monitor the organisation and severity of the salivary gland infiltrate has been hampered by the lack of sensitive tools to describe TLS extent and features.

Objectives: To investigate CXCL13 and CXCL12 serum and tissue expression and to find any possible association with clinical, histological and laboratory features.

Methods: We studied histological features of the minor salivary glands (MSG) and sera of respectively fifty and seventy (table 1) unselected consecutive patients with pSS (AECG criteria). Concentration of CXCL13 and CXCL12 were evaluated by ELISA in patient sera and eleven healthy controls (HC). Paraffin embedded MSG were studied by haematoxylin/eosin and anti-CD3, anti-CD20, anti-CD21 staining. Images analysis was used to calculate focus score (FS), mean foci area, percentage of infiltration (%i), segregated foci (%SF), %GCs and lymphoepithelial lesions (%LEL). GCs from MSG and tonsils were microdissected and quantitative PCR was used to test CXCL12 and CXCL13 transcripts.

Results: Histological analysis unveiled strong correlations between the mean foci area with the %i and the presence of SF; positive correlations were also observed between the %i and both the FS and %SF. This was significantly higher in patients exhibiting SF. The % of SF positively correlated with FS, presence of % GC and %LEL that also correlated with the %i and the %SF (image). Mean CXCL13 and CXCL12 serum levels were significantly higher in pSS compared to HC [(124.12±119.73 pg/ml vs 8.9±15.4 pg/ml (p=0.001) and 34.6±54.2 pg/ml vs 2.5±8.3 pg/ml (p=0.05), respectively]. CXCL13 was significantly higher in patients with SF, with GCs and LEL and correlated with the mean foci area, the %i, the FS and the percentage of LEL. Higher CXCL13 levels were associated with the presence of antibodies and other biological findings including hyperglobulinemia. Higher CXCL13 levels were also able to discriminate patients with lymphoma (p=0.009). CXCL12 levels correlated with the FS, %i and % of LEL. Transcript analysis showed no difference in the expression of CXCL13 between MSG and tonsil GC, whilst CXCL12 was found significantly higher in MSG (p<0.0001).

PATIENTS	SERUM ANALYSIS	HISTOLOGICAL ANALYSIS
Demographic features	Mean±SD	Mean±SD
Mean age	54.6±15.5	54.8±12.5
Mean age at diagnosis	51.7±12.2	52.1±12.2
Laboratory/Clinical features	Number/70 (%)	Number/50 (%)
ANA	50/70 (71.4)	37/50 (74)
Anti-Ro/SSA antibodies	38/70 (54.3)	16/50 (32)
Anti-La/SSB antibodies	20/70 (28.6)	13/50 (26)
Rheumatoid factor	20/70 (28.6)	12/50 (24)
Hypergammaglobulinemia	23/70 (32.9)	15/50 (30)
Cryoglobulins	2/70 (2.9)	2/50 (4)
Monoclonal component	8/70 (11.4)	8/50 (16)
Hypocomplementemia	9/70 (12.9)	5/50 (10)
Leucopenia	12/70 (17.1)	5/50 (10)
Xerostomia	61/70 (87.1)	46/50 (92)
Xerophthalmia	60/70 (85.7)	46/50 (92)
Gland swelling	24/70 (34.3)	15/50 (30)
Lymphoma	8/70 (11.4)	0/50

Conclusions: Our results suggest the utility to expand the parameters of histologic evaluation of MSG, whilst reinforcing the role of the FS as reliable instrument to reflect the severity of inflammation. Analysis of MSG infiltration and foci segregation was able to identify subjects with increased proliferative risk. We demonstrated that serum CXCL13, is a biomarker of histological severity and is able to stratify patients with lymphoma. The high levels of CXCL12 in MSG GC suggest a differential biology of TLS in the SG, probably implicated in aberrant B cell clone survival.

Disclosure of Interest: None declared

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FRI0290

PRO-INFLAMMATORY CYTOKINES PROMOTE GLOBAL AND GENE-SPECIFIC CHANGES IN DNA METHYLATION IN SALIVARY GLANDS FROM SJÖGREN'S SYNDROME PATIENTS

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Background: Salivary glands (SG) from Sjögren's syndrome (SS)-patients show chronic inflammation and altered unfolded protein response (UPR). Pro-inflammatory cytokines induce epigenetic changes including DNA methylation, a dynamic and complex process where cytosines of CpG sites are methylated (5mC) by DNA methyltransferases (DNMT), and then hydroxymethylated (5hmC) by TET enzymes.

