Lupus and Sjögren's syndrome distinct disease endotypes clustered based on activity scores and immune profiles

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INTRODUCTION:

Sjögren's syndrome (SS) is a chronic autoimmune disorder affecting approximately 0.1–0.4% of the general population with a female-to-male ratio of 9:1 usually diagnosed in the fourth and fifth decades of life [1]. Clinically, SS is typified by ocular and oral dryness developed as a consequence of the autoimmune process. It may occur either alone, as primary (p)SS, or secondary to other autoimmune disease, often rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) or systemic sclerosis, secondary (s)SS. There is an increased risk of developing non-Hodgkin's B cell lymphoma [2]. Altered B cell subpopulations have also been shown to correlate with disease activity [2]. $CD4^+$ T helper (Th) cells play a crucial role in the pathogenesis of pSS. T cells were initially identified as the predominant cells within the salivary gland infiltrates and have been shown to be involved in the generation and perpetuation of inflammatory infiltrates in salivary glands in SS [3-4].

Aims

1.) Identify the peripheral B and T cells abnormalities in patients with pSS, secondary SS associated with systemic lupus erythematosus (SLE) and SLE alone in comparison to healthy donors.
2.) Correlate immune phenotypes with clinical features and serological parameters.
3). Identify distinct patients' endotypes relevant for therapeutic strategies.

METHODS:

Table1: Extensive disease activity, clinical and serological characteristics of subjects groups.

	pSS (n=28)	SLE (n=32)	SS/SLE (n=15)	Significance
Disease duration, (years) mean (range)	11 (3 -23)	17 (0.25 - 39)	24 (3 - 43)	pSS vs. SS/SLE p***0.0007
Positive salivary gland biopsy N (%)	14 (50)	n/a	7 (47)	ns
Disease scores and serology				
ESSPRI SCORE, median [IQR]	3 [3 - 6]	n/a	5 [2 - 6]	ns
ESSDAI, median [IQR]	2 [0 - 3]	n/a	2 [1 - 4]	ns
SSDDI, median [IQR]	1 [1 - 2]	n/a	1 [0 - 1]	ns
Global BILAG, median [IQR]	n/a	0 [0 - 2]	0 [0 - 1]	ns
Positive anti-Ro antibodies N (%)	23 (82)	18 (56)	10 (67)	ns
Positive anti-La antibodies N (%)	14 (50)	9 (28)	2 (15)	ns
RF N (%)	16 (57)	4 (13)	4 (27)	ns
Positive ANA N (%)	23 (82)	25 (78)	8 (53)	ns
Anti dsDNA N (%)	0 (0)	18 (56)	6 (40)	pSS vs. SLE p****0.0001, pSS vs. SS/SLE p***0.0019
Peak titer anti-dsDNA, mean	0	253 (2 -1338)	344 (3 - 1698)	ns
Positive anti-Sm N (%)	0 (0)	6 (19)	0 (0)	ns
Positive anti-RNP antibodies N (%)	0 (0)	8 (25)	1 (7)	pSS vs. SLE p*0.0473
C3, g/L (normal values 0.9-1.8) mean (range)	1.2 (0.60 - 3.0)	1.1 (0.65 - 1.58)	1.0 (0.63 - 1.81)	ns
CRP, > 5 mg/l N (range)	2 (0.6 - 10.7)	8 (0.6 - 23.6)	3 (0.6 - 12.9)	ns
ESR, mm/hr (normal range 0-20), mean (range)	14 (2 - 49)	27 (2 - 120)	26 (2 - 81)	ns
IgM, g/L (normal range 0.4-2.3) mean (range)	1.2 (0.42 - 3.38)	1.1 (0.10 - 6.27)	1.1 (0.40 - 1.83)	ns
IgG, g/L (normal range 7-16) mean (range)	16.0 (6.87 - 27.86)	12.8 (4.1 - 18.23)	14.7 (9.53 - 33.7)	ns
IgG, > 20g/L N (%)	4 (14)	1 (3)	2 (13)	ns
Hb, g/L (normal range 115-155.) mean (range)	125.3 (96 - 143)	118.9 (9.8 - 150)	104.6 (11.4 - 141)	ns
WBC, $X10^3/mm^3$ (normal range 3-10) , mean (range)	5.6 (2.93 - 10.05)	7.0 (3.27 - 12.51)	7.5 (2.09 - 14.24)	ns
Lymphocytes, $X\ 10^9/L$, (normal range 1.2-3.65) mean (range)	1.3 (0.53 - 2.69)	1.8 (0.49 - 6.81)	2.2 (0.80 - 4.17)	ns
Neutrophils, $X\ 10^9/L$, (normal range 2-7.5) mean (range)	3.6 (1.0 - 7.86)	4.5 (1.62 - 9.81)	5.2 (0.99 - 12.52)	pSS vs. SS/SLE p*0.0398
Lipid profile				
N (%) patients with normal cholesterol	9 (32)	13 (41)	11 (73)	ns
N (%) patients with abnormal triglycerides (>2.2)	0 (0)	4 (13)	1 (7)	ns

