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 1 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.

Acknowledgements: NIL.

Disclosure of Interests: None declared.

DOI: 10.1136/annrheumdis-2023-eular.2669

POS0290 NOVEL ANTI-MITOCHONDRIAL ANTIBODIES IDENTIFY PATIENTS WITH PROPENSITY TO THROMBOGENESIS IN SYSTEMIC LUPUS ERYTHEMATOSUS

Keywords: Autoantibodies, Biomarkers

M. Barguil Macedo[1], J. Chapa[1], T. Wang[1], A. Bengtsson[2], C. Lood[1]. [1]*University of Washington, Division of Rheumatology, Seattle, United States of America*; [2]*Lund University, Division of Rheumatology, Stockholm, Sweden*

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 kDa, 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.

REFERENCES:

[1] Bello N, Meyers KJ, Workman J, et al. Systematic Literature Review and Meta-analysis of Venous Thromboembolism Events in Systemic Lupus Erythematosus. Rheumatol Ther. 2022 Dec 6. doi: 10.1007/s40744-022-00513-1.

[2] Moore RE, Wang T, Duvvuri B, et al. Anti-mitochondrial antibodies predict erosive disease development in rheumatoid arthritis. Arthritis Rheumatol. 2022 Dec 29. doi: 10.1002/art.42428.

Acknowledgements: NIL.

Disclosure of Interests: None declared.

DOI: 10.1136/annrheumdis-2023-eular.3873

POS0291 IDENTIFICATION OF SUBSETS OF SLE PATIENTS RESPONSIVE TO IBERDOMIDE BY TRANSCRIPTOMIC ANALYSIS OF BASELINE SAMPLES

Keywords: Artificial Intelligence, Systemic lupus erythematosus, Clinical Trials

P. Bachali[1], Y. Hu[2], N. Delev[3], P. Schafer[2], P. Lipsky[1]. [1]*AMPEL BioSolutions, Research and Development, Charlottesville, United States of America*; [2]*Bristol Myers Squibb, Research and Development, Princeton, United States of America*; [3]*Bristol Myers Squibb, Research and Development, Princeton, United States of America*

Background: Iberdomide is a high affinity cereblon ligand that promotes ubiquitylation and proteasomal degradation of Ikaros (IKZF1) and Aiolos (IKZF3) transcription factors and, thereby altering specific aspects of immune responsiveness. Iberdomide has been shown to be efficacious in a randomized controlled trial in patients with generalized SLE (NCT03161483) and to be specifically effective in patients with high baseline expression of the interferon gene signature (IGS)[1,2].

Objectives: The goal of this study was to identify subsets of SLE patients responsive to iberdomide more effectively by analyzing baseline gene expression profiles.

Methods: Baseline whole blood samples from 276 female SLE patients from the phase 2b iberdomide trial were utilized for this analysis. These patients had a >/= 6 month history of SLE and disease activity determined by SLEDAI-2K >/=6. Patients were randomized to placebo, or one of three doses of iberdomide (0.15, 0.3 or 0.45 mg per day). Clinical response was determined by the SLE Responder Index 4 (SRI-4) at 24 weeks. RNAseq was performed and analyzed by Gene Set Variation Analysis (GSVA) using 32 informative gene modules and K-means clustering.

Results: Whole blood K-means clustering of the GSVA scores yielded 5 clusters or endotypes (Figure 1). Cluster 0 had the fewest molecular abnormalities, whereas Cluster 1 had the most disturbances in immune function, including enrichments in the interferon gene signature (IGS), immunoproteasome, IL-1/inflammasome pathway, and neutrophil/granulocyte genes. Clusters 2-3 had intermediate degrees of abnormal enrichment in specific gene modules. Cluster 4 had high IGS, immunoproteasome, plasma cells/Ig chains, and IL-23 complex genes. No differences were noted between the subsets with regard to steroid or hydroxychloroquine use and differed only slightly in disease activity as measured by SLEDAI-2K. In general, cluster 1 had the most severe clinical laboratory measures with the highest anti-dsDNA antibodies and lowest C3 and C4. Clinical responses to iberdomide were confined to clusters 1 and 4. Effect sizes of responses in these groups approximated 30%. Other clusters had higher placebo responses and no additional response to iberdomide.

