disease activity. Unfortunately, no reliable simple or standardised assays to quantify IFN α are available in routine clinical practice. The new single-molecule array (Simoa) assay, also called digital ELISA, enables direct IFN α quantification at attomolar (i.e. fg/mL or 10^{-15} moles/mL) concentrations corresponding to 5,000-fold-increased sensitivity over commercial ELISAs.

Objectives: We hypothesised that serum-IFN α levels determined with this new standardised assay would be a better biomarker of SLE activity than the Farr test, still considered the "gold standard" for this purpose. The primary objective of this study was to characterise the relationship between digital ELISA-determined serum-IFN α concentrations and clinically assessed SLE activity. We also compared that assay to a functional, sensitive biological assay (bioassay), based on IFN α antiviral properties, used routinely in our institution for 30 years.

Methods: IFN α concentrations in serum samples from 150 consecutive SLE patients and 68 healthy donors in a cross-sectional study were determined with the digital ELISA and the bioassay. For SLE patients, clinical characteristics, Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), therapeutic regimen, Farr assay, C3 levels and other usual biological parameters were recorded on the day of the blood draw.

Results: Based on healthy blood donors, the abnormal serum-IFN α level threshold value was 136 fg/mL. Next, using receiver operating characteristics curves for an SLE-patient series, widely heterogeneous for disease activity and organ involvement, the threshold IFN α value associated with active disease was 266 fg/mL. The digital ELISA-assessed serum-IFN α level was a better biomarker of disease activity than the Farr test: its specificity, positive likelihood ratio and positive-predictive value better discerned active SLE and a flare. The digital ELISA was more sensitive than the bioassay to detect low-abnormal serum-IFN α concentrations and patients with low disease activity. In multivariate analyses, abnormal digital ELISA-determined IFN α concentrations were significantly associated with SLE-specific fever, active mucocutaneous lupus, active lupus nephritis and anti-Sm Abs but no other anti-ribonucleoprotein Abs (i.e., anti-Ro/SSA 52, anti-Ro/SSA 60, anti-La/SSB and anti-RNP).

Conclusions: Direct serum-IFN α determination with a highly sensitive assay might improve monitoring of clinical SLE activity and selection of the best candidates for anti-IFN α treatment.

Disclosure of Interest: None declared

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FRI0263

CHARACTERISATION OF EPITHELIUM-ASSOCIATED FCRL4+ B CELLS FROM PAROTID GLANDS OF PATIENTS WITH SJÖGREN'S SYNDROME USING SINGLE CELL RNA SEQUENCING

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Background: A subset of B cells expressing the inhibitory Fc receptor-like protein 4 (FcRL4) is found in salivary gland lesions of patients with primary Sjögren's

syndrome (pSS). FcRL4 +B cells are associated with ductal epithelial cells forming lymphoepithelial lesions (LEL), in particular within parotid glands¹. Furthermore, FcRL4 is expressed by mucosa-associated lymphoid tissue (MALT) lymphoma B cells.

Objectives: We aimed to investigate, by single cell and bulk RNA sequencing, how the gene expression profile of FcRL4 +B cells differs from FcRL4-negative naive and memory B cells in salivary gland tissue from pSS patients. We hypothesised that FcRL4 +B cells contribute to LEL formation and are prone to lymphomagenesis.

Methods: Parotid gland biopsies of 5 pSS patients without MALT lymphoma were obtained. Single cell suspensions were prepared by mechanical disruption and enzymatic digestion. The cells were incubated with anti-CD19, anti-CD27 and anti-FcRL4 antibodies, and sorted as single cells or 5 cells per well based on the following definitions: CD19 +CD27-FcRL4- ('naive'), CD19 +CD27+FcRL4- (memory) and CD19 +FcRL4+ (FcRL4+). Library preparation was done using an in-house SMARTseq2 protocol and sequencing was done on an Illumina HiSeq2500.

Results: Samples from 4 pSS patients passed quality control and were included. A total of 160 single cells and 360 cells in bulk were included in the analysis. Genes identified by differential expression were subjected to gene pathway analysis. Both in single cell and bulk samples, multiple genes coding for integrins, such as *ITGAX* (CD11c), were significantly upregulated in FcRL4 +B cells. Gene Ontology pathways that showed the highest upregulation in FcRL4 +B cells (both single cell and bulk) were receptor binding, GTPase and protein kinase pathways. Analysis of bulk samples further revealed that expression levels of genes encoding for Src tyrosine kinases, genes involved in the NF- κ B pathway, *CXCR3*, and *TNFRSF13B* (TACI), among others, were significantly upregulated in FcRL4 +B cells, compared with either naive or memory B cells. Expression levels of *CD40* were significantly decreased in FcRL4 +B cells.

Conclusions: FcRL4 +B cells in salivary glands of pSS patients show upregulation of genes involved in homing and cell adhesion, consistent with their tissue location close to the epithelium. FcRL4 +B cells also show increased levels of transcripts that induce inflammation and B cell survival. These cells exhibit all characteristics of chronically stimulated CD11c+memory B cells, and we speculate that FcRL4 +B cells contribute significantly to the epithelial damage seen in the glandular tissue of pSS patients.

