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Fig. 1: Immunohistological staining of ADAM12 for synovial tissues

Fig. 2: ADAM12 relative mRNA expression and the proliferation of RASF after siADAM12 transfection

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SAT0024

JAK-INHIBITION WITH BARICTINIB INHIBITS ACTIVATION OF NLRP1/CASPASE1/GSDMD PYROPTOSIS PATHWAY IN RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS

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Background: Synovial fibroblasts (SFs) play a major role in the pathogenesis of rheumatoid arthritis (RA) and develop an aggressive phenotype destroying cartilage and bone, thus termed RASFs.

Objectives: We aim to examine the presence of GSDMD-mediated pyroptosis and its role in activated RASFs.

Methods: Fibroblasts were isolated from RA synovium obtained from knee replacement surgeries. NLRP1, Caspase-1, GSDMD expression in synovial tissue and TNF-treated RASFs were assessed by qPCR and Western blot. Interleukin (IL)-1 was measured by ELISA in supernatant after pretreated with TNF and baricitinib. LDH release was measured using the CytoTox 96 Non-Radioactive

Cytotoxicity Assay Kit. Endogenous NLRP1, Caspase-1, and GSDMD was knocked down using small interfering RNA.

Results: The expressions of NLRP1, pro-Caspase-1, Caspase-1 p10, GSDMD and its pyroptosis-inducing fragment GSDMD-N were greater in RA synovium than OA synovium. TNF-induced NLRP1, pro-Caspase-1, Caspase-1 p10, GSDMD, and GSDMD-N expression at the transcript and protein level in a time-dependent manner ($P < 0.05$). Meanwhile, the release of LDH and IL-1 were significantly increased in RASFs after treated with TNF. We also confirmed the presence of pyroptosis in electron microscopy. Furthermore, blocking the JAK pathway with baricitinib significantly reduced TNF-induced pyroptosis at the transcriptional, protein and activity levels ($P < 0.05$). Finally, blocking the JAK pathway, we observed a reduction of IL-1 bioactivity in RASFs ($P < 0.05$).

Conclusion: Our results demonstrate an important role of GSDMD-mediated pyroptosis and shed lights on a potential pyroptosis-targeted treatment. Meanwhile, JAK inhibition alleviates inflammasome-induced pyroptosis by blocking pyroptosis pathway in RASFs.

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SAT0025

NEUROMEDIN U SUPPRESSES COLLAGEN-INDUCED ARTHRITIS THROUGH ACTIVATION OF ILC2

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Background: Reduction and dysregulation of ILC2 was linked to delayed resolution of arthritis. The neuropeptide Neuromedin U (NMU) has been reported to rapidly activate ILC2 and initiate a Th2 type immune response through NMUR1 expressed on the surface of ILC2. However, one previous study reported that NMU promoted autoantibody-mediated arthritis.

Objectives: The aim of this work was to investigate the effect of NMU on collagen-induced arthritis (CIA) mice and the potential mechanisms.

Methods: CIA was induced in C57BL/6 WT and C57BL/6^{Nmu} deficient mice on day 1. WT mice were treated i.p. daily by NMU-23 (20ug/mice) or by PBS for 10 days from day 1 to 5 and day 21 to 25. The clinical scores of CIA mice were assessed every two days from day 22 and determined on a scale of 0–4 for each paw. The proportion of ILC2 as well as Th1, Th2, Th17 and Treg in spleen, mesenteric lymph node (mLN) and joints of arthritic mice were analyzed by flow cytometry on day 42.