Objectives: To determine DNA methylation in promoters of specific UPR genes and levels of 5mC, 5hmC, DNMT and TET enzymes in labial-SG from SS-patients and cytokine effects on global DNA methylation and DNA methylation of specific gene promoters in human SG cells

Methods: SG biopsies from 23 SS-patients and 15 controls were analysed. 5mC and 5hmC levels were assessed by immunofluorescence (IF), quantified independently in epithelial and inflammatory cells and correlated with focus score. mRNA levels of DNA methyltransferases (DNMT1, DNMT3a, and DNMT3b), and dioxygenases TET1, TET2 and TET3 were determined by RT-qPCR. The *in situ* protein levels of these enzymes were evaluated by IF. Specific DNA methylation of IRE1α, XBP-1, GRP78, ATF4 and ATF6α gene promoters was evaluated by MS-HRM. Human SG cells (HSG) and 3D-acini were incubated with 1 or 10 ng/mL TNF-α and IFN-γ for 24 hour. Levels of 5mC, 5hmC, methylation of specific gene promoters and transcript levels of UPR molecules, DNMTs and TETs were determined.

Results: LSG epithelial cells from SS-patients showed significant increase of DNA hydroxymethylation and decrease of DNA methylation. Their 5hmC levels were positively and 5mC levels inversely correlated with focus score. Inflammatory cells showed high levels of 5mC and DNMTs and low levels of 5hmC. Increased mRNA levels of DNMT1, DNMT3a, and TET2 and a significant decrease of TET1 and TET3 were observed. Protein levels of TET2 were significantly higher in LSG epithelial cells from SS-patients. The above results were reproduced in HSG cells where cytokine stimulation increased TET2 and 5hmC and decreased 5mC levels. SS-patients SG and 3D-acini stimulated with cytokines, revealed an inverse correlation between gene promoter DNA methylation and transcript levels of IRE1α, XBP-1, GRP78, ATF4 and ATF6α.

Conclusions: Pro-inflammatory cytokines promoted increase of 5hmC and decrease of 5mC in SG epithelial cells likely by inducing TET2 expression. Global DNA hypomethylation have also been observed in other autoimmune diseases, where some specific genes appear to be hypermethylated. Our results showed a concordance between the methylation of UPR gene promoters and its transcriptional regulation, which was modulated by cytokines. High DNMTs protein levels observed in inflammatory cells are consistent with high levels of 5mC suggesting that increased DNMTs transcript levels in LSG from SS-patients come from inflammatory cells. 5mC has been associated with transcriptional repression, while 5hmC with transcriptional activation, therefore, we postulate that changes of DNA hydroxymethylation resulting from altered levels of TET2 could have an etio-pathogenic role in Sjögren's syndrome.

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FRI0291

CHARACTERISATION OF MONOCYTE POPULATIONS IN PERIPHERAL BLOOD OF SLE PATIENTS

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Background: Systemic lupus erythematosus (SLE) is an autoimmune diseases characterised by dysregulation of immune cell function with numerous clinical manifestations. Along with B and T cells, myeloid cells play an important role in disease pathogenesis. Circulating monocytes are recruited to sites of inflammation where they play an active role in mediating tissue inflammation and injury.

Objectives: To understand the changes in circulating monocyte phenotypes in SLE patients.

Methods: Peripheral blood was collected from 25 female SLE patients who were autoantibody positive (dsDNA/Ro/La/SM), with SLEDAI between 2 and 6 (all patients on hydroxychloroquine, 6 patients on steroids, and 3 on MMF); TR BIO Inc, Hawthorne, NY). Gender matched healthy controls (27 HC) were obtained from BI volunteers. Using multicolor flow cytometry, the percent changes in the expression of HLA-DR/DP/DQ, CD163, CD40, CCR1, CX3CR1 and CD11b on CD14⁺, CD14⁺CD16⁺, and CD16⁺ monocytes were evaluated. In a smaller subset of these patients (8 SLE and 15 HC), circulating endothelial cell (CECs) phenotyping was performed by evaluating: CD45, CD133/1, CD106, CD105, CD31, CD46, CD34, and CX3CL1. Human 38-plex cytokine/chemokine multiplexes (Luminex, Millipore) were used to evaluate serum analytes. To understand the regulation of cytokines and chemokines from the monocyte population, HC monocytes were sorted to 95% purity, stimulated with disease-relevant TLR ligands, and profiled for their analytes. Statistical analyses were performed using GraphPad Prism, version 7, GraphPad Software Inc. and determined by the Mann-Whitney t-test.

Results: SLE patients had a significant increase in MFI for CD14⁺CD11b⁺ and CD16⁺CD11b⁺ and percentages as well as MFI for CD14⁺CD163⁺, CD16⁺CD163⁺, and CD14⁺CD16⁺CD163⁺ as compared to controls. An increase in the percentage of CX3CR1⁺ CD14⁺ and CD14⁺CD16⁺ monocytes were observed, see table 1. Concurrently, an increase in CX3CL1 percentage was observed in immature circulating endothelial cells (ICECs) (p<0.0366) as compared to HC. Increases in monocyte/macrophage-associated cytokines and