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.

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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 (con- homogenized by a glass Douncer, followed by sequential centrifugation. Upon DNase-mediated removal of extracellular DNA, ultra-pure mitochondria (concealed by flow cytometry, qPCR, and Western blot), labeled with MitoTracker, were incubated with sera (diluted 1:100) from SLE patients or healthy individ- uals. 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 anonymity 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 anticoagu- lant (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 reac- tivity 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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POS0292

TRANSCRIPTOMIC PROFILING OF SJÖGREN’S SALIVARY GLANDS IDENTIFIES FOLLICULAR AND EXTRA-FOLLICULAR GENE SIGNATURES ASSOCIATED WITH RHEUMATOID FACTOR SEROPOSITIVITY

Keywords: Autoantibodies, Sjögren syndrome

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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 (MALFL). Neoplastic MALFL B-cells express highly hypermutated B-cell receptors bearing RF immunoreactivity in up to 50% of the cases, whereas their infiltration and proliferation is dependent on ELS or is also induced by extra-follicular 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 taxing.

Objectives: To perform transcriptomic profiling of SS minor SG tissue characterised by different degrees of inflammatory aggregate organization to and 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 specific chronic sialoadenitis (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 transcriptomic 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, FCLR3, FCLR4, IL1R1, 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 pathways being upregulated in ELS– SG, 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 immunoactivity within the SG of SS patients and could be involved in B-cell lymphomagenesis.

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POS0293

METABOLIC PROFILING IN ANTIPHOSPHOLIPID SYNDROME: STRATIFIED PATIENTS ACCORDING TO THEIR THROMBOTIC RISK

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

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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 ubiqinol (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 metabolic profiles to disease features, unsupervised machine learning clustering analyses were developed. Separately, 33 APS patients treated with Ubiquinol (Qred, reduced CoQ10, 200mg/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 metabolic 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...