biological markers. Using unbiased magnitude-based hierarchical clustering of biomarker expression levels, we isolated a patient subset (n=9) with distinctively heterogeneous patterns of expression of the 17 biological parameters, compared to the other (n=101) patients who were more homogeneous. The smaller subgroup had significantly higher levels of MIF, CCL2, CCL19 and CXCL10, but the larger subgroup had stronger associations between biological parameters and SLEDAI-2k, based on leave-one-out cross-validated regression analysis. In this subgroup, when we constructed a time-dependent regression model, compared to the equivalent time-agnostic regression model, the biological parameters had significantly stronger predictive power for disease activity, suggesting a time-dependent relationship. To disentangle the effect of magnitude versus temporal correlation, we used dynamic time-warping analysis to align longitudinal clinical and laboratory profiles. This revealed a further subset (n=69) in whom a time-dependent regression model showed significantly stronger associations between biological parameters and disease activity, despite no significant difference in simple magnitude. This subgroup was characterised by lower rates of flare, lower disease activity and lower damage scores, suggesting that this patient cluster is highly clinically meaningful.

Conclusions: Using aggregated longitudinal clinical data and samples, we demonstrated significant subgroups of time-dependent relationships between disease activity and biological markers among patients with SLE. These results imply the association between biological parameters and disease activity may exist in a multi-dimensional time-dependent pattern. Longitudinal SLE data presents potential opportunities to identify patient-stratifying biomarker patterns that are concealed when time is not considered. This finding has significant implications for the design of SLE biomarker studies.

Disclosure of Interest: None declared

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SAT0034

POSSIBLE INVOLVEMENT OF BAFF AND MATRIXMETALLOPROTEINASE-9 IN THE ACTIVATION OF MONOCYTES OF PATIENTS WITH PRIMARY SJÖGREN'S SYNDROME

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Background: B cell activating factor belonging to TNF family (BAFF) is well known as a factor which regulates proliferation, differentiation and survival of B cells, and plays a pivotal role in the pathogenesis of primary Sjögren's syndrome (pSS). In our previous study, we found that BAFF significantly enhanced IL-6 production by pSS monocytes and the amount of IL-6 produced by BAFF-stimulated monocytes was positively and significantly correlated with the expression level of a BAFF receptor (BR3). These data collectively suggest that the BAFF signalling through BR3 is involved in activation of monocytes to promote production of inflammatory cytokines, such as IL-6. Matrix metalloproteinase-9 (MMP-9) is well known as one of the enzymes involved in degradation of extracellular matrix (ECM) and mainly produced by activated T cells and monocytes. It has been reported that the concentration of MMP-9 in saliva was significantly higher in pSS patients as compared to healthy controls (HC). Therefore, it is conceivable that MMP-9 is involved in the pathogenesis of pSS through degradation of ECM of salivary glands, which consequently results in decrease in saliva, one of the clinical manifestations of pSS.

Objectives: To explore the relationship between BAFF and MMP-9 in the pathogenesis of pSS.

Methods: Peripheral monocytes from pSS patients (n=37) and HC (n=19) were prepared by using CD14 +microbeads and cultured *in vitro* in the presence or absence of recombinant human soluble BAFF (rhsBAFF) for 96 hours. The amounts of IL-6 and MMP-9 in the culture supernatants were measured by ELISA. Signal transduction pathways were investigated by exposing rhsBAFF-stimulated pSS monocytes to several inhibitors against NF- κ B (BAY11-7082 and BAY11-7085) and PI3 kinase (LY294002). FACS analysis of whole blood samples was performed to investigate the expression levels of BR3 and MMP-9 in monocytes. The expression level of MMP-9 was also analysed by quantitative RT-PCR (qPCR). Serum levels of BAFF and MMP-9 were measured by an electrochemiluminescence assay.