$CD19^+$ B cells, $CD4^+$ and $CD8^+$ T cell subpopulations representative plots from peripheral blood mononuclear cells (PBMCs).

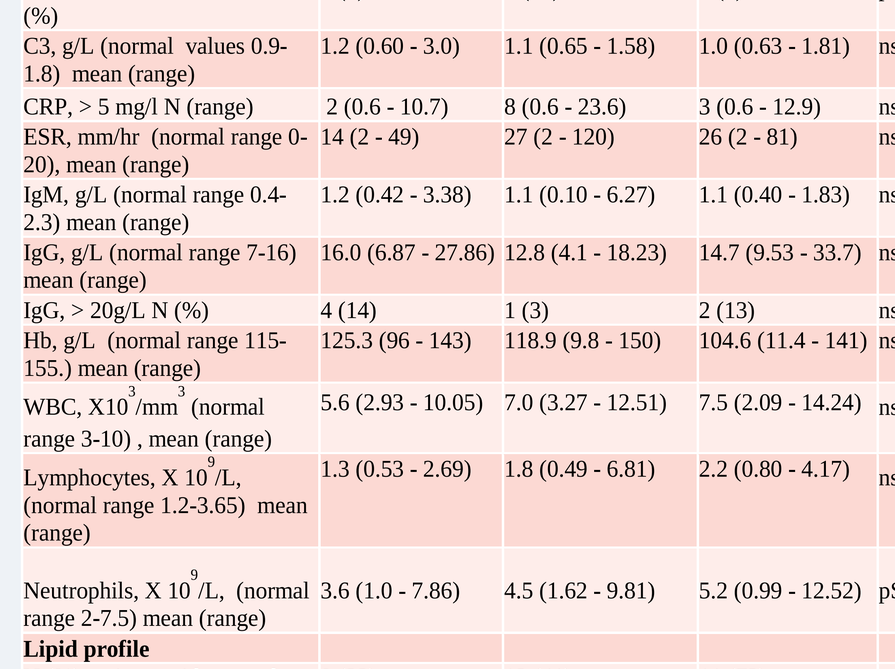
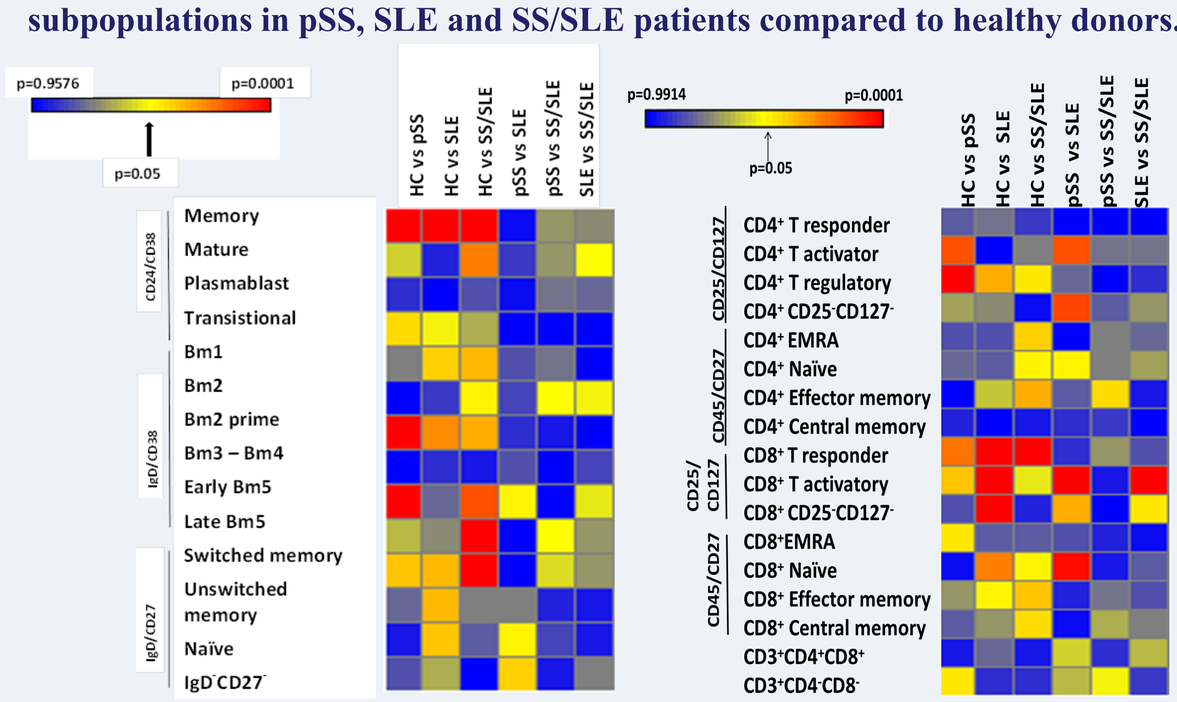


