disease activity group; 9 from remission and 19 from HC) the level of IL-6 in serum was evaluated.

Results: Patients with RA in comparison to HC have had a lower *FCER1G* methylation (0.98 [0.73-1.46] vs 1.96 [1.44-3], $p<0.00001$; median [interquartile range]) and miR-106b (0.79 [0.49-1.68] vs 1.54 [0.88-2.51], $p=0.008$) and miR-17 (1.26 [0.41-2.04] vs 2.44 [2.09-3.47], $p=0.0001$) expressions. No difference in methylation between high and remission RA groups was found. MiR-106b and miR-17 expressions were different between RA patients with high disease activity and remission ($p=0.009$ and $p=0.003$, respectively), however a high disease activity group was not different to HC ($p=0.82$ and $p=0.12$, respectively). Detailed results are presented in Table 1. A strong correlation between IL-6 levels and *FCER1G* methylation ($r_s = -0.46$) was found.

Table 1. Methylation and expression between patients divided by disease activity in compare to controls.

	High disease activity, n=29	Remission, n=21	HC, n=24
<i>FCER1G</i> methylation	1.11 [0.83-1.52]	0.96 [0.61-1.18]	1.96 [1.44-3]
miR-106b expression	1.36 [0.63-1.76]	0.54 [0.19-1.19]	1.54 [0.88-2.51]
miR-93 expression	0.63 [0.49-1.21]	0.59 [0.15-1.5]	1.03 [0.65-1.38]
miR-17 expression	1.46 [1.05-2.54]	0.34 [0.11-1.26]	2.44 [2.09-3.47]

Data are given by median [interquartile range]. *FCER1G*, Fc receptor gamma chain gene; HC, healthy controls; miR, micro-RNA; RA, rheumatoid arthritis patients.

Conclusion: *FCER1G* methylation was found as a new epigenetic marker of RA, which is independent of disease activity and may be associated with IL-6 production. Plasma miR-17 and miR-106b can be considered as a novel molecular biomarkers of disease severity in RA.

FcRγ may plays a significant role in RA pathogenesis and *FCER1G* gene methylation was found as a new, epigenetic and promising marker of RA.

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AB0097

TISSUE CYTOKINES AS NEW DIAGNOSTIC BIOMARKER OF BONE METABOLISM DISORDERS IN RHEUMATOID ARTHRITIS

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Background: Bone mineral density and proteins/peptides determination in blood and urine as markers of bone resorption and formation are currently used to diagnose osteoporosis (OP) and metabolic bone diseases. Recent evidence suggests that in RA changes in the secretion of hormones of white adipose tissue can be revealed [1,2,3,4].

Objectives: To study the clinical and diagnostic value of serum fetuin A, nesfatin, hemerin, leptin, adiponectin, resistin, visfatin determination in RA patients complicated by OP.

Methods: We examined 88 women with documented diagnosis of RA and mean disease duration of 6.56±0.88 years. We used EULAR/ARA 2010 criteria to diagnose the patients. Female patients with II degree of disease activity (DAS28), Steinbrocker stage II (erosive), rheumatoid factor- and anti-cyclic-citrullinated peptide antibody-positive were prevalent. We excluded patients who had surgery or developed an infection within the last 8 weeks, pregnant and breast-feeding women, those with severe heart, liver or kidney disease, immune deficiency, leucopenia or chronic infection.

A control group of 45 healthy females aged of 25 and 59 years were included in the study. There were no reported findings of joint pain and RA symptoms in the group. The groups were adjusted for age ($p>0.05$) and showed no statistically significant differences.

We measured serum fetuin A, nesfatin, hemerin, leptin, adiponectin, resistin, visfatin levels (μg/ml) using ELISA commercial test systems. We used spectrophotometer with wavelength of 450nm to detect the test results («Multiskan» immunoenzyme analyzer, Finland). We plotted a curve using computer software. We diagnosed OP using dual-energy X-ray absorptiometry with LUNAR DPX PRO (GE, USA).