Results: Serum levels of BAFF and MMP-9 in pSS patients were significantly higher than those of HC, and the levels showed positive and significant correlation. FACS analysis of whole blood samples demonstrated that MMP-9 was mainly expressed in monocytes and that the expression level was significantly higher in pSS than in HC. ELISA and qPCR revealed that stimulation of pSS monocytes with rhsBAFF drastically enhanced the expression of MMP-9 as compared to normal monocytes. Remarkably, the amount of MMP-9 produced by the cells was positively and significantly correlated with the expression level of BR3 in pSS monocytes, suggesting that BAFF-signalling is involved in the production of MMP-9 by the cells. Moreover, the elevated production of MMP-9 was significantly

suppressed by specific inhibitors against NF- κ B and PI3 kinase in a dose dependent manner.

Conclusions: The present study suggests that BAFF stimulates monocytes through BR3 to promote MMP9 production and may contribute to ECM degradation. Our study also suggests that NF- κ B and PI3 kinase are involved in the pathway.

Disclosure of Interest: None declared

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SAT0035

THE EFFECTS OF VISFATIN, RESISTIN AND IL-17 ON SYNOVIAL FIBROBLASTS FROM DIFFERENT RHEUMATIC DISEASE BACKGROUNDS

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Background: Although rheumatoid arthritis (RA) and psoriatic arthritis (PsA) have several features in common, they also possess distinct differences. We hypothesised that RA and PsA synovial fibroblasts (SF), known key effector cells in the pathophysiology of inflammatory arthritis, differentially respond to various stimuli including adipokines and cytokines and that this may contribute to those differences. For example, IL-17 (also found in synovial tissue) is of particular therapeutic significance in PsA but not as effective in RA. Thus far, IL-17 in its isoform IL-17A has been the major therapeutic target in PsA but IL-17F also plays a role in the IL-23/IL-17 axis of inflammatory diseases.

Objectives: Therefore, we analysed the responses of SF from patients with PsA, RA or no rheumatic disease to IL-17A/F±TNF- α and the adipokines visfatin and resistin, which show strong expression in the synovium of inflammatory arthritis.

Methods: SF were isolated from patients with PsA, RA or non-rheumatic disease controls (N), each undergoing joint surgery. PsASF, RASF and NSF were stimulated with human recombinant IL-17A/F, TNF- α , visfatin, and resistin. A neutralising anti-IL-17A antibody was used to verify specificity of the IL-17A effects. Secretion of the proinflammatory cytokine IL-6 was used as the initial readout parameter and was quantified using a commercial immunoassay.

Results: Stimulation with visfatin caused a strong increase in IL-6 secretion in all SF types (n=3 each), while resistin had no effect. Differences in responses were not statistically significant between the SF types studied. IL-17A at concentrations found in serum or synovial fluid did not induce IL-6 secretion in any of the SF. Dose-response curve analysis showed that considerably higher concentrations of IL-17A, which may occur locally in tissue, are required for the induction of IL-6 secretion. An anti-IL-17A antibody abolished the effect, thus showing that the effect is specific for IL-17A. The effects of IL-17A and IL-17F on IL-6 secretion by PsASF could be strongly amplified by a co-stimulation with TNF- α (IL-17A: 5-fold vs 113-fold; IL-17F: 1.7-fold vs 39-fold; TNF- α alone: 12-fold). The effects were stronger for IL-17A than for IL-17F with or without TNF co-stimulation. No effect of IL-17F alone was observed on NSF (n=1).

Conclusions: SF from RA and PsA patients were not differentially affected by the adipokines visfatin and resistin or IL-17A when used at serum or synovial fluid concentrations suggesting inflammatory cells to be the primary target of anti-IL-17 therapy. In its use as a therapeutic target, the attribute of IL-17F affecting PsASF would potentially increase the beneficial effects.

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SAT0036

NICOTINE PROMOTES MMP-3 AND RANKL SECRETION THROUGH OVEREXRESSED NICOTINIC ACETYLCHOLINE RECEPTOR A7 IN RHEUMATOID ARTHRITIS FIBROBLAST-LIKE SYNOVIOCYTES

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Background: Smoking has been reported not only an established environmental risk factor for developing rheumatoid arthritis (RA), but also a predictor of radiographic progression. Nicotine, the major constituent of cigarette smoke, has been demonstrated inhibitory effect on proinflammatory cytokines through its receptor nicotinic acetylcholine receptor $\alpha 7$ (AChR $\alpha 7$) in RA fibroblast-like synoviocytes (FLS). However, its effects on other function of RA-FLS remain elusive.