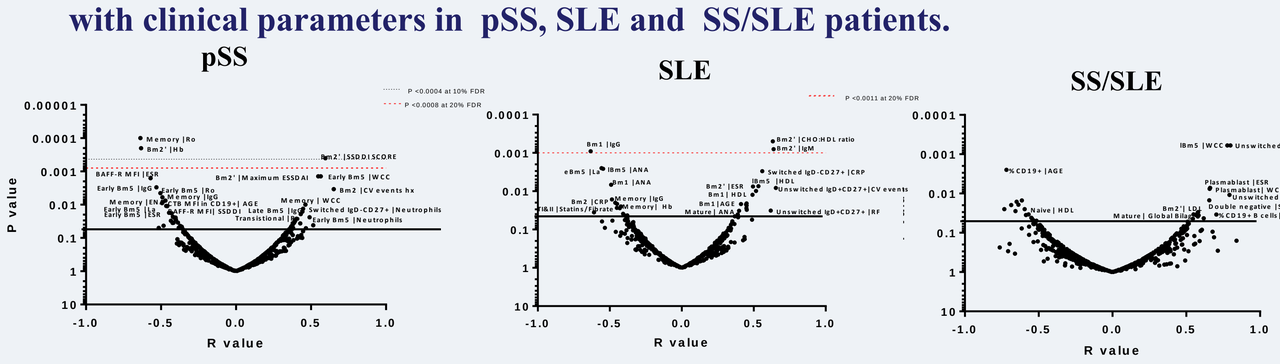
Figure 1: Representative flow cytometry dotplots showing $CD19^+$ B cells and $CD4^+/CD8^+$ T cell subpopulations.

RESULTS:

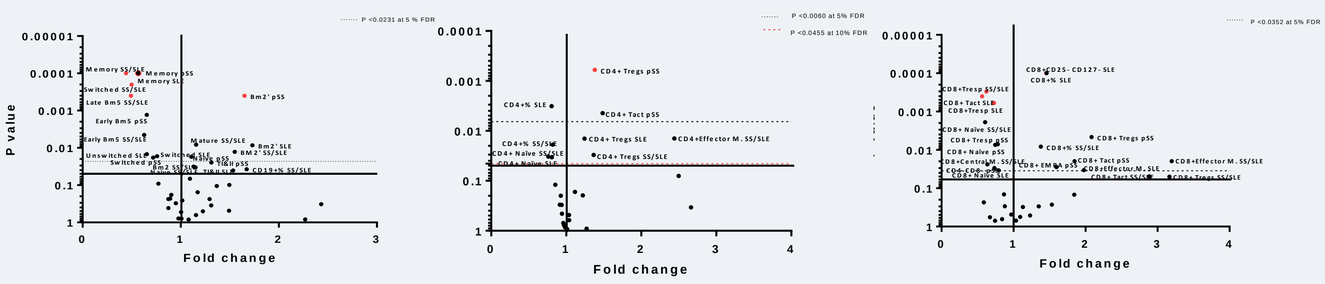
1) Heat-maps showing distinct $CD19^+$ B cells, and $CD4^+$ and $CD8^+$ T cell subpopulations in pSS, SLE and SS/SLE patients compared to healthy donors.