Conclusion: K-means clustering of GSVA scores from baseline samples of the iberdomide trial successfully clustered patients into endotypes that exhibited differences in response to iberdomide treatment, with the greatest responses observed in patients with the highest IGS, immunoproteasome, plasma cell, inflammasome, and IL-23 pathways. Gene expression based subsetting (endotyping) may be useful to enrich trials for responsive patients.

REFERENCES:

[1] Merrill JT et al. N Engl J Med 2022; 386:1034

[2] Lipsky, PE et al Ann Rheum Dis 2022-222212

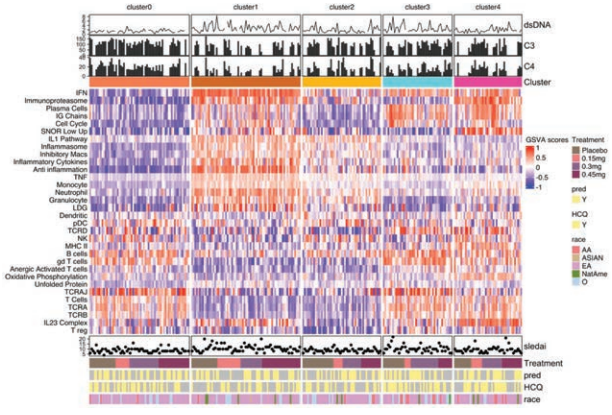

Figure 1. K-means clustering of GSVA scores from baseline gene expression profiles effectively identifies 5 subsets of SLE patients.

Acknowledgements: Research supported by BMS.

Disclosure of Interests: Prathyusha Bachali: None declared, Yanhua Hu Shareholder of: BMS, Employee of: BMS, Nikolay Delev Shareholder of: BMS,

Employee of: BMS, Peter Schafer Shareholder of: BMS, Employee of: BMS, Peter Lipsky: None declared.
DOI: 10.1136/annrheumdis-2023-eular.4878

POS0292 TRANSCRIPTOMIC PROFILING OF SJOGREN'S SALIVARY GLANDS IDENTIFIES FOLLICULAR AND EXTRA-FOLLICULAR GENE SIGNATURES ASSOCIATED WITH RHEUMATOID FACTOR SEROPOSITIVITY

Keywords: Autoantibodies, Sjögren syndrome

E. Pontarini[1], D. Lucchesi[2], K. Goldmann[2], M. Lewis[2], Q. Song[3], E. Thomas[3], L. Y. Hao[3], K. Sivils[3], C. Pitzalis[2], M. Bombardieri[2]. *[1]William Harvey Research Institute, Queen Mary University of London, Experimental Medicine and Rheumatology, London, United Kingdom*; *[1]William Harvey Research Institute, Queen Mary University of London, Experimental Medicine and Rheumatology, London, United Kingdom*; *[3]Janssen Pharmaceuticals, Immunology Translational Science, Spring House, PA, United States of America*