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FRI0264

ROLE OF P-GP IN PATHOLOGIC CONVERSION OF TH17 CELLS IN LUPUS NEPHRITIS LEADING TO GLUCOCORTICOID RESISTANCE

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Background: Th17 cells and cytokine IL-17 are mainly involved in autoimmunity. Recently IL-17/IFN- γ double-positive Th17 cell were found to be allied with inflammatory diseases. P-glycoprotein (P-gp) +ve Th17 cells are refractory to steroid. P-gp on lymphocyte effluxes out steroid and prevents its action. We conducted this study with hypothesis that P-gp positive IL-17/IFN- γ double +ve Th17 are one of key pharmacokinetic and pharmacodynamic modulator liable for steroid resistance in lupus nephritis (LN).

Objectives: We planned to study the frequency of P-gp expressing pathogenic Th17 cells in steroid responsive and non-responsive steroid patients.

Methods: We analysed the frequency of pathogenic IL-17/IFN- γ double-positive Th17 lymphocytes and P-gp expression on their surface by flowcytometry in responsive (n=52; mean age 34.06 $\pm$ 10.84) and non-responsive (n=25; mean age 37.29 $\pm$ 13.73) patients. We also included 10 age and sex matched healthy controls. All patients were biopsy proven LN.

Results: We found a significant increase in the frequency of Th17 (p=0.001); Th17 (p=0.006) and IL-17/IFN- γ double positive Th17 (p=0.006) and IL-17/IFN- γ double-positive Th17 (<0.001) cells in non-responsive as compared to responsive patients and healthy controls (p<0.001). Of the total Th1; Th17 and pathogenic Th17; 78.45%; 72.37% and 95.8% cells expressed P-gp on their surface in non-responsive; 45.0%; 30.27% and 30.1% cells expressed P-gp in responsive group; and 30.91%; 15.51% and 15.62% in healthy control respectively.

Conclusions: Higher frequency of IL-17/IFN- γ doublepositive Th17 cell with P-gp expression may be associated immunological and pharmacological factor for steroid resistance in LN.

Disclosure of Interest: None declared

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FRI0265 ANGIOGENIC T CELLS IN PRIMARY SJÖGREN'S SYNDROME: A DOUBLE-EDGED SWORD

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Background: Angiogenic T cells (Tang) have been recently identified within colonies of endothelial progenitor cells (EPCs) as mediators of endothelial repair. Both Tang and EPCs are reduced in rheumatoid arthritis and this contributes to persistent endothelial damage and eventually increased cardiovascular risk. In primary Sjögren's syndrome (pSS), EPCs are expanded but no data are currently available about Tang.

Objectives: Aim of this study was to assess Tang (CD3 +CD31+CXCR4+) in peripheral blood (PB) and target organs of pSS as well as the association with EPCs (CD34 +CD133+VEGFR-2+) and clinical and serological features of the disease.

Methods: Thirty-six pSS patients and 20 sex- and age-matched healthy donors (HD) were enrolled. Phenotypic analysis of peripheral blood mononuclear cells was performed by flow cytometry using FITC, Pe, Pe-Cy7 or AlexaFluor647 labelled anti-human CD3, CD31, CXCR4, CD4, CD8, CD28, CD34, CD133, VEGFR-2, and IL-17 antibodies. Minor salivary gland (MSG) biopsies from 8 pSS patients were studied and compared to samples from 12 patients with sicca symptoms and either non-specific chronic sialadenitis (NSCS) or normal parenchyma (n=6 each). MSG sections were subjected to immunofluorescence staining to assess the presence of CD3 +CD31+CXCR4+Tang cells and the expression of the CXCR4-ligand CXCL12/SDF-1 chemokine.

Results: Circulating Tang were expanded in pSS compared to HD and were directly correlated to EPCs. Both Tang and EPCs directly correlated with disease activity as calculated with the EULAR Sjögren's syndrome disease activity index (ESSDAI). Over 60% of Tang lacked CD28 revealing a senescent phenotype. Only a small proportion of Tang displayed either CD4 or CD8, the majority of Tang being therefore CD4-CD8- double negative (DN). A subset of Tang produced IL-17 and the highest proportion of IL-17-producing cells was observed among DN cells. Immunofluorescence analyses revealed the exclusive presence of infiltrating Tang cells along with increased expression of CXCL12/SDF-1 in pSS MSGs compared to either NSCS or normal MSGs.

Conclusions: Circulating Tang cells are expanded in pSS, display a senescent phenotype, are mainly CD4-CD8- DN and produce IL-17. Moreover, Tang cells home to and infiltrate MSGs in pSS, presumably through the SDF-1/CXCR4 chemotactic axis. Our data suggest that besides their positive effect together with EPCs in endothelial repair, Tang cells may contribute to disease pathogenesis.

