onset of symptoms. Thus iTreg have significant positive effects on PIL, which may have consequences for future approaches in treating SLE.

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FRI0260

POLYMORPHISMS OF STAT4 AND MIR146A PREDICT THE ACHIEVEMENT OF 5 YEARS REMISSION IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with a complex pathogenesis in which genes and environmental factors interact leading to a protean clinical picture. Treat-to-target recommendations have identified 'remission' as a target in SLE, since achievement of remission improves the outcome and is associated with decreased damage progression. Nonetheless, predicting factors for the achievement of remission are lacking. It is likely that genes associated with SLE pathogenesis may influence the disease course.

Objectives: Thus, our aim was to analyse previously identified loci associated with SLE in a cohort of SLE patients to evaluate their influence on remission achievement.

Methods: We recruited 117 Italian SLE patients. A panel of 34 SNPs in 19 genes involved in immune response, autophagy and inflammation, was selected. SNPs genotyping was performed by allelic discrimination assay by TaqMan assays (Applied Biosystems, Foster City, CA, USA) and ABI PRISM 7000. The main clinical/laboratory features (including injury index and disease activity) were collected on an electronic platform. Remission was defined according to Zen et al.¹ and evaluated over 5 years. A genotype/phenotype correlation analysis was performed.

Results: The variant alleles of rs7574965 (STAT4) ($p<0.001$) and rs2910164 (MIR146a) ($p=0.031$) were significantly associated with lack of achievement of 5 years remission in SLE. Specifically, patients carrying the C allele of MIR146a were less likely to achieve 5 years remission ($p=0.01$, OR 0.235, 95% CI 0.074–0.752) as well as to achieve remission after 1, 2 and 3 years of evaluation ($p=0.002$, $p=0.001$, $p=0.002$, respectively). Among the clinical and laboratory features, 5 years remission was less likely to be achieved by patients who had arthritis in their clinical history ($p=0.007$), and who tested positive for anti-dsDNA ($p=0.005$). In a multinomial logistic regression analysis, arthritis ($p=0.022$, Exp(B)=0.255, 95% CI 0.079–0.820), anti-dsDNA ($p=0.003$, Exp(B)=0.166, 95% CI 0.051–0.537) and MIR146a rs2910164 gene variant ($p=0.046$, Exp(B)=0.250, 95% CI 0.064–0.974) were confirmed to be independent risk factors for unachieved 5 years remission (table 1).

Abstract FRI0260 – Table 1

REMISSION	Exp (B)	Exp (B) 95% CI	
		Lower	Upper
Arthritis	0255	0079	0,82
anti-dsDNA	0166	0051	0537
STAT4	0354	0,11	1139
mir146A	0,25	0064	0974

Conclusions: We describe for the first time the contribution of STAT4 and MIR146a SNPs as predicting factors for the achievement of 5 years remission in SLE. No genetic study has been performed so far in SLE, while a genetic profile of patients may be useful to predict the disease outcome.

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[1] Zen, et al. Ann Rheum Dis. 2017 Mar;76(3):562–565.

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FRI0261

ASSESSMENT OF AUTOPHAGY FUNCTION IN SYSTEMIC LUPUS ERYTHEMATOSUS IN RESPECT OF HYPERLIPIDEMIA AND IMMUNOSUPPRESSIVE DRUGS

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Background: Autophagy is an orchestrated homeostatic process to eliminate unwanted proteins and damaged organelles. Lipid turnover, as well, is controlled by autophagy through a process described as lipophagy. Defective lipophagy has been already linked to important metabolic disorders such as fatty liver, obesity and atherosclerosis.

Objectives: Assessment of autophagy focusing on lipids regulation in untreated newly diagnosed systemic lupus Erythematosus (SLE) patients and after three months of treatment with immunosuppressive drugs.

Methods: Subjects in this study have been divided into three groups. Group 1 included 60 newly diagnosed SLE patients before receiving any treatment, group2 included the same subjects of group 1 after three months of treatment with immunosuppressive drugs and group 3 included 30 healthy donors of matched age and sex as a control group. For each subject, disease activity was assessed by (SLE-DAI) score, lipid profile was measured in addition to evaluation of lipids uptake, enhanced phagocytosis and intracellular killing ability of monocytes and neutrophils using Sudan Black B stain and Nitroblue tetrazolium stain mixed with latex particles coated with antibodies. Microscopic pictures were captured and quantified by ImageJ.

Results: 95% of patients were females(57/60) with mean of age(39.7±8.6). Mean of SLEDAI score in group 1 was (18.6±3.4) decreased in group 2 (3 months after treatment) to (10.4±4.2). There was a positive correlation between total cholesterol, LDL and triglycerides and disease activity(SLEDAI score) ($r=0.677$, $r=0.603$ and $r=0.718$; respectively). On the contrary, There was a negative correlation between HDL and disease activity($r=-0.396$). Furthermore, there was a negative correlation between lipid content of cells and intracellular killing and disease activity($r=-0.258$ and $r=-0.324$; respectively). After 3 months, 100% of patients were taking Corticosteroids and Hydroxychloroquine(60/60).18.3% of patients received Azathioprine(11/60), 40.0% received Cyclophosphamide(24/60) and 15% received Mycophenolate(9/60) besides Corticosteroids and Hydroxychloroquine. Comparing group 2 to group 1, there was significant increase in cholesterol, LDL and triglycerides ($p=0.027$, $p=0.021$ and $p=0.017$; respectively) while HDL showed insignificant difference($p=0.0740$). Lipid content in cells and intracellular killing significantly decreased($p=0.0322$ and $p=0.0271$; respectively).

Conclusions: Autophagy is deficient in patients with SLE aggravated by immunosuppressive drugs so they are more susceptible to infections and dyslipidemia. Consequently, lipid lowering drugs are definitely required to decrease comorbidity.

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FRI0262

MONITORING DISEASE ACTIVITY IN SYSTEMIC LUPUS ERYTHEMATOSUS WITH DIGITAL ELISA QUANTIFICATION OF SERUM INTERFERON- α

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Background: To date, anti-dsDNA-Ab titration, better achieved with the Farr test, has been used to monitor global disease activity in systemic lupus erythematosus (SLE). Indeed, anti-DNA-Ab-positivity is associated with overall SLE activity. However, the sensitivity and specificity of that association are relatively low. The close association between Interferon alpha (IFN α) expression and SLE activity suggests that monitoring this cytokine might help physicians better evaluate

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.

REFERENCE:

[1] Haacke, et al. J Autoimmun. 2017;81:90–8.

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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.