Results: NMU-23 dramatically inhibited clinical onset and severity of arthritis in treated WT mice compared with control mice. Interestingly, NMU-deficient mice also developed significantly less severe arthritis compared with WT control (Fig 1). Flow cytometry analyses showed that the proportion of ILC2, which defined as Lin-CD45+CD127+KLRG1+ICOS+ST2+, was elevated in the joint but not in the spleen and mLN of arthritic mice treated with NMU-23. In contrast, the proportion of ILC2 was significantly lower in the spleen of NMU-deficient mice than WT control. The percentage of Th2 cells in the spleen and mLN tend to be higher in NMU-23 treated mice, but there is no statistical significance. Surprisingly, Th1 cells were increased in the mLN of NMU-23 treated and NMU-deficient mice compared with control whereas Th17 was comparable among groups. In addition, the proportion of Treg was decreased in the joint of NMU-23 treated and NMU-deficient mice compared with control mice.

Conclusion: Our preliminary results show that repeated injection of NMU-23 during induction (early) and development (late) stage of CIA strongly suppressed clinical onset and severity of arthritis, which might be ascribed to activation of ILC2 in the joint. Further study is needed to explore other cellular and molecular mechanisms in the effect.

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Figure 1. Arthritis score among 3 group of mice.

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SATURDAY, 06 JUNE 2020

Rheumatoid arthritis - prognosis, predictors and outcome

SAT0026

HIGHER LEVELS OF NATURAL ANTI-PHOSPHORYLCHOLINE ANTIBODIES ARE ASSOCIATED WITH LOWER RISK OF INCIDENT CARDIOVASCULAR EVENTS IN YOUNGER PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: The increased cardiovascular (CV) risk in rheumatoid arthritis (RA), especially in seropositive RA, is not fully explained by traditional risk factors. Immuno-inflammatory mechanisms and autoantibodies could be involved in the pathogenesis of atherosclerotic disease. Recent studies have suggested that anti-phosphorylcholine antibodies (anti-PC) of IgM subclass counteract the generation of senescent and IL-17+ T-cells, have atheroprotective effects and may play a role in formation and stabilization of atherosclerotic plaque.

Objectives: To investigate the association between IgM anti-PC antibodies with cardiovascular (CV) morbidity in patients with RA in age and sex groups and by serostatus.

Methods: The study population was derived from the BARFOT early RA cohort, recruited in 1994-1999. The outcome was CV events i.e. AMI, angina pectoris, coronary intervention, ischemic stroke and TIA tracked through the Swedish Hospital Discharge and the National Cause of Death Registries. The RA-disease measures and traditional risk factors were assessed according to the protocol. Sera collected at inclusion and the 2-year visit were analyzed with ELISA to determine levels of anti-PC IgM (Athera CVDefine kit, Athera Biotechnologies AB). The Kaplan-Meier estimates and Cox proportional-hazards regression models were applied. Analysis was stratified by median level of IgM anti-PC and performed within strata of age, sex and RA-autoantibodies.

Results: In all, 654 patients with early RA, 68% women, mean (SD) age 55(14.7) years, DAS28 5.2(1.3), 60% RF-positive and 60% ACPA-positive without prevalent CVD were included in this analysis. The level of IgM anti-PC at baseline was median (IQR) of 60.9(36.4-94.9) and at 2 years 56.0(32.3-84.2) U/ml. During follow-up of > 10 years, 141 incident CV events (21.6%) were registered. The levels of anti-PC both at inclusion and after 2 years of observation were lower in participants who experienced CV event than in those who did not, $p=0.020$ and $p=0.012$. The CV event-free survival differed between patients with levels of anti-PC above median compared with those with levels below, $p=0.003$ by log-rank test. The risk for incident CV event showed a 0.6-fold hazard (95% CI, 0.4-0.8) among patients with higher anti-PC levels as compared with those with lower levels, $p=0.003$. In the age groups, the risk for incident CV event was lower in patients aged <55 years at inclusion than in those who were older, hazard ratio (HR) 0.40 (0.17-0.94), $p=0.036$. This result persisted when adjusted for sex and all traditional risk factors, HR 0.36 (0.14-0.92), $p=0.032$. Also, the risk for incident CV events was lower in patients with higher anti-PC levels in females, HR 0.61 (0.39-0.45), and double RF- and ACPA- negative patients, 0.44 (0.21-0.90), in crude analyses. The favourable effect of anti-PC at baseline and the CV outcome was not observed in ages >55 years, males, ACPA+ and RF+ patients. There were no significant association between anti-PC level at 2 years and outcome.