Results: At the first stage, the level of pro-inflammatory cytokines was studied in a group of healthy individuals. Then, the reference values of these indicators were measured as $M \pm 26$. Patients with OP and RA had significantly higher levels of serum pro-inflammatory cytokines ($p<0.001$). For example, mean serum Adiponectin levels in RA patients who had normal bone density and had no OP were 35.21±0.6 μg/ml. Mean serum Adiponectin levels in RA/OP patients with low bone mineral density were 52.42±0.69 μg/ml. Adiponectin levels of 44 μg/ml and higher were associated with osteoporosis. Adiponectin levels of 43.9 μg/ml and lower were associated with normal bone density. Other pro-inflammatory cytokines have demonstrated similar dynamics of level serum.

Conclusion: Thus, we revealed that fetuin A, nesfatin, hemerin, leptin, adiponectin, resistin, visfatin levels depend on osteoporosis presence in RA patients. The test may be used to reduce the risk of low-energy fractures and to improve the quality of life in RA.

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AB0098

THE EFFECT OF MALT1-DEFICIENCY ON THE EFFECTOR PHASE OF EXPERIMENTAL AUTOIMMUNE ARTHRITIS

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Background: The paracaspase Malt1 is a cysteine protease, which forms a complex leading to the activation of the in proinflammatory transcription factor NF-κB in lymphocytes with CARMA1 and Bcl10. Previously, we showed that the myeloid equivalent of CARMA1, Card9 is important in neutrophils in Fcγ receptor-mediated cytokine release together with Bcl10 and Malt1. In line with these findings, we observed a significant decrease in the severity of autoantibody-triggered arthritis in the absence of Card9 and Bcl10.

Objectives: Our aim was to directly investigate whether the genetic deficiency of Malt1, the third component of the complex altered the process of the K/BxN serum transfer arthritis (that resembles to the effector phase of rheumatoid arthritis).

Methods: We used wild type and Malt1^{-/-} mice for our experiments. Autoantibody-mediated arthritis was induced by a single intraperitoneal injection of K/BxN serum. Clinical signs of joint inflammation were scored on a scale based on the cardinal inflammatory clues for two weeks. Ankle thickness was measured by a spring-loaded caliper.

Results: Similar to the deficiency of the other two components of the complex, Malt1^{-/-} mice showed a partial, but significant decrease in the macroscopic joint inflammation compared to arthritic serum-treated wild type animals during the entire experimental process. In line with this phenomenon, Malt1^{-/-} animals had reduced autoantibody-triggered ankle thickening.

Conclusion: Our results show that Malt1 seems to be an important molecule in the development and progression of experimental autoantibody-induced arthritis in mice, highlighting the role of the molecule as a potential therapeutic target in the future.

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AB0099

METHOTREXATE REDUCES THE INVASIVE ACTIVITIES OF PRIMARY RA SYNOVIAL FIBROBLASTS

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Background: Rheumatoid Arthritis synovial fibroblasts (RASFs) are key player in tissue destruction via the production of a wide range of chemical reactions in the joint with high growth rate and resistance to mortality [1]. Methotrexate (MTX) is a dihydrofolate reductase inhibitor that attenuates inflammation within joints resulting in reduced cartilage and bone damage and is the anchor therapy for RA. Its mechanisms of action are thought to differ from its anti-proliferative effects and are known to include increased adenosine release (2), but may also involve alterations in intracellular methyl donor status resulting in alteration in DNA methylation and gene expression.

Objectives: To investigate the effects of MTX on RASFs auto-aggressive activities, including invasion, migration, proliferation and apoptosis.

Methods: RASF were derived from knee biopsies of RA patients taken at arthroscopy (n=9). Matrigel chambers were used to measure invasive activities. The cells were incubated with DMSO (control), 1µM or 10µM MTX for 96 hours. Wound healing (scratch assays) were used to measure migration. Proliferation and apoptosis was determined using BrdU and caspase-3/7 assays respectively. Significance was determined via repeated measures ANOVA using SPSS software.

