FRI0036 ASSOCIATION BETWEEN THE 18FDG-PET IMAGING AND THE PATHOLOGICAL FINDINGS OF RHEUMATOID SYNOVITIS

C. Okura, Y. Yonemoto, K. Okamura, T. Suto, M. Tachibana, H. Chikuda. *Orthopaedic Surgery, Gunma University Graduate School of Medicine, Maebashi, Japan*

Background: It has been reported that 18FDG-PET (PET) is useful in the evaluation of RA and for monitoring the effects of treatment on the disease activity, so its utility for the evaluation of arthritis is expected. However, the mechanism underlying the uptake of FDG into the inflamed joint is still unclear.
Objectives: The aim of this study was to investigate the associations among the amount of FDG uptake in RA joints, the inflammatory findings with regard to the pathology of the synovium and the clinical findings.
Methods: We performed PET in 18 RA patients who underwent Total Knee Arthroplasty surgery in our hospital just prior to surgery. We calculated the FDG uptake as the standardized uptake value (SUV)max and scored it using the Rooney score, with the degree of inflammation of the synovial tissues used for the pathological evaluation. We evaluated the associations among the SUVmax, Rooney score, CRP level, ESR and MMP-3 level just before surgery.
Results: The subjects were 18 cases with 20 joints, which were in four females and 14 males. At the time of surgery, the average age of the patients was 66.7±7.9 years old, and the mean disease duration was 20.8±14.0 years. Significant correlation was not observed between SUVmax and total Rooney score (r =0.056, p=0.814). But there were strong correlations between SUVmax and some of the individual items in Rooney score, including the "Synoviocyte hyperplasia", and also between SUV max and "Diffuse infiltrates of lymphocytes" (r=0.512, p=0.021 and r=0.581, p=0.007, respectively).
Conclusions: The accumulation of FDG was associated with the extent of "synoviocyte hyperplasia" and "diffuse infiltrates of lymphocytes". It is estimated that accumulation of FDG is associated with relatively early stage of active inflammation of RA.
References:
[1] McQueen FM, et al. Clin Rheumatol: 2007.
[2] Beckers C, et al. Eur J Nucl Med Mol Imaging; 2006.
[3] Okamura K, et al. Rheumatology (Oxford); 2012.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.7021

FRI0037 MER-MEDIATED EFFEROCYTOSIS TEMPERS ARTHRITIS BY PREVENTING NEUTROPHILS TO GO INTO SECONDARY NECROSIS AND SPILL THEIR INFLAMMATORY CONTENT IN THE JOINT

C.E.J. Waterborg, S. Beermann, M.B. Bennink, M.I. Koenders, P.L.E.M. van Lent, F. van den Hoogen, P.M. van der Kraan, F.A.J. van de Loo. *Experimental Rheumatology, Radboudumc, Nijmegen, Netherlands*

Background: Rheumatoid arthritis is characterized by an inflammatory response in synovial joints, showing a predominant influx of neutrophils. These cells are cytotoxic and contribute to matrix degradation. In addition, they are implicated as a source of citrullinated auto-antigens, leading to the production of anti-citrullinated protein antibodies. Neutrophils have a relative short life span and many of them undergo apoptosis. If they are not cleared, they undergo secondary necrosis and release their cell content. A key mediator in the resolution of inflammation and the uptake of apoptotic cells, or efferocytosis, is the receptor tyrosine kinase Mer.
Objectives: To elucidate the local role of Mer during gonarthritis.
Methods: Macrophages were transduced by adenoviruses encoding the Mer ligand *Pros1* or *Luciferase*. One day after the collagen booster injection in mice, Mer-specific antibodies or IgG antibodies, or adenoviruses overexpressing *Pros1* or *Luciferase* were injected intravenously. Mice were euthanized at day 30 or 36, respectively. The KRN serum transfer arthritis model was induced by two intraperitoneal injections of arthritic K/BxN serum in either Mer-deficient or wild-type (WT) mice, or in mice that overexpress *Pros1* or *Luciferase* in their knee joints. Mice were euthanized at day 7 or day 14, respectively. From all mice, serum was taken for cytokine profiling and knee joints were isolated for either synovial gene expression or histology and immunohistochemistry.
Results: Adenoviral overexpression of the Mer ligand *Pros1* resulted in reduced production of pro-inflammatory cytokines and chemokines by macrophages, compared to *Luciferase*. In addition, local *Pros1* overexpression resulted in reduced expression of pro-inflammatory and pro-destructive mediators by synovial cells of arthritic mice. Systemic and local *Pros1* overexpression diminished joint pathology, reduced the number of cleaved Caspase 3-positive apoptotic cells and secondary necrotic neutrophils. Conversely, inhibiting Mer-mediated efferocytosis by either Mer-specific antibodies or *Mertk* gene ablation resulted in aggravation of arthritis compared to controls, as evidenced by increased inflammation and tissue destruction. Additionally, Mer-inhibited mice had increased numbers of apoptotic cells in their knee joints, and higher serum levels of IL-16C, a cytokine released by secondary necrotic neutrophils.
Conclusions: Together, these results demonstrate that Mer locally plays a unique protective role in knee joint disease by enhancing resolution of arthritis. Our data suggest that promoting and/or restoring Mer-mediated uptake of apoptotic cells in the arthritic joint might be therapeutically beneficial.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.4902