Objectives: To explore the effect of nicotine on matrix metalloproteinases (MMPs) and RANKL expression from RA-FLS and its possible intracellular signalling mechanism.

Methods: Synovial tissues were obtained from 45 patients with active RA as well as 11 osteoarthritis (OA) and 11 noninflammatory orthopaedic arthropathies (Orth.A) patients for control. The expression of AChR α 7 in synovial membrane and cultured FLS were detected by immunohistochemistry staining and Western blot. RA-FLS were treated *in vitro* with different concentration of nicotine and its effect on RA-FLS viability was evaluated by cell counting kit-8. After nicotine pretreatment on TNF- α stimulated RA-FLS, the expression of MMP-2, MMP-3, MMP-9, TIMP-1, TIMP-2, RANKL, and OPG in culture supernatant were measured by ELISA, while the change of AP-1 pathway including c-Fos and c-Jun were detected by quantitative real-time PCR and Western blot.

Results: Immunohistochemical analyses showed intense endochylema staining for AChR α 7 mainly in lining layer. The percentage of both lining and sublining AChR α 7 positive cells were significantly higher in RA than that in OA or Orth.A (figure 1A). Further western blot showed significantly higher expression of AChR α 7 in RA-FLS than that in Orth.A-FLS ($p=0.003$, figure 1B). Nicotine (0.1 μ M~50 μ M) showed no cytotoxicity on RA-FLS proliferation. Pretreatment with 50 μ M nicotine for 24 hours significantly promoted the secretion of MMP-3 and RANKL but inhibited TIMP-1 secretion in TNF- α stimulated RA-FLS (all $p<0.05$, figure 1C). Further, 25 μ M and 50 μ M nicotine treatment for 24 hours upregulated both mRNA and protein expression of c-Fos but not c-Jun which indicated that nicotine might activate AP-1 signalling pathway by c-Fos (all $p<0.05$, figure 1D).

Abstract SAT0036 – Figure 1. Effects of nicotine on the secretion of MMPs, RANKL and OPG as well as AP-1 signalling pathway activation in RA-FLS. **A** Immunohistochemistry staining for AChR α 7 in RA synovium. The data were representative as median and interquartile range from 45 RA, 11 OA and 11 Orth. A patients. **B** The expression of AChR α 7 in RA-FLS was measured by Western blot. **C** After nicotine treatment for 24 hours, the levels of MMP-3, TIMP-1 and RANKL in culture supernatant of TNF- α stimulated RA-FLS were measured by ELISA. **D** After nicotine treatment for 24 hours, the expression of c-Fos and c-Jun in RA-FLS was measured by quantitative real-time PCR and Western blot. The data were representative as means $\pm$ SD from six different RA patients, * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Conclusions: Nicotine can promote MMP-3 and RANKL expression through overexpressed AChR α 7 in RA-FLS which might be involved in the pathogenesis of osteogenesis and bone destruction in RA.

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SAT0037 IGURATIMOD AMELIORATES BLEOMYCIN-INDUCED ALVEOLAR INFLAMMATION AND PULMONARY FIBROSIS IN MICE BY SUPPRESSING EXPRESSION OF MMP-9

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Background: Interstitial lung disease (ILD) occurs in 15% of patients with connective tissue diseases (CTD). It contributes significantly to morbidity and mortality, and is difficult to manage⁽¹⁾. Despite advances in management strategies, CTD-ILD have a significant adverse effect on quality of life and is still a leading cause of mortality, highlighting an urgent need for optimal treatment regimens. It is known that inflammatory cytokines played an important role in alveolitis and the development of pulmonary fibrosis⁽²⁾. Iguratimod (IGU) has a reported effect on

preventing the inflammatory processes by inhibiting the production of various inflammatory cytokines⁽³⁾.

