COMPLEX IMMUNOPHENOTYPING STRATIFIES PATIENTS WITH PRIMARY AND SECONDARY SJÖGREN'S SYNDROME INTO DISTINCT CLINICALLY RELEVANT GROUPS WITH POTENTIAL THERAPEUTIC IMPLICATIONS

Nicolyn Thompson[1], Akash Gandhi, Rebecca Radmore, Vivek Gupta, George Robinson[1], David A Isenberg[1,2], Elizabeth C Jury[1#], Coziana Ciurtin[1,2#]
[1]Centre for Rheumatology, Division of Medicine, University College London, London, UK,
[2]University College London Hospital, London, UK
Share senior authorship

Corresponding Author
Dr Coziana Ciurtin, PhD, FRCP, Department of Rheumatology, University College London, 3rd Floor Central, 250 Euston Road, London, NW1 2PG, email c.ciurtin@ucl.ac.uk; phone +44 020 3447 9035.

Background: Despite recent advances in understanding the pathogenesis of primary Sjögren's syndrome (pSS), treatment of patients remains mostly empiric and symptom-based. Clinical trials with various biologic agents showed minimal or no effect in patients with pSS, suggesting that better stratification of patients based on the immune cell subsets that drive the disease pathogenesis is needed for better selection of therapeutic targets.

The aims of this study were:
1) Stratify patients with pSS, secondary SS associated with lupus (SS/SLE) and SLE based on in-depth immune phenotyping.
2) Identify shared immune endotypes underlying the pathobiology of three different autoimmune phenotypes (pSS, SLE and SLE/SS).

Methods: Peripheral blood was collected from pSS (n=55), SLE (n=38), SS/SLE (n=15) patients and age/sex-matched healthy controls (HCs) (n=34). Demographic, clinical and serological data were collected and in-depth phenotyping was undertaken on B and T-cell subsets by flow-cytometry.

Results: Patients with pSS, SLE and SS/SLE had both unique and shared defects in immune cell phenotype. Hierarchical clustering of CD19$^+$ B cells, CD4$^+$ and CD8$^+$ T cells across the three disease groups identified five distinct clusters of patients with shared immune cell defects spanning diagnosis boundaries. The main immune signatures driving the patient clustering were reduced memory (CD24$^+$ CD38$^-$), and switched memory (CD27$^+$ IgD$^-$) and increased Bm2' (transitional) B cells and CD4$^+$ Tregs (CD25^{high}CD127^{low}) populations across all three disease groups. While activated CD4$^+$T cells (CD25$^+$ CD127$^+$) were increased only in pSS patients; CD8$^+$ activated T cells were significantly increased in pSS and SS/SLE patients, but reduced in SLE patients.

Table 1 Significant differences observed in immune cell subpopulations expression across 5 distinct endo-clusters identified by unsupervised hierarchical clustering * p<0.05, ** p<0.01 *** p<0.001 by unpaired T-test or one-way ANOVA followed by Tukey's multiple comparisons post-test).

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5

CD19+ B cells	**Bm1** $p=0.002$ **Bm2'** $p=2.3^{-9}$ **Early Bm5** $p=0.0003$ **Late Bm5** $p=0.002$ **Total CD19+** $p=2.2^{-5}$	**Bm2** $p=0.0018$ **Early Bm5** $p=0.0018$		**Early Bm5** $p=0.02$ **Late Bm5** $p=0.01$	**Bm2** $p=0.003$ **Early Bm5** $p=0.01$ **Late Bm5** $p=0.04$
CD4+ T cells	**Total CD4+** $p=0.007$ **Tregs** $p=4.8^{-6}$	**Total CD4+** $p=9.0^{-5}$ **Activated CD4+** $p=0.006$ **Tregs** $p=0.02$	**Naïve** $p=0.04$ **Central memory** $p=0.01$	**Tregs** $p=0.003$	**Total CD4+** $p=2.1^{-6}$ **Naïve** $p=0.005$ **Central memory (CM)** $p=0.02$ **Effector memory (EM)** $p=1.69^{-7}$ **EMRA** $p=0.0001$ **Double negative (DN)** $p=0.009$
CD8+ T cells	**Total CD8+** $p=0.008$ **CD25-CD127-** $p=0.002$ **CD4-CD8-** $p=0.01$	**Total CD8+** $p=0.0004$ **Activated** $p=0.01$ **CD4-CD8-** $p=0.002$	**CD4-CD8-** $p=0.01$		**Total CD8+** $p=9.8^{-8}$ **Naïve** $p=5.7^{-9}$ **CM** $p=5.4^{-6}$ **EM** $p=4.6^{-8}$ **EMRA** $p=8.9^{-5}$ **Act** $p=0.03$ **CD25+CD127-** $p=0.008$ **DN** $p=0.04$

Conclusion: Our preliminary analysis proposes the possibility to re-classify patients based on their underlying pathobiology, which is likely to have diagnostic and therapeutic implications. Furthermore, we illustrate that there are distinct subpopulations that are differentially expressed or shared across these diseases. This suggest that better characterisation of patient immune signatures could lead to optimised diagnostic and therapeutic approaches ('stratified personalised medicine' approach).

REFERENCES

1.) Voulgarelis and Tzioufas, Pathogenetic mechanisms in the initiation and perpetuation of Sjögren's syndrome. Nat Rev Rheumatol. 9, 529-37 (2010).
2.) Roberts, M. et al. Primary Sjögren's Syndrome Is Characterized by Distinct Phenotypic and Transcriptional Profiles of IgD+Unswitched Memory B Cells. Arthritis & Rheumatatism; 9, 2558-2569 (2014).
3. Vitali, C., G. Palombi, and P. Cataleta, Treating Sjogren's Syndrome Insights for the Clinician. Ther Adv Musculoskelet Dis, 2010. 2(3) p. 155-66.
4. Volchenkov, R., et al., In vitro suppression of immune responses using monocyte-derived tolerogenic dendritic cells from patients with primary Sjogren's syndrome. Arthritis Res Ther, 2013. 15(5) p. R114.
5. Quartuccio, L., et al., Resistance to rituximab therapy and local BAFF overexpression in Sjögren's syndrome-related myoepithelial sialadenitis and low-grade parotid B-cell lymphoma. The open rheumatology journal, 2008. 2 p. 38-43.
6. Kheirallah, S., et al., Rituximab inhibits B-cell receptor signaling. Blood, 2010. 115(5) p. 985-994.