FRI0038 RELEASE OF PEPTIDYLARGININE DEIMINIASE 2 FROM ACTIVATED NEUTROPHILS

M.A. Steffensen, D. Damgaard, M. Bassi, C.H. Nielsen. *Institute for Inflammation Research, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark*

Background: Extracellular citrullination catalyzed by peptidylarginine deiminase (PAD) is thought to play a central role in the pathogenesis of rheumatoid arthritis. Neutrophils are a major reservoir of PAD2 and PAD4. Cellular release of PAD2 and PAD4 is usually considered a consequence of cell death.
Objectives: We aimed to determine if PAD2 can be released from live, activated neutrophils as an active process.
Methods: Whole blood cells were purified from healthy blood were stimulated with phorbol 12-myristate 13-acetate (PMA). To capture PAD2 released from neutrophils and detect it by flow cytometry, we used biotinylated anti-CD15 and anti-PAD2 (mAb DN6) mAbs linked by streptavidin, and a different PE-labelled anti-PAD2 amtibody (mAb DN2). In addition, intracellular PAD2 was quantified by intracellular staining with PE-anti-PAD2. PAD2 released from leukocytes and subcellular fractions of human granulocytes were assessed for content of PAD2 using an in-house luminex-based assay.
Results: On incubation of whole blood cells with PMA, PAD2 was detectable in the supernatants after 30 minutes, and levels increased thereafter in parallel with increasing cell death. However, using PAD2 catch reagent, we found that live neutrophils released PAD2 in the 30 minutes after stimulation. Intracellular staining for PAD2 showed that the content of PAD2 in live neutrophils decreased correspondingly. Upon subcellular fractioning of granulocytes, the majority of PAD2 was found in cytosol and, in 25 times lower quantities, in the fraction containing plasma membranes and secretory vesicles. Sparse amounts of PAD2 were also observed in gelatinase granules.

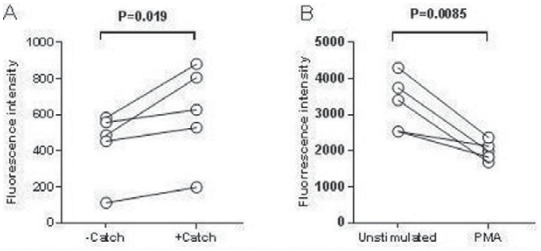

Figure 1: Release of PAD2 by live neutrophils following activation. (A) Whole blood cells were stimulated with PMA for 30 minutes in presence of a catch reagent consisting of co-conjugated anti-CD15 and anti-PAD2 antibodies, and PAD2 caught on the surface of CD15- granulocytes was detected by flow cytometry using PE-conjugated anti-PAD2 antibody (+Catch). Cells not exposed to catch reagent (-Catch) were included as controls. (B) Whole blood cells were incubated with or without PMA for 30 minutes, and the content of PAD2 in granulocytes was assessed using flow cytometry with a PE-conjugated anti-PAD2 antibody (DN2). In both A and B, granulocytes were gated on the basis of forward and side light scatter characteristics, and dead cells staining positive for a near-IR dead-cell stain were excluded.