Objectives: To investigate the potential therapeutic efficacy of IGU on mouse model of bleomycin (BLM) induced pulmonary fibrosis.

Methods: A total of 75 C57BL/6 mice were randomly and evenly divided into control group, BLM (5 mg/kg) group, BLM +IGU (90 mg/kg) group, BLM +methylprednisolone (MP, 10 mg/kg) group and BLM +pirfenidone (PF, 100 mg/kg) group. The mice were sacrificed on day7, day 14, day 28 respectively. The lung tissue was examined by Hematoxylin and eosin staining and Masson staining to evaluate the degree of alveolitis and fibrosis, and plasma cytokines were measured.

Results: Histopathological results showed that IGU attenuated BLM-induced alveolar inflammation and decreased collagen deposition in lung tissue from day 7 till day 28. Both the pathological alveolitis scores and fibrosis scores in drug treated group (IGU group, MP group and PF group) were decreased dramatically compared with BLM group on day7, day 14 and day 28 respectively ($p<0.05$). There were no statistical significances among those three groups. Cytokine profile showed that IGU decreased the level of tumour necrosis factor- α (TNF- α), interleukin (IL)-1, IL-6 and matrix metalloproteinase (MMP)-9 which were up-regulated by BLM on day 7, day 14 and day 28 respectively ($p<0.05$). Furthermore, there is a strong correlation between the severity of the pulmonary fibrosis and the plasma MMP-9 levels ($r=-0.836$, $p<0.001$).

Conclusions: IGU was effective in suppressing BLM-induced alveolar inflammation and pulmonary fibrosis, and it has the equal anti-inflammatory and anti-fibrotic effects as MP or PF. The therapeutic effects by IGU may be attributed to the inhibition of MMP-9 either directly or indirectly. Therefore, IGU presents as a promising agent for pulmonary fibrosis treatment.

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SAT0038 MEMBRANE-ASSOCIATED AMPD2, A NOVEL REGULATOR IN SHIFTING THE BALANCE BETWEEN EXTRACELLULAR ATP AND ADENOSINE?

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Background: Extracellular ATP and adenosine are potent immunomodulatory molecules that accumulate in states of inflammation. ATP/ADP are released from damaged or stressed cells and sequentially catabolized to AMP which is then catabolized to adenosine by the action of the ectonucleotidases CD39 and CD73, which drives a shift from an ATP-driven proinflammatory environment to an anti-inflammatory milieu induced by adenosine. Intracellularly, AMPD2 which encodes one of three known AMP deaminase homologs executes AMP deamination to IMP thereby reducing the formation of adenosine. Here, we postulate that this mode of action is also present on the cell surface of immune cells, which may lead to an increased state of inflammation such as found in chronic inflammatory diseases.

Objectives: Therefore, we analysed surface AMPD2 expression and its modulation on distinct human cell lines and primary human immune cells.

Methods: To this end, surface AMPD2 expression was evaluated on cell lines (THP1, Jurkat, HMEC1, and HEK293), human PBMCs and isolated monocytes by flow cytometry. Moreover, co-expression of surface AMPD2, CD73 and CD39 has been analysed on PBMCs and isolated monocytes. Association of surface AMPD2 and cell death was visualised using annexin V and 7-AAD staining and examined by flow cytometry. In addition, expression of AMPD2 was analysed by immunoblot of precipitated AMPD2 from membrane fractions and by mass spectrometry after precipitation from membrane fractions and from biotinylated surface-molecules using the surface AMPD2 positive cell line HEK293.

Results: Here, we demonstrate that (i) surface AMPD2 is present on T cells and monocytes in PBMCs from healthy donors, (ii) that LPS enhances surface expression of AMPD2 in PBMCs on both cell populations after 24 hour, (iii) that LPS also enhances surface expression of AMPD2 in isolated monocytes, (iv) that surface AMPD2 does not correlate to the expression of the ectonucleotidases CD39 and CD73 and (v) that all cell lines analysed are capable of expressing surface