2) Volcano plots of significant positive and negative correlations of immune cells with clinical parameters in pSS, SLE and SS/SLE patients.

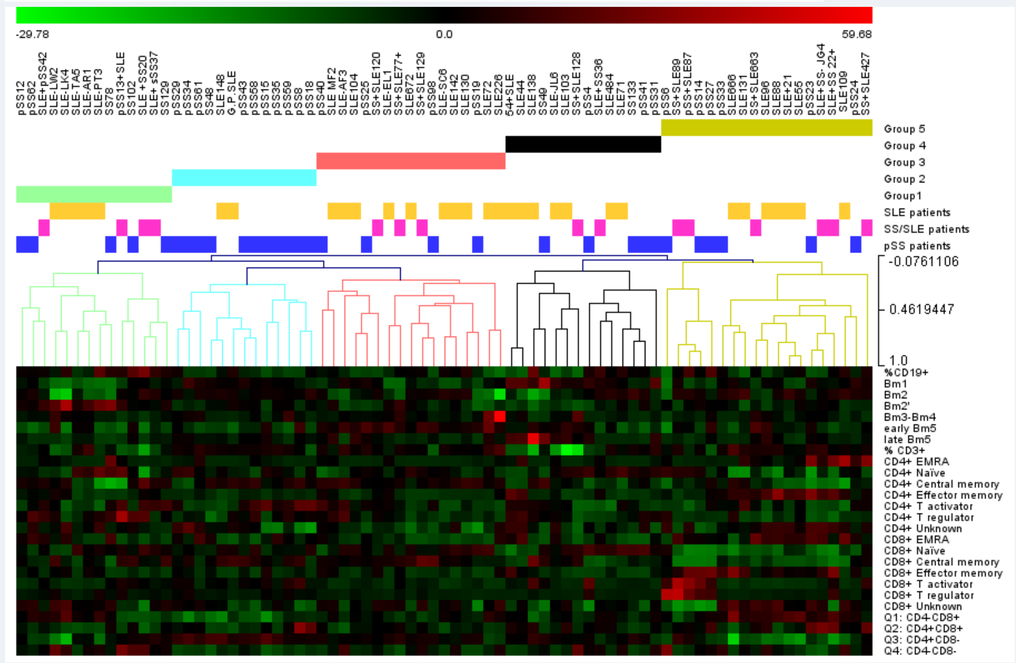


3) Fold-change graphs of immune cells expression in patients comparison to healthy donors.



4) Hierarchical clustering of immune cells in patients with pSS, SLE and SS/SLE found 5 distinct endotypes.

ESSDAI=2	ESSDAI=2	ESSDAI=0	ESSDAI=2	ESSDAI=3
SSDDI=2	SSDDI=1	SSDDI=0	SSDDI=2	SSDDI=1
BILAG=2	BILAG=0	BILAG=0	BILAG=0	BILAG=0



Group-specific hierarchical clustering of immune cells in patients with pSS, SLE and SS/SLE, group 1= light green, group 2 = cyan, group 3 = light red, group 4 = black and group 5 = gold.

DISCUSSION/CONCLUSIONS:

- This is the first comprehensive immunophenotype analysis performed patients with pSS, SLE and SS/SLE
- We identified significant reduction in memory B cells fold-changed in all disease groups, reduction in $CD4^+$ naïve T cells in SLE and SS/SLE and reduction in T responders in all disease $CD8^+$ in comparison to healthy donors.
- The most significant T cell abnormalities were found in patients with SLE, however a significant correlation between lipid raft expression as marker of cell activation and disease activity score (ESSDAI) was found only in pSS patients.
- SS/SLE have the most striking B cell phenotype abnormalities than patients with pSS or SLE (increased Bm2 cells and decreased early and late Bm5 cells).
- The five distinct disease endotype clustering showed distinct immune profile in patients with overlapping autoimmune conditions which is particularly relevant for stratification of therapy.