Conclusion: These results suggest that higher levels of IgM anti-PC are associated with a lower risk of incident CV events over 10 years in younger patients. The favourable atheroprotective effect of IgM anti-PC may be a part of explanation of lower risk of atherosclerotic disease in younger persons, females and in those with seronegative RA.

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SAT0027

DEVELOPMENT AND VALIDATION OF A NOMOGRAM COMBINING CLINICAL AND HISTOPATHOLOGICAL SYNOVIAL FEATURES FOR PREDICTING EARLY TREATMENT RESPONSE IN NAIVE TO TREATMENT RHEUMATOID ARTHRITIS

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Background: Rheumatoid Arthritis (RA) is characterized by synovial tissue (ST) heterogeneity at disease onset in terms of inflammatory degree and microanatomical organization being related to treatment response.

Objectives: To develop a multiparametric tool for baseline treatment response prediction including disease characteristics and histopathologic features of ST biopsies, using a large single center (SYNGem Unit) naive to treatment RA cohort.

Methods: 240 naive to treatment RA who underwent US-guided ST biopsy, at the first clinical evaluation, were enrolled. Clinical and immunological characteristics were recorded for each patient. All ST FFPE specimens were stained with H&E and classified by a pathologist, blinded to clinical characteristics, using the Krenn score [1] to assess the degree of ST inflammation. All naive to treatment RA were treated according to the T2T scheme and DAS remission rate at 6-12 months was recorded. On the basis of the regression analysis, a nomogram was constructed that incorporated the significant factors predicting the "achievement of DAS-Remission at 6 months follow-up" in naive RA. The performance of the nomogram was assessed by discrimination and calibration.

Results: Univariate analysis showed that RA who achieved early (6 months) DAS-remission had, at baseline, significantly lower total Krenn score ($p<0.001$), shorter symptoms duration ($p=0.005$) and lower disease activity ($p<0.001$) than RA not achieving this clinical outcome. ROC curve analysis revealed that RA having, at baseline, a total Krenn score <4.5 [(AUC)95%CI.: 0.67(0.60-0.74), $p<0.001$] achieved more likely DAS-remission at 6 months (53.1%) than RA with total Krenn score ≥ 4.5 (28.9%, $p<0.001$). Interestingly, RA whose ST was biopsied within 3 months from joint symptoms beginning showed significantly lower ST inflammation as total Krenn score than RA whose ST was analyzed among 3-12 months ($p=0.04$) or after 12 months ($p=0.002$) since symptoms beginning. However, in terms of follicular structure presence, the microanatomical organization of the synovial inflammatory infiltrate did not differ comparing RA whose ST was biopsied within 3 months from joint symptoms beginning (44.4%) and RA whose ST was biopsied among 3-12 months (47.6%, $p=0.74$) or after 12 months (52.7%, $p=0.33$) since symptoms beginning.

Logistic regression analysis revealed that, at baseline, being VERA, not having HDA and having a total Krenn score <4.5 were synergistic factors of DAS-remission achievement at 6 months [OR:10.5(95%CI:2.28-48.01); $p<0.05$]. Based on the regression analysis, a nomogram integrating baseline clinical (disease activity and duration) and histological (total Krenn score) characteristics was developed in which the value of each of the variables was given a point score. A total score was calculated by adding each single point score and, by projecting the value of the "total points" score to the "probability" line up to 87.5%.

Conclusion: Krenn score is a reliable tool for the semi-quantitative assessment of ST inflammation on US-guided ST biopsies being contingent to baseline disease characteristics and can be integrated within a nomogram to better predict the therapeutic response in naive to treatment RA.

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