Results: Incubation with MTX resulted in significantly reduced invasive activity compared with DMSO control; 1µM (35%, p=0.006) and 10µM (58%, p=0.002) in paired samples. However MTX did not have significant effects on RASF migration, apoptosis or proliferation at either concentration.

Conclusion: Our data reveals that MTX reduces the invasive potential of RASFs in vitro, this effect may contribute to the clinical efficacy of this agent. Further investigation will involve epigenome-wide methylation to determine if the DNA methylome of RASFs is altered by MTX.

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AB0100

PHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF SYNOVIAL FLUID-DERIVED FIBROBLAST-LIKE SYNOVIOCYTES IN RHEUMATOID ARTHRITIS

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Background: Fibroblast-like synoviocytes (FLS) are central cellular components in persistent inflammatory joint diseases such as

rheumatoid arthritis (RA). Pathological subsets of FLS have been identified from synovial tissue. However, the synovial tissue obtained from arthroplasty procedures is acquired at late disease stages and the cellular yield obtained from synovial tissue biopsies is fairly low. Collectively, challenging the robustness of human RA *in vivo* and *in vitro* models. FLS obtained from the synovial fluid (SF-FLS) are proposed as an alternative source of FLS, but a detailed phenotypical and functional characterization of FLS subsets from the synovial fluid has not been performed.

Objectives: The aim of this study was to determine the phenotypical and functional characteristics of synovial fluid-derived fibroblast-like synoviocytes in rheumatoid arthritis.

Methods: In the present study, paired peripheral blood mononuclear cells (PBMC) and SF-FLS from patients with RA were obtained (n=7). FLS were isolated from the synovial fluid by a strict trypsinization protocol¹ and their cellular characteristics and functionality were evaluated at passage 4. Monocultures (SF-FLS) and autologous co-cultures (SF-FLS and PBMC) were established from five patients with RA and subsequently evaluated by flow cytometry, Western blotting and multiplex immunoassays. Human cartilage-sponges (n=3) with SF-FLS and without SF-FLS (n=3) were co-implanted subcutaneously in SCID mice (n=15), mice with only cell-free human cartilage-sponges were used as controls (n=12). After 45 days, the implants were evaluated using stained sections to determine the SF-FLS invasion score based on perichondrocytic cartilage degradation. Data are expressed as median (25-75 percentile). P-values <0.05 were considered statistically significant.

Results: The homogeneous subpopulations of FLS, isolated from the synovial fluid, were negative for CD34 and CD45 [98.9%, (97.5-99.7)] and positive for Thy-1 and PDPN [94.6%, (79.9-97.4)]. Without stimulation, RA SF-FLS showed high and comparable levels of NFκB related pathway proteins and secreted multiple pro-inflammatory cytokines and chemokines dominated by IL-6 [2648 pg/mL, (1327-6116)] and MCP-1 [2458 pg/mL, (692-8719)]. SF-FLS increased their ICAM-1 and HLA-DR expression after encountering autologous PBMCs (p<0.01), (p<0.05). Further, SF-FLS and PBMC interacted synergistically in a co-culture model of RA and significantly increasing the secretion of several cytokines (IL-1β, IL-2, IL-6, (p<0.01)) and a chemokine (MCP-1, (p<0.01)). The invasion score of the human SF-FLS *in vivo* was at primary site, [1.6, (1.3-1.7)] and contralateral implantation site [1.5, (1.1-2.2)]. The invasion score of the human SF-FLS-containing implants both at primary and contralateral site were significantly higher compared with cartilage-sponges evaluated from SF-FLS-free control mice (p<0001).

Conclusion: This phenotypical and functional characterization of SF-FLS, acquired and activated at the site of pathology, lays a foundation for establishing *in vivo* and *in vitro* FLS models. These FLS models will be beneficial in our understanding of the role of this cellular subset in arthritis and for characterization of drugs specifically targeting this pathological RA FLS subset.

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