.2014.01.014.
- [3] Roubille C, Richer V, Starnino T, et al. The effects of tumour necrosis factor inhibitors, methotrexate, non-steroidal anti-inflammatory drugs and corticosteroids on cardiovascular events in rheumatoid arthritis, psoriasis and psoriatic arthritis: a systematic review and meta-analysis. *Ann Rheum Dis* 2015;74:480–9. doi: 10.1136/annrheumdis-2014-206624.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.3234

FRI0040 CHARACTERIZATION OF ANTI-CARBAMYLATED SYNOVIAL PROTEIN ANTIBODIES (ANTI-CARPS) IN RHEUMATOID ARTHRITIS PATIENTS

C. Regueiro¹, A. Montes¹, M.D. Boveda², S. Amhaz-Escanlar³, E. Perez-Pampin⁴, A. Mera-Varela⁴, A. Gonzalez¹. ¹Experimental and observational Rheumatology; ²Unit of Diagnosis and Treatment of Congenital Metabolic Diseases; ³Orthopedic Surgery and Traumatology; ⁴Rheumatology Unit, Instituto Investigación Sanitaria- H. Clínico Universitario de Santiago, Santiago de Compostela, Spain

Background: Anti-carbamylated protein antibodies (anti-CarP) are a new type of autoantibodies specific of patients with RA. Reactivity has generally been tested with *in vitro* carbamylated proteins from fetal calf serum (FCS) as anti-CarPF. It is likely that other sources of antigens could be more suitable than FCS unless the reactivity of the autoantibodies is directed primarily against the carbamylated residues independently of the protein.

Objectives: To evaluate the anti-CarP antibodies against *in vitro* carbamylated synovial tissue proteins (anti-CarPS) and to compare them with anti-CarPF antibodies.

Methods: A pool of synovial tissue proteins from 3 osteoarthritis patients was *in vitro* carbamylated and used as antigen for ELISA. Anti-CarPS antibodies were determined in 520 sera from patients with RA and 278 healthy controls, which have been previously tested for anti-CarPF antibodies (1). Logistic regression analysis was used to evaluate the association of the antibodies with SE alleles, PTPN22, smoking and erosions. Relationship between antibodies titers was analyzed with Spearman rank correlation (r_s) and concordance between positivity of the different antibodies was analyzed with the Goodman and Kruskal γ .

Results: Similar percentages of patients were positive for anti-CarPS (31.7%) and for anti-CarPF antibodies (29.4%). However, many patients were discordant (28.9%). Notably, the concordance between anti-CarPS and anti-CCP antibodies was higher than the concordance between anti-CarPS and anti-CarPF antibodies ($\gamma = 0.73$, 95% CI: 0.60–0.86 vs. $\gamma = 0.60$, 95% CI: 0.47–0.73, respectively). Moreover, titers of anti-CarPS antibodies were more correlated with anti-CCP than with anti-CarPF antibodies ($r_s = 0.41$, 95% CI: 0.33–0.48 vs. $r_s = 0.29$, 95% CI: 0.21–0.37, respectively). Accordingly, presence of anti-CarPS antibodies showed a clearer trend to be associated with the genetic and environmental RA risk factors than anti-CarPF antibodies, although only the association with smoking was significant (smoking OR=2.0, $p = 0.02$ vs. OR=1.1, $p = 0.8$, respectively; OR=1.3 vs. OR=1.1 for HLA-SE and OR=1.3 vs. OR=0.9 for PTPN22). There were no differences in the association with erosions, which was significant for both antibodies analyzed separately (OR=2.3, $p = 6.8 \times 10^{-4}$ for anti-CarPS and OR=2.4 $p = 2.0 \times 10^{-4}$ for anti-CarPF) and together (OR=1.8, $p = 0.02$ and OR=2.0, $p = 0.005$, respectively).

Conclusions: Our results indicate that the proteins used as antigens in the determination of anti-CarP antibodies are relevant for the obtained positivity and titer. The differences could affect the pattern of characteristics associated with these antibodies, making it necessary to identify the specific carbamylated autoantigens targeted by them *in vivo*.

