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POS0174 IMMUNOPHENOTYPE OF SJÖGREN'S SYNDROME AND SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS IDENTIFIED TWO ENDOTYPES WITH POTENTIAL THERAPEUTIC IMPLICATIONS

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Background: Primary Sjögren's syndrome (pSS) and systemic lupus erythematosus (SLE) are chronic autoimmune rheumatic diseases (ARDs) that share a strong female gender bias, as well as genetic, clinical and serological characteristics. Although significant progress has been made in improving treatment and patient related outcomes in pSS and SLE, there is a need for improved early diagnosis, adequate therapy monitoring, treatment of refractory manifestations and strategies to address co-morbidities. However, the results of many clinical trials are disappointing, and nobiologic treatments are licensed in pSS, while few are available for SLE patients with refractory disease.

Objectives: Identifying shared immunological features between patients with pSS and SLE that could lead to better treatment selection using a stratification approach.

Methods: Immune-phenotyping of 29 immune-cell subsets in peripheral blood from patients with pSS (n=45), SLE (n=29) and secondary SS associated with SLE (SLE/SS) (n=14) with low disease activity or in clinical remission, and sex-matched healthy controls (n=31), was performed using flow cytometry. Data were analysed using logistic regression and multiple t-tests and supervised machine learning (balanced random forest-BRF, sparse partial least squares discriminant analysis-sPLS-DA). Patients were stratified by k-means clustering. Clinical trajectories were analysed over 5 year follow-up.

Results: Comparing the immune profile of pSS and SLE patients using a variety of statistical and machine learning (ML) approaches, identified very few statistically significant differences between the two cohorts despite patients having a different clinical presentation and diagnosis. Thus, we hypothesised that immune-based subtypes could be shared between pSS, SLE and SLE/SS patients. Unsupervised k-means clustering was applied to the immunological features of the combined patient cohorts and two distinct patient endotypes, were identified: Group-1 (n=49; pSS=24, SLE=19, SLE/SS=6) and Group-2 (n=39; pSS=21, SLE=10, SLE/SS=8). Significant differences in immune-cell phenotypes across B-cell and T-cell subsets were identified by logistic regression, BRF (AUC=0.9942, assessed by 10-fold cross-validation) and sPLS-DA analysis. Comparison of the multiple analysis approaches identified eight common immune-cell subsets, including total and memory CD4+ and CD8+ T-cell subsets but no B-cell subsets. Using this common immune-signature the stratification between the groups was maintained and slightly improved (AUC=0.9979 and accuracy 96.16%). Interestingly, patients in Group-2 had elevated disease activity measures at baseline and over a 5-year trajectory compared to Group-1. Finally, correlation analysis identified correlations between disease activity markers and the top ranked immune features from the ML models.

Conclusion: The identified immune-cell signatures could reflect the underlying disease pathogenesis that spans diagnostic criteria and could be used to select patients for targeted therapeutic approaches.

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POS0175 ASSOCIATION BETWEEN BIOMARKERS AND THERAPEUTIC PATHWAY IN PATIENTS WITH SLE

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Background: SLE is heterogeneous in clinical presentation, underlying immunology, and response to therapy. Patients with severe or resistant disease receive cyclophosphamide or rituximab. Although effective, late use of these therapies entails cumulative damage.

Emerging predictors of response to rituximab include demographics, IFN-Scores and autoantibodies. For such predictors to change treatment strategy we need to understand their association with the effectiveness of other therapeutic options at earlier decision points.

Prospective studies of treatment pathways are difficult to perform, requiring large populations followed for many years. We therefore investigated this question by analysing retrospective treatment pathways since diagnosis in patients whose biomarker status is subsequently known.

Objectives: To use interferon scores and other markers to;

- [1] identify SLE patients more likely to need escalation of therapy to either rituximab or cyclophosphamide, and
- [2] determine if this occurred sooner than in others.

Methods: In patients with established SLE, attending Leeds SLE clinic, we collected demographics, clinical characteristics and blood at a single timepoint. Two previously validated IFN-Scores[1,2] (IFN-Score A and IFN-Score B) were measured using Taqman. Autoantibodies were measured using immunoprecipitation. Treatment history covering all antimalarials, oral and intravenous immunosuppressants (agent, start and stop dates and reasons) since diagnosis was collected. Univariable and multivariable Cox proportional hazards models were used to test variables as predictors of the primary endpoint: time to cyclophosphamide/rituximab.

