Background: A decline of urine protein-to-creatinine ratio (UPCR) to < 0.5 is associated with better long-term preservation of kidney function in lupus nephritis (LN). UPCR < 0.5 defines complete response in guidelines and clinical trials when achieved after 1 or 2 years. Biomarkers of early response are needed to guide early treatment changes. We studied longitudinal urine proteomic profiles in LN to identify early predictors of proteinuric response.

Objectives: Identify early predictors of proteinuric response in LN using longitudinal urine proteomic profiles.

Methods: We quantified 1200 biomarkers (Kiloplex, RayBiotech) in urine samples collected on the day of (73%) or within 3 weeks (27%) of kidney biopsy and week 12, 24, or 52 in LN patients (ISN class III, IV, V, or mixed) with proteinuria > 1 g/d. Response was defined at one year from renal biopsy: Complete = UPCR < 0.5, serum creatinine (sCr) < 125% of baseline, prednisone ≤ 10mg/d; Partial = UPCR < 50% from baseline but > 0.5, sCr < 125% of baseline, but prednisone allowed to 15mg/d; Non responder = not meeting previous definitions.

Results: A total of 127 patients were included: 48 (38%) with pure proliferative LN (class III or IV), 41 (32%) with mixed LN (III or IV + V), and 38 (30%) with pure membranous LN. Response was complete in 34 (27%), partial in 29 (23%), and none in 64 (50%). There were no urinary biomarkers at baseline that predicted response. We then analyzed the changes in urinary proteins at 3 months compared to baseline. Patients who responded at 1 year showed an early decline in 51 urinary proteins led by CD163, IL-16, and CD206 (macrophage mannose receptor) (Figure 1A) which matched the proteomic signature associated with histological activity (Figure 1B). No changes were observed in nonresponders. The decline of several urinary biomarkers at 3 months outperformed a decline in UPCR (clinical standard) in predicting the 1 year response. In particular, a decline of CD163 predicted 1 year response in ROC analysis with an area under the curve (AUC) of 83% compared to an AUC of 75% for UPCR decline. In proliferative LN, urinary biomarkers displayed superior performance with an AUC of 91%, 86%, and 78% for the decline of CD206, CD163, and UPCR, respectively (Figure 1C-D). Pathway enrichment analysis identified leukocyte activation, neutrophil degranulation, and matrix degradation as the main pathways reduced at 3 months in responders.

Conclusion: An early decline in urinary biomarkers of histological activity is associated with proteinuric response at 1 year. These findings indicate that effective immunosuppression induces by 3 months an immunological response in the kidney that can be noninvasively monitored in the urine. Biomarkers of immunological response outperformed early decline of UPCR, the standard of care, in predicting 1-year proteinuric response, especially in proliferative LN.
analysis. Our data provide clues to the molecular pathways contributing to the glandular and systemic manifestations of pSS and to potential therapeutic targets for different pSS subgroups.

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Figure 1. Two-dimensional UMAP for a random subset of 50000 B cells divided by group. Different colors label FlowSOM metaclusters. Clustering was performed on 1.5x10^6 B cells and 7 surface markers. Black dots represent PIC-specific cells. A significant increase for both total and PIC-specific B cells was observed in the pink cluster for pAPS patients compared to HCs (p<0.01). Main clusters: green – naive, brown – switch memory, red: plasmablasts, pink – pre-switch memory (IgD+/CD24+/CD27+CD11c+).

Figure 2. A multi-dimensional approach reveals a dysregulated systemic lupus erythematosus immune rheostat with an abnormal immunoregulatory response and reduced CTLA4 expression in effector T cells.

Keywords: Adaptive immunity, Autoantibodies, Anti-phospholipid syndrome

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Background: Antiphospholipid syndrome (APS) is an autoimmune disease defined by arterial or venous events or pregnancy complications in association with autoantibodies against phospholipids. Despite these autoantibody-antigens, B lineage cells remain poorly studied in APS.[1] Besides triple-positivity of autoantibodies and the occurrence of lupus anticoagulant, there are no biomarkers for risk assessment in APS.[2] Characterization of autoantigen-specific B cells hold promise for better understanding APS pathogenesis which may guide targeted immune therapies including identification of biomarkers.