Disclosure of Interest: None declared

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FRI0266 CD16+ MONOCYTE SUBSETS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS, PRIMARY ANTIPHOSPHOLIPID SYNDROME, AND ANTIPHOSPHOLIPID SYNDROME WITH LUPUS ARE ASSOCIATED WITH SPECIFIC CLINICAL/SEROLOGICAL FEATURES OF THESE DISEASES

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Objectives: This study, developed within the IMI-JU project PRECISESADS framework, aimed to determine the enrichment on CD14 +and CD16+monocyte subpopulations in SLE, APS and APS +SLE patients, and to investigate their role in the pathogenesis of these diseases.

Methods: The frequencies of monocyte subpopulations in the peripheral blood of 54 healthy donors and 46 SLE patients included in the PRECISESADS study (preliminary data) were determined by flow cytometry. A second cohort of 21 APS +SLE patients and a third cohort of 19 APS patients were included. Clinical profile,

proinflammatory circulating mediators, and peroxide levels were analysed. Carotid intima media thickness (CMT) was evaluated as atherosclerosis marker

Results: The CD14 +CD16+ (non-classical) monocyte subset was reduced in SLE patients. Their frequencies were negatively associated with the positivity for anti-dsDNA, Anti-SSB, anti-SSA and anti-U1RNP autoantibodies, as well as with renal involvement, which might reflect a recruitment process of this subset in renal tissues. Correlation studies further indicated a link between the reduced frequency of non-classical monocytes and increased levels of circulating inflammatory mediators. Conversely, SLE patients with a previous history of thrombosis displayed a significant increase in circulating CD14 +CD16+monocytes in relation to those without previous thrombotic manifestations. These results prompted us to evaluate the proportion and profile of this inflammatory subtype in parallel cohorts of SLE +APS and APS patients, on which thrombosis constitute the main clinical disorder.

SLE +APS patients showed enrichment in intermediate (CD14 ++CD16+) and non-classical monocytes, associated with the positivity for anti-dsDNA antibodies and the presence of atheroma plaques. Correlations among the frequency of those monocyte subsets and inflammatory mediators, and circulating cytokines, were also demonstrated. Yet, SLE +APS patients with renal involvement displayed reduced proportion of circulating intermediate and non-classical monocytes, suggesting that, as in the case of SLE patients, circulating CD16 +cells might have migrated to the kidney.

In APS patients we also saw enrichment of the CD16 +inflammatory subsets, associated to recurrent thrombotic events and a pathologic CMT, pointing out that such subsets are associated with CVD. The scores of various markers related to autoimmunity, oxidative stress and prothrombotic molecules further correlated with the proportions of intermediate and non-classical monocytes.

Conclusions: Circulating CD16 +monocytes might constitute an important subpopulation of proinflammatory cells, whose frequency might identify APS, APS +SLE and SLE patients suffering thrombosis, atherosclerosis and organ involvement.

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FRI0267 CHARACTERISATION OF THE MOLECULAR PROFILE OF ALTERED GENES AND PATHWAYS IN MONOCYTES OF ANTIPHOSPHOLIPID SYNDROME PATIENTS RELATED TO THEIR ATHEROTHROMBOTIC STATUS. EFFECTS OF IN VIVO UBIQUINOL SUPPLEMENTATION

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Objectives: This study was undertaken to: 1. Characterise monocytes molecular profile of altered genes and pathways involved in the pathology of APS. 2. To evaluate the role of antiphospholipid antibodies in the regulation of these processes. 3. To investigate the short-term effects of *in vivo* ubiquinol (reduced coenzyme Q10 [Qred]) supplementation on the modulation of genes related to inflammation and thrombosis in this autoimmune disorder.

Methods: Monocytes from peripheral blood of 60 subjects, including 30 APS patients and 30 healthy donors (HDs) were purified by negative immunomagnetic selection (Miltenyi). Total RNA was extracted from 6 subjects –as exploratory cohort- and microarray studies were performed in an Agilent G4112F platform (Whole Human Genome Microarray 44 k). Functional categorization of the altered gene signature and molecular pathways and networks was carried out using the Ingenuity Pathway Analysis Software (IPA). The most differentially expressed genes were validated by RT-PCR in monocytes purified from all the subjects recruited to the study. Correlation and association studies were performed with clinical and analytical variables. The effects of antiphospholipid antibodies (aPL) were also evaluated by *in vitro* studies. The short-term effects of *in vivo* Qred supplementation on the monocyte gene profile were further analysed.

Results: Gene expression array identified 518 altered genes in monocytes from APS patients in relation to the control group (p<0,05 and fold change >2). IPA analysis showed that the main canonical pathways integrated by these genes were leukocyte adhesion, diapedesis and extravasation signalling, interleukin and cytokine signalling, as well as oxidative stress production and antioxidant response. This analysis further identified that the most relevant diseases in which these altered genes are involved were inflammatory and cardiovascular diseases (44%), as well as reproductive (42%), neurological (11%), renal (1%) and ophthalmic diseases (2%). The alteration of several of these genes was validated by RT-PCR and protein analysis, and associated to clinical parameters of APS patients, including thrombotic recurrences and early atherosclerosis. *In vitro* studies