Results: 124 patients were included. Follow up since diagnosis was Median (IQR) 37.5 (27.4-52.3) years, total therapies per patient was 3 (1-4), therapies per year was 0.2 (0.1-0.3). 54/124 (44%) patients required cyclophosphamide/rituximab.

Results of Cox regression are shown in the table 1. There was a significant association between high expression of IFN-Score B and requirement for cyclophosphamide/rituximab as well as trends for age in the first quartile (<28 years), IFN-Score A and anti-Sm/U1RNP antibodies. Both age <28 and IFN-Score B retained trends as independent predictors in a multivariable model.

Table 1.

Characteristic	CYC/ RTX=Yes n(%)	CYC/ RTX = No n(%)	Univariable Hazard ratio (95% CI)	Univariable p value	Multivariable Hazard Ratio (95% CI)	Multivariable p value
Age						
-Q1: under 28	22 (71%)	9 (29%)	2.25 (0.99,5.12)	0.054	1.74 (0.95,3.18)	0.074
-Q2: 28 – 38	12 (39%)	19 (61%)	1.03 (0.41,2.56)	0.949	Not included	Not included
-Q3: 38 – 52	12 (39%)	19 (61%)	1.52 (0.62,3.72)	0.360	Not included	Not included
-Q4: over 52	8 (26%)	23 (74%)	reference	reference	Not included	Not included
Ethnicity						
-Caucasian	42 (48%)	46 (52%)	1.17 (0.58,2.35)	0.660	Not included	Not included
-AfroCaribbean, South Asian, East Asian, Other	10 (36%)	18 (64%)	reference	reference		
IFN Score A, mean (SD)	-2.59 (-2.67)	-3.84 (-2.71)	1.10 (0.98,1.24)	0.113	Not included	Not included
IFN Score B, mean (SD)	-2.61 (-1.16)	-3.53 (-1.76)	1.31 (1.05,1.64)	0.017	1.23 (0.98,1.55)	0.079
Anti-Sm/U1RNP Ab positive	17 (61%)	11 (39%)	1.50 (0.84,2.67)	0.171	Not included	Not included
Anti-Ro(60) Ab positive	14 (45%)	17 (55%)	0.88 (0.47,1.65)	0.686	Not included	Not included

Conclusion: Patients developing SLE before age 28 with raised interferon scores may be forecast to develop severe disease, fail oral therapies, and require rituximab. While this retrospective analysis may be confounded by a survivorship bias, our findings are consistent with other literature on these variables as predictors of severe disease. Future work will analyse more biomarkers in a larger and more varied patient population.

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POS0176

COMBINING CXCL5 WITH CONVENTIONAL THERAPY PROVIDES DURABLE IMMUNOSUPPRESSION IN MURINE LUPUS NEPHRITIS

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Background: Lupus nephritis (LN) is a condition arising from abnormal immune responses to internal organs. Patients with LN suffer from severe morbidity and mortality^{1,2}. Despite the aggressive regimen, 20-40% of patients do not respond to current conventional therapy. CXCL5, as a potent chemoattractant and activator of neutrophils³, demonstrates strong immunosuppression in the pre-clinical mouse model of graft versus host disease (GvHD)⁴ and lupus nephritis (LN) by intravenous administration.

Objectives: In this study, we aim to evaluate whether the therapeutic effect of conventional therapy could be further improved by combination therapy with CXCL5.

Methods: Ten doses of exogenous CXCL5 (3ug/kg, biweekly) together with conventional therapy (methylprednisolone (MP, intravenous (IV) injection with 8.3mg/kg/day at day-1, day-2 and day-3) + cyclophosphamide (CP, IV injection with 0.5g/BSA at day-4, monthly for 5 doses)) were administered to 8-week-old Fas^{pr} mice by IV injection. Mice were monitored for 64 weeks. Splenic immune profile at 3 weeks post treatment (PT) was measured by flow cytometry. Circulating cytokine profile were detected by Luminex technology. Renal function was evaluated by urinary spot albumin creatinine ratio. In situ renal immune cell infiltration and complement 3 deposition were detected by Haematoxylin and Eosin (H&E) and immunohistochemistry staining.