Conclusions: In conclusion, PAD2 can be released from live, activated neutrophils, which may contribute to extracellular citrullination and thereby play a role in driving inflammatory processes in RA patients with immune responses to citrullinated proteins.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.5964

FRI0039 ENDOTHELIAL DYSFUNCTION IN RHEUMATOID ARTHRITIS: WHICH EFFECT OF METHOTREXATE? A STUDY IN ADJUVANT INDUCED ARTHRITIS MODEL

R. Bordy [1], P. Totoson [1], F. Verhoeven [2], C. Prati [1,2], J. Moretto [1], M. Tournier-Nappey [1], C. Demougeot [1]. *[1]Pepite Ea4267, Université de Franche-Comté; [2]Rhumatologie, CHRU Besançon, Besançon, France*

Background: Rheumatoid arthritis (RA) is associated with increased cardiovascular (CV) risk [1] secondary to endothelial dysfunction (ED) [2]. There is accumulating evidence that methotrexate (MTX), first intention DMARD, reduces CV risk in RA [3], but the mechanisms involved are still unknown.
Objectives: The aim of this study was to determine the effect of MTX on endothelial function in arthritis and to investigate its effect on endothelial pathways.
Methods: Experiments were conducted in the adjuvant-induced arthritis (AIA) model in Lewis rat. At onset of arthritis, rats were treated by a sub-cutaneous injection of MTX (1 mg/kg/week) or phosphate buffer saline (vehicle) for 3 weeks. Arthritis score was daily monitored. At the end of treatment, thoracic aorta was harvested to measure the relaxation to acetylcholine on pre-constricted aortic rings in the presence or not of inhibitor of nitric oxide (NO) synthase (L-NAME), arginase (nor-NOHA), EDHF (Apamin/Charybdotoxin), or a superoxide dismutase analog (Tempol). The effect of norepinephrine (NE) and sodium nitroprusside

(SNP) was studied on endothelium-denuded aortic rings. The effect of MTX on hind paw radiographic score, serum lipids and plasma pro-inflammatory cytokines (TNFα and IL-1β) 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. Regueiro[1], A. Montes[1], M.D. Boveda[2], S. Amhaz-Escanlar[3], E. Perez-Pampin[4], A. Mera-Varela[4], A. Gonzalez[1]. *[1]Experimental and observational Rheumatology; [2]Unit of Diagnosis and Treatment of Congenital Metabolic Diseases; [3]Orthopedic Surgery and Traumatology; [4]Rheumatology Unit, Instituto Investigacion Sanitaria- H. Clinico 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 sinovial 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 alelles, *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 (γ =0.73, 95% CI: 0.60–0.86 *vs.* γ =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. Chandrupatla[1], C.J. van der Laken[1], R. Vos[2], M. Verlaan[2], R. van Kooij[2], A.D. Windhorst[2], Q. Chen[3], P.S. Low[3], A.A. Lammertsma[2], G. Jansen[1], C.F.M. Molthoff[2]. *[1]Amsterdam Rheumatology and immunology Centre – location VUmc; [2]Radiology and Nuclear Medicine, VU University Medical Center, Amsterdam, Netherlands; [3]Department 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 [^{18}F]fluoro-PEG-folate positron emission tomography ([^{18}F]fluoro-PEG-folate PET) can be used to visualize arthritis *in vivo* [2]. In addition, [^{18}F]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 [^{18}F]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. [^{18}F]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 [^{18}F]fluoro-PEG-folate PET, *ex vivo* tissue distribution [^{18}F]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 [^{18}F]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 ($p<0.01$).

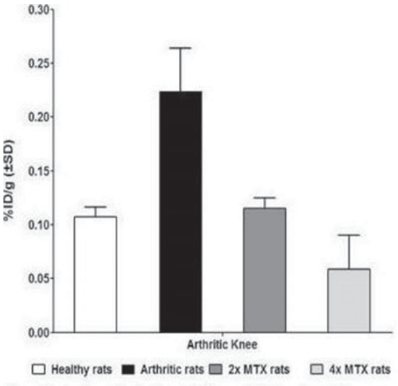

Figure. Ex vivo tissue distribution of [^{18}F]fluoro-PEG-folate in arthritic knee of healthy (white bars) (n=3), arthritic (black bars) (n=3), 2x-MTX (dark grey) (n=4) and 4x-MTX (light grey) (n=4) treated rats at 60 min post tracer injection. Results are expressed as mean percentage injected dose per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

Conclusions: This study in arthritic rats underscores the potential and usefulness of [^{18}F]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