Background: The presence of circulating rheumatoid factor (RF) and the formation of ectopic lymphoid structures (ELS) in labial salivary glands (SG) of patients with Sjögren's Syndrome (SS) have been reported as independent risk factors associated with the development of SG B-cell MALT lymphoma (MALT-L). Neoplastic MALT-L B-cells express highly hypermutated B-cell receptors bearing RF immunoreactivity in up to 50% of the cases, but whether their maturation and proliferation is dependent on ELS or is also induced by extrafollicular responses in the SG is currently unclear. The definition of ELS and their association with circulating autoantibodies has so far relied on SG histopathology which bears significant limitations. Conversely, molecular pathology analysis through whole-tissue RNA Sequencing (RNASeq) has allowed a better definition of disease heterogeneity and disease taxonomy.
Objectives: To perform transcriptomic profiling of SS minor SG tissue characterised by different degrees of inflammatory aggregate organization and to identify transcriptomic clusters and gene signatures associated with peripheral and histological biomarkers of disease.
Methods: Labial SG were obtained from 99 patients including SS with and without ELS (respectively ELS+ and ELS-, as assessed by immunohistochemistry) and non-specific chronic sialadenitis (Sicca). Total RNA was extracted, complementary DNA libraries were prepared and sequenced. Differentially expressed genes (DEG), deconvolution and pathway analysis were performed.
Results: Unsupervised gene clustering by differential expression between sicca and SS confirmed a clear transcriptome segregation between the two diagnoses. As expected, in SS SG expression of genes associated with inflammation and adaptive immune responses was upregulated (e.g. CCR7, CD19, CR2, CXCL13, CXCL9, CXCR5, FCRL3, FCRL4, IL21R, MS4A1, PAX5, SLAMF6, TLR10) (Figure A). Bulk RNAseq cell deconvolution confirmed immune cell enrichment (Th, B and plasma cells) in SS SG, especially those ELS+. A three-way comparison among sicca, ELS+ and ELS- SG, showed only a few genes specifically associated to ELS- and sicca, whereas most of the DEGs were either ELS+ specific or common to all SS (Figure B). Results of pathway analysis on ELS+ and SS-associated DEGs showed very similar profiles characterised by adaptive and interferon-associated pathway activation. Weighted Gene Co-Expression Network analysis showed higher activation of anti-viral pathways in ELS-SG, where ELS+ SG were enriched in adaptive immune pathways. Principal component analysis on SS SG mRNA showed that a significant proportion of variability was associated with the presence of RF in the serum of patients independently of the presence of ELS. Surprisingly, transcriptome clustering was closer between ELS+/RF+ and ELS-/RF+ SG than the clustering of ELS+/RF- with ELS-/RF- SG. Accordingly, SG of RF+ patients showed significant upregulation of B cell-associated genes (e.g. CXCL13, MS4A1). However, while SG transcriptomes of ELS+/RF+ were characterised by genes associated with germinal centre formation (e.g IL21, AICDA, LTB), transcriptomes from ELS-/RF+ SG displayed a unique set of DEGs such as BCL2, TRIM22, XAF1, DDX58, DDX60 and IFIT1. Conversely, a similar analysis performed based on anti-Ro/SSA and/or anti-La/SSB did not yield any significant differences in gene expression.
Conclusion: A comprehensive bulk RNA sequencing analysis of SS and sicca patients SG showed that higher tissue inflammation with features of functional germinal centres is associated to the presence of both RF and ELS, rather than ELS alone. Furthermore, the existence of a RF-driven SG transcriptome independent of the presence of ELS suggest that both follicular and extra-follicular responses support the selection of B-cells with RF immunoreactivity within the SG of SS patients and could be involved in B-cell lymphomagenesis.

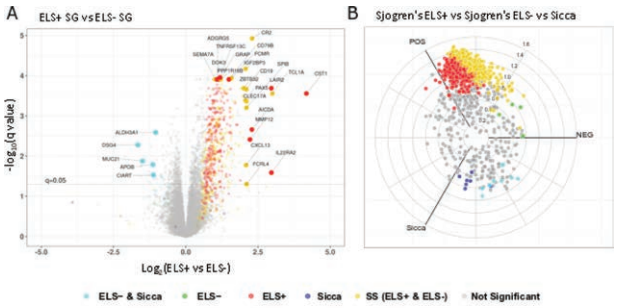

Figure Legend

(A) Volcano plots showing DEGs between ELS+ SG vs ELS- SG labial salivary gland biopsies. Coloured dots represent genes with associated q-value <0.05. Colors indicate pairwise comparisons (FDR < 0.05) between ELS+ SG, ELS- SG and Sicca groups. (B) 3-way volcano plot of differentially expressed genes comparing salivary gland RNA sequencing. Vectors for pathotype mean Z score per gene were projected onto a polar coordinate space. Sjogren's SG ELS+, Sjogren's SG ELS- and Sicca SG vectors are mapped to 3 axes (POS, NEG and Sicca respectively), using polar coordinates in the horizontal plane. Genes with adjusted p value for likelihood ratio test < 0.05 (z axis) were considered significant (non-significant genes colored gray). Colors demonstrate pairwise comparisons (FDR < 0.05) between the groups as in Figure A.