References:

[1] Montes A, et al. *PLoS One* 2016;11:e0161141.

Acknowledgements: Supported by PI14/01651 and RD12/0009/0008 of the ISCIII (Spain) with participation of FEDER.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.4789

FRI0041 IN VIVO MONITORING OF ANTI-FOLATE THERAPY IN ARTHRITIC RATS USING [18F]FLUORO-PEG-FOLATE AND PET

D.M.S.H. Chandrupatla¹, C.J. van der Laken¹, R. Vos², M. Verlaan², R. van Kooij², A.D. Windhorst², Q. Chen³, P.S. Low³, A.A. Lammertsma², G. Jansen¹, C.F.M. Molthoff². ¹Amsterdam Rheumatology and immunology Centre – location VUmc; ²Radiology and Nuclear Medicine, VU University Medical Center, Amsterdam, Netherlands; ³Department of Chemistry, Purdue University, West Lafayette, United States

Background: Macrophages play a key role in the pathophysiology of rheumatoid arthritis (RA). The folate receptor β (FR- β) is expressed on these macrophages [1] and [¹⁸F]fluoro-PEG-folate positron emission tomography ([¹⁸F]fluoro-PEG-folate PET) can be used to visualize arthritis *in vivo* [2]. In addition, [¹⁸F]fluoro-PEG-folate PET could be a highly interesting tool for therapeutic monitoring of MTX therapy, the corner stone for RA therapy.

Objectives: To study the potential of [¹⁸F]fluoro-PEG-folate PET for monitoring response to MTX in a rat model of RA.

Methods: Arthritic rats [3] (n=3–6 per group) received interventions with either MTX [i.p., 1mg/kg; 2 times (group A) or 4 times (group B)] or PBS (control group) with a time interval of 3–4 days. Healthy rats didn't receive any arthritic induction or MTX therapy. [¹⁸F]fluoro-PEG-folate PET-CT were acquired for one hour after tracer injection [2]. Scans were analysed using the region of interest method and standardized uptake values (SUVs) were determined (50–60 min time frame). Sixty minutes after the PET scan, *ex-vivo* tissue distribution was performed and the amount of radioactivity measured in a gamma counter (expressed as percentage of the injected dose/gram tissue (%ID/g)) [2]. For histopathology (Haematoxylin-Eosin (HE) and immunohistochemistry with macrophage specific antibodies ED1 (~CD68) and ED2 (~CD163) were applied. Synovial macrophages were counted in predefined areas of the knees [3].

Results: PET scans clearly visualized significantly lower SUVs (1.5-fold, $p < 0.01$) in arthritic knees of both MTX-treated groups, approximating the levels observed in healthy rats. Corroborating [¹⁸F]fluoro-PEG-folate PET, *ex vivo* tissue distribution [¹⁸F]fluoro-PEG-folate demonstrating a 2- and 4-fold decrease (group A and B, respectively) in tracer uptake in arthritic knees after MTX therapy (0.12 and 0.06 for groups A and B, and 0.22%ID/g for controls, respectively) (Figure). This reduction in uptake of [¹⁸F]fluoro-PEG-folate in arthritic knees was also associated with a significant decrease in ED1 and ED2 positive synovial macrophages in arthritic knees (~4 fold) for both treated groups compared with control rats knees ($p < 0.01$).

Figure. Ex vivo tissue distribution of [¹⁸F]fluoro-PEG-folate in arthritic knee of healthy (white bars) (n=3), arthritic (black bars) (n=3), 2x-MTX (dark grey) (n=4) and 4x-MTX (light grey) (n=4) treated rats at 60 min post tracer injection. Results are expressed as mean percentage injected dose per gram (%ID/g ± SD).

Conclusions: This study in arthritic rats underscores the potential and usefulness of [¹⁸F]fluoro-PEG-folate PET as tool to monitor of MTX therapy and potentially other anti-folate treatments of RA.

References:

- [1] Van der Heijden et al. *Arthr & Rheum* (2009).
- [2] Gent et al. *Arthr Res Ther* (2013).
- [3] Chandrupatla et al. *BioMed Res Int* (2015).

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.4803