Objectives: The study characterized B cell subsets among patients with primary (pAPS) and secondary APS (sAPS) compared to healthy controls. In particular, we analyzed phosphatidylycholine (PIC)-specific B cells for their frequency of occurrence and immunophenotype related to disease characteristics.

Methods: We analyzed PBMCs from 20 HCs, 25 pAPS and 16 sAPS patients. Using multi-dimensional flow cytometry, we analyzed the expression of 15 surface markers on B cells including the frequency of PIC-specific B lineage cells. We established a novel assay to detect antigen-specific B cells against PIC as a potential correlate of antiphospholipid antibodies as previously reported for autoreactive B1 cells in mice. [3] The obtained cellular subsets and autoreactive cells were subjected to the FlowSOM algorithm in R to identify B cell clusters through an unbiased strategy.

Results: pAPS and sAPS patients showed increased frequencies of atypical CD21low as well as CD21low/CD11c+ B cells, most prominent within the IgD+/CD27+ memory compartment (CD21low: p<0.01, CD21low/CD11c+: p<0.001). We found higher frequencies of total PIC-specific B cells compared to HCs among pAPS in contrast to sAPS patients. In HCs, PIC-specific B cells were found mainly among naïve B cells, while they were significantly enriched within IgD+/CD27+ pre-switch memory B cells in pAPS patients (p<0.01). Most notably, high frequencies of PIC-specific IgD+/CD27+ pre-switch memory B cells were associated with a high-risk APS profile according to the EULAR classification criteria (p<0.05). Unsupervised FlowSOM clustering identified eight distinct B cell clusters. Remarkably, PIC-reactive memory B cells mainly resided in a unique cluster of IgD+/CD27+/CD11c+ memory B cells in pAPS. The data indicate that there is ongoing induction of autoantigen-specific atypical memory B cells apparently induced outside the germinal centers escaping negative selection.

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Figure 2. A multi-dimensional approach reveals a dysregulated systemic lupus erythematosus immune rheostat with an abnormal immunoregulatory response and reduced CTLA4 expression in effectort T cells.

Keywords: Adaptive immunity, Systemic lupus erythematosus, Biomarkers

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Background: Systemic Lupus Erythematosus (SLE) immunopathogenesis involves a complex network of regulatory and effecter cells with its balance constituting an immune rheostat that maintains immune homeostasis. Thus, the disease must be interrogated holistically to identify mechanistically and clinically relevant immune cell subsets for its pathogenesis. There is a crucial unmet need to examine the mechanism of tolerance loss in lupus and identify novel targets for potential therapeutic intervention to improve outcomes.

Objectives: To characterise the SLE immune holistically and address our hypothesis that SLE is driven dually by an impaired immunoregulatory axis and perturbed immune effector system.

Methods: Forty-one peripheral blood mononuclear cell (PBMC) samples from 26 adult SLE patients and 27 age-matched healthy controls were studied with a 43 markers mass cytometry panel. The SLE patients (23 females) had a median age of 40 (interquartile range [IQR]: 28 to 54) years with a median SLEDAI 2K score of 4 (IQR: 0 to 6). Quality check, batch-effect correction, cell clustering, annotation and visualisation after t-distributed stochastic neighbour embedding (tSNE) dimensional reduction was done using our extended multidimensional immunophenotyping pipeline ([1]. Frequencies are expressed as a percentage of total CD45+ PBMC or ratio and described with median and IQR. Statistical significance is defined as p<0.05 (Mann-Whitney U test with Bonferroni correction).

Results: Our multi-parametric approach reveals multiple derangements in all the major immune lineages but predominantly in the CD4+ T cell population (Figure 1). Interestingly, there were no significant differences in the memory (CD45RO+CD25+CD4+) and naïve (CD45RA+CD25-) Treg (CD3+CD4+CD25+Foxp3+) subsets between SLE and controls. Instead, an enrichment of an activated memory Treg-like T subset (CD3+CD4+CD45RO+CD25 Foxp3+CTLA4+CD122+) was found in SLE (LEG SLE}