Results: Comparing to control mice (dPBS: 0% at 28 weeks PT), mice survival was improved to 100% at 40 weeks PT and 55.6% at 64 weeks PT by combination therapy of CXCL5 and conventional therapy (MP + CP) ($p < 0.0001$). The accumulation of autoantibody (anti-dsDNA) and proteinuria were reduced 61.2-fold at 32 weeks PT ($p = 0.004$) and 83.5-fold at 28 weeks PT ($p = 0.03$) respectively. Both autoantibody and proteinuria were maintained at low level for 64 weeks. The classification of LN was significantly reduced at 10 weeks PT and equivalent to the classes we observed in pre-onset mice ($p = 0.004$). Although combination therapy was not able to promote Tregs, it reduced both innate (neutrophils and macrophages) and adaptive (T_H1 , T_H2 and T_H17 cells and B cells) immunities significantly. Concomitantly, the serum level of endogenous CXCL5 was boosted up by exogenous administration from 74.2 ± 53.9 pg/ml to 254.1 ± 147.1 pg/ml at 8 weeks PT ($p = 0.05$) and this relative high concentration was maintained for 48 weeks.

Conclusion: Combining CXCL5 with conventional therapy provides effective and durable immunosuppression in murine LN and it may provide a new option for LN therapy.

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POS0177

POTENTIAL INVOLVEMENT OF IL-40 AND IL-40-PRODUCING CELLS IN PRIMARY SJOGREN'S SYNDROME (pSS) AND pSS-ASSOCIATED LYMPHOMA

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Background: The pathogenesis of pSS relies on a complex interplay between both innate and adaptive immune responses in which B cells play a pivotal role. Their chronic aberrant hyperactivation may drive clonal escape and consequent lymphomagenesis [1]. In the last few years, B cells have emerged as potential effector cells, able to release a wide range of cytokines that actively contribute to shape the microenvironment they act in. Recently, IL-40, a novel B cell associated cytokine encoded by an uncharacterized gene (C17orf99; chromosome 17 open reading frame 99) was described. Naïve B cells can express IL-40 at both tissue and peripheral level and the stimulation of B cells with IL-4 and TGF- β significantly increases IL-40 release. In addition, human B cells lymphomas are able to constitutively produce IL-40 [2]. Taking into account this emerging evidence and considering the well-known role of IL-4 and TGF- β in pSS pathogenesis, as well as the association with lymphomas, we decided to focus our attention on IL-40 in pSS patients.

Objectives: The aim of the present study was to investigate IL-40 expression in the salivary glands of patients affected by pSS and pSS-associated non-Hodgkin's lymphoma (NHL).

Methods: Minor salivary gland biopsies were obtained from 22 patients with pSS and 12 patients with non-specific chronic sialoadenitis (nSCS), included as controls. Paraffine-embedded samples of parotid glands from patients with a previous diagnosis of pSS-associated NHL (n=10) were selected from the biopsy bank of the Pathology Unit of the Ospedale Cervello (Palermo, Italy). Quantitative gene expression analysis by TaqMan real-time PCR and immunohistochemistry (IHC) for IL-40, IL-4, TGF- β 1 was performed on salivary glands from patients and controls. The cellular sources of IL-40 among infiltrating inflammatory cells were determined by fluorescence-activated cell sorting (FACS) analysis and immunofluorescence (IF). Serum IL-40 levels were measured by ELISA in both patients (n=10) and controls (n=9).

Results: IL-40 was significantly increased at both protein and mRNA level in the inflamed salivary glands of patients with pSS where a positive strong correlation between the IL-40 mRNA levels and the focus score (FS) was evidenced. The expression of IL-40 in parotid glands of pSS-associated NHL was also markedly increased (Figure 1). IL-40 expression correlated with the presence of IL-4 and TGF- β ; both cytokines were significantly increased in pSS at mRNA and protein level. Among infiltrating immune cells, CD19⁺ B cells resulted the major source of IL-40. However, we identified CD4⁺, CD8⁺ T cells and CD68⁺ macrophages as additional producers of IL-40 in both FACS and IF analysis. The ELISA test also showed a significant increase of serum IL-40 concentration in pSS patients (p value = 0.0190), compared to controls.

Conclusion: Our preliminary results suggest that IL-40 may play a role in the pathogenesis of pSS and pSS-associated NHL. To the best of our knowledge, this is the first demonstration of the overexpression of this cytokine in salivary gland tissue and sera in pSS. Moreover, we demonstrated that IL-40 is produced by several cellular types, such as T cells and macrophages, and is not exclusively released by B cells. Further studies are necessary to clarify IL-40 pathways and functions in order to unravel IL-40 possible role in pSS development.