REFERENCES: NIL.
Acknowledgements: NIL.
Disclosure of Interests: Elena Pontarini: None declared, Davide Lucchesi: None declared, Katriona Goldmann: None declared, Myles Lewis: None declared, Qingxuan Song Employee of: Janssen Pharmaceuticals, Inc., Spring House, PA, USA, Elizabeth Thomas Employee of: Janssen Pharmaceuticals, Inc., Spring House, PA, USA, Ling-Yang Hao Employee of: Janssen Pharmaceuticals, Inc., Spring House, PA, USA, Kathy Sivils Employee of: Janssen Pharmaceuticals, Inc., Spring House, PA, USA, Costantino Pitzalis: None declared, Michele Bombardieri Speakers bureau: UCB, Janssen, Consultant of: GSK, Janssen, MedImmune, Grant/research support from: Janssen, MedImmune.
DOI: 10.1136/annrheumdis-2023-eular.5849

POS0293 METABOLOMIC IN ANTIPHOSPHOLIPID SYNDROME STRATIFIED PATIENTS ACCORDING TO THEIR THROMBOTIC RISK

Keywords: Artificial Intelligence, Cardiovascular disease, Anti-phospholipid syndrome

C. Lopez-Pedrera[1], M. A. Aguirre[1], I. Sanchez-Pareja[1], T. Cerdó[1], L. Muñoz-Barrera[1], P. Seguí Azpilcueta[2], C. Merlo[1], P. Ortiz Buitrago[1], D. Ruiz[3], M. C. Ábalos-Aguilera[1], N. Barbarroja Puerto[3], R. Ortega Castro[1], C. Perez-Sanchez[1]. *[1]IMIBIC/Reina Sofia Hospital/University of Cordoba, Rheumatology, Córdoba, Spain*; *[2]IMIBIC/Reina Sofia Hospital/University of Cordoba, Radiology, Córdoba, Spain*; *[3]IMIBIC/Reina Sofia Hospital/University of Cordoba, Medical and Surgical Science, Córdoba, Spain*

Background: Antiphospholipid syndrome (APS) is a systemic autoimmune disorder inducing hypercoagulable state that causes arterial, venous, or microvascular thrombosis, atherosclerosis, and pregnancy morbidity. Timely diagnosis and truthful monitoring of disease are decisive to improve the accuracy of therapy.
Objectives: 1. To map metabolic signatures in sera of APS patients and investigate its association with clinical features of the disease. 2. To investigate the short-term effects of *in vivo* ubiquinol (reduced coenzyme Q10 [Qred]) supplementation.
Methods: Serum nuclear magnetic resonance (NMR) metabolomics (>250 metabolites, Nightingale) covering glycolysis metabolites, amino acids and 130 lipid measures was performed on a cohort of primary APS patients (n=150) and 43 healthy donors (HD). Extensive clinical and analytical profile of recruited subjects was performed. NMR data were analyzed in MetaboAnalyst 5.0 software platform. To evaluate the contribution of metabolomic profiles to disease features, unsupervised machine learning clustering analyses were developed. Separately, 33 APS patients treated with Ubiquinol (Qred, reduced CoQ10, 200 mg/day) as an adjuvant treatment to standard therapy for one month, were also studied.
Results: Fifty-three metabolites were significantly altered in APS patients compared to HCs, involving decreased atheroprotective HDL subsets, sphingomyelins, phospholipids, and histidine, and increased proatherogenic VLDL subsets and fatty acids (MUFA, omega-3). Unbiased hierarchical clustering of metabolomic data identified two patient groups, presenting different metabolomic profiles. Clinically, although no differences were found in terms of age, gender, disease duration, or treatments, patients belonging to cluster 1 (C1) were characterized by higher thrombotic risk status (aGAPSS over 12), and prevalence of triple positivity for antiphospholipid antibody profile than C2 patients. Besides, C1 patients showed preponderance of arterial thrombosis, and comprised APS patients with