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 PATHOGENIC 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.