Background: Despite optimal treatment, lupus nephritis (LN) remains associated with irreversible kidney damage[1]. A better understanding of the mechanisms underlying LN pathogenesis is needed to develop better treatment targets. As part of the Accelerating Medicines Partnership (AMP), we discovered that urinary PR3, a myeloid degranulation product, correlated with histological activity implicating neutrophil/mönocyte degranulation in proliferative LN, the most aggressive type[2]. PR3 is a serine protease that can mediate kidney damage. Mature neutrophils with classical polylobate nuclei are rare in LN kidney biopsies. However, recent evidence displayed how immature, degranulating myeloid cells have been implicated in the pathogenesis of LN[3], but their role in mediating kidney damage is not completely understood.

**Objectives:** To investigate PR3+ cells in LN kidney, their association with histological-pathological features, and define their immunophenotype.

**Methods:** We performed multiplexed histology using serial immunohistochemistry (sHiC)[4] on archival LN kidney biopsies to quantify the expression of PR3 and multiple cell lineage markers (20-plex). Image analysis including deconvolution, cell segmentation,glomerular annotation, and quantitative histology was performed using Indica HALO. The analysis was limited to renal cortex.

**Results:** A total of 11 patients with LN who underwent a clinically indicated kidney biopsy were enrolled: 6 (55%) with pure proliferative LN (ISN/RPS class III or IV) and 5 (45%) with pure membranous LN. PR3+ cells were identified in all LN biopsies (range 343-7625 per sample). Most PR3+ cells did not show a polylobate nucleus. The majority of PR3+ cells were in the tubulointerstitium (Figure 1A). However, accounting for the smaller glomerular area, there was a higher density of PR3+ cells in the glomeruli (Figure 1A-C). PR3+ cell abundance was higher in proliferative LN, especially in the glomeruli (Figure 1A-C). Glomerular PR3+ cell density very strongly correlated with histological activity measured by the NIH Activity Index (Pearson’s r=0.97; p=5×10−6; Figure 1D). Preliminary serial IHC analysis showed that PR3+ cells coexpress MPO and variably coexpress CD66b and CD14, but not neutrophil elastase, CD3, or CD20.

**Conclusion:** PR3+ cells are abundant in LN. PR3+ cells are increased in proliferative LN and are strongly associated with histological activity thereby characterizing a more aggressive phenotype. This population densely infiltrated the glomeruli emphasizing a potential role in the endothelial pathogenic process. In preliminary analysis, kidney infiltrating PR3+ cells were not polymorphonuclear, did not express neutrophil elastase, and variably expressed CD14 suggesting a phenotype consistent with degranulating monocyte or an immature myeloid population. We previously showed the association between urinary PR3 and histological activity suggesting that intrarenal PR3+ cells are actively degranulating and therefore likely inducing kidney damage. These findings nominate PR3+ cells as a potential therapeutic target. Spatial transcriptomics and proteomic studies are ongoing to define the lineage and function of these cells.

**REFERENCES:**


Conclusion: Distinct histological features of LN are paralleled by specific signatures of circulating soluble mediators. Within LN, proliferative LN is associated with higher circulating levels of inflammatory cytokines, notably, type I IFNs. Furthermore, a decline in the titers of several immune mediators correlated with LN activity was associated with treatment response, suggesting a possible role in LN pathogenesis. These signatures and trajectories provide insight into LN pathogenesis, heterogeneity, and biomarker development.

REFERENCES: NIL.

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POS0290

NOVEL ANTI-MITOCHONDRIAL ANTIBODIES IDENTIFY PATIENTS WITH PROPENSITY TO THROMBOSIS IN SYSTEMIC LUPUS ERYTHEMATOSUS

Keywords: Autoantibodies, Biomarkers

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Background: Venous and arterial thrombotic events are leading causes of mortality in systemic lupus erythematosus (SLE), amounting to up to a quarter of deaths[1]. Other than traditional risk factors, SLE-specific factors, including chronic inflammation, have also been shown to contribute to thrombosis development. Nonetheless, the four times higher relative risk of thrombosis compared to healthy individuals is not fully accounted to the presence of conventional cardiovascular risk factors and anti-phospholipid antibodies[1]. As such, novel biomarkers of thrombosis are needed in early identification and preventative management of SLE patients.

Objectives: To characterize novel antibodies directed against mitochondrial constituents, and investigate their capacity to stratify patients based on thrombotic events.

Methods: Highly purified mitochondria were isolated from HepG2 cells (ATCC HB-8065TM) as previously described[2]. Briefly, cells were harvested and homogenized by a glass Douncer, followed by sequential centrifugation. Upon DNase-mediated removal of extracellular DNA, ultra-pure mitochondria (confirmed by flow cytometry, qPCR, and Western blot), labeled with MitoTracker, were incubated with sera (diluted 1:100) from SLE patients or healthy individuals. BINDing of IgG to the mitochondrial outer membrane was detected using a secondary FITC-conjugated anti-human-IgG antibody, and analyzed by FACS. In some experiments, mitochondria were treated with trypsin (0.05%) prior to incubation with sera. Finally, reactivity towards mitochondrial protein lysate was confirmed using WB. Samples and clinical data from SLE patients (n=92) and healthy individuals (n=80) were provided from Division of Rheumatology, Lund University, Sweden. Statistical analyses were performed on SPSS 22.0. Mann-Whitney test was performed, with a p-value of 0.05 or below deemed as significant. Data protection and patient anonymization were ensured in accordance with regulation by Research Advisory Boards.

Results: Based on our novel flow cytometry assay, quantifying anti-mitochondrial antibodies (AMA) targeting the outer mitochondrial membrane, a large proportion (40.8%) of SLE patients were deemed positive for AMA using the 95th percentile of healthy controls as a cut-off. Presence of AMA was associated with severe SLE manifestations, including history of nephritis (OR = 3.3, p=0.02), anti-phospholipid syndrome (APS; OR = 5.7, p=0.02), and venous thromboembolism (OR = 6.7, p=0.008), the latter which was not seen in our cohort for isolated presence of anti-cardiolipin (aCL) antibodies (OR = 2.2, p=0.17), neither for lupus anticoagulant (OR 5.0, p=0.08) or anti-beta-2 glycoprotein 1 (OR 1.1, p=0.93). Pre-treatment of mitochondria with trypsin induced loss of binding of sera-derived antibodies (p<0.01), suggesting that AMA were targeting protein components of mitochondria, and not phospholipids, as is the case of aCL. WB confirmed presence of reactivity towards mitochondrial protein antigens, in particular of 35 and 60 kD, though reactivity towards other protein antigens, including 17 kDa ones, were also seen in some patients, with the latter being associated with APS (p=0.008).

Conclusion: SLE patients positive for novel AMAs targeting mitochondrial outer membrane proteins develop severe lupus manifestations, including venous thromboembolism. Future studies are warranted to further characterize the novel antibodies, as well as determine their prognostic value.

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Figure 1. K-means clustering of GSVA scores from baseline gene expression profiles effectively identifies 5 subsets of SLE patients.