Conclusion: anti-PC IgG1 is negatively associated with disease activity and atherosclerosis in SLE. One potential underlying mechanism could be increased phagocytosis and clearance of dead cells. Our findings raise the possibility of prevention and/or treatment of SLE (and atherosclerosis) to raise anti-PC levels, by passive or active immunization.

REFERENCES:

- [1] Frostegard J. Immunity, atherosclerosis and cardiovascular disease. *BMC Med.* 2013;11:117.
- [2] Anania C, Gustafsson T, Hua X, Su J, Vikstrom M, de Faire U, Heimbürger M, Jogestrand T and Frostegard J. Increased prevalence of vulnerable atherosclerotic plaques and low levels of natural IgM antibodies against phosphorylcholine in patients with systemic lupus erythematosus. *Arthritis Res Ther.* 2010;12:R214.

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THU0217

SERUM THYMIC STROMAL LYMPHOPOIETIN (TSLP) AS A BIOMARKER OF B-CELL LYMPHOPROLIFERATION IN SJÖGREN'S SYNDROME

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Background: Thymic stromal lymphopoietin (TSLP) has been demonstrated to be expressed on salivary glands (SG) biopsies of patients with primary Sjögren's syndrome (pSS), mainly in more advanced degrees of lymphoproliferation and in B-cell MALT lymphoma tissues in pSS (1).

Objectives: To study serum TSLP in a larger number of pSS patients, to confirm its correlation with the already studied tissue expression, and then to investigate a possible role of TSLP not only as a tissue biomarker, but also as a peripheral blood biomarker.

Methods: Serum TSLP levels were assessed by ELISA in sera collected from ninety-one anti-SSA-positive pSS patients (females n=86, 94.5%; mean age 57.2 years, range 25-80), fulfilling the 2016 ACR-EULAR pSS classification criteria, 80 matched healthy blood donors (HBDs) and 21 patients with non-autoimmune sicca syndrome (nSS).

pSS patients were then stratified according to the degree of lymphoproliferation (2), well previously defined by tissue studies in the same patients, as follows: pSS fully benign (fbSS), pSS with myoepithelial sialadenitis (MESA), and pSS B-cell MALT lymphoma (NHL); the difference in serum TSLP levels was studied between these three subgroups.

In addition, prospective serum samples, collected both at the time of MESA diagnosis and later at the time of NHL development in the same patient, were studied in 3 cases.

All the pSS patients were naïve to immunosuppressants, biotechnological drugs, chemotherapies, and were not receiving steroids at the time of sample collection.

The most relevant features of pSS linked to a heavier mucosa-associated lymphoid tissue (MALT) involvement and lymphoproliferation, i.e. persistent parotid swelling and mixed cryoglobulinemia, were recorded, as well as the pathologic evidence of ectopic germinal centres (GCs) in SG biopsy. EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) was calculated for all the pSS patients.

Results: Serum TSLP was significantly higher in pSS (mean 47.19 pg/mL, range 0-324.89) compared to nSS (mean 2.74 pg/mL, range 0-15.9) ($p<0.0001$) and to HBDs controls (mean 0.59 pg/mL, range 0-11.09: very low levels being detected only in 4/80) ($p<0.0001$). The significance was the same ($p<0.0001$) also after excluding NHL pSS patients. Serum TSLP showed a progressive, significant increase from patients with fbSS (n=65; mean 26.54 pg/mL; range 0-75.11) to MESA (n=14; mean 69.72 pg/mL; range 20.62-140.8) (MESA vs fbSS $p<0.0001$) and finally to NHL (n=12; mean 151.96 pg/mL; range 58.16-324.89) (NHL vs fbSS $p<0.0001$; NHL vs MESA $p=0.009$).

In prospective sera, TSLP levels significantly increased in all the 3 pSS patients from MESA to NHL.

pSS patients showing a heavier involvement of MALT, i.e. with persistent parotid swelling, mixed cryoglobulinemia, or GCs in SG biopsy, showed

significantly higher TSLP serum levels compared to pSS patients without this heavier MALT involvement.

A significant correlation between higher TSLP serum levels and increasing ESSDAI was finally found ($p<0.0001$).

Conclusion: Serum TSLP could represent a novel biomarker of pSS-related B-cell lymphoproliferation, characterized by a heavier MALT involvement. The validation of the present monocentric results is currently ongoing within the EU HarmonicSS Project.

REFERENCES:

- [1] Gandolfo S. et al, *Arthritis Rheumatol.* 2017; 69 (suppl 10).
- [2] De Vita S. et al. *Blood Cells Mol Di.* 2001;27:757-66.

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THU0218

IDENTIFICATION, VERIFICATION AND VALIDATION OF NEW BIOMARKER CANDIDATES FOR MONITORING ACTIVITY IN PSORIATIC ARTHRITIS AND OTHER IMMUNE-MEDIATED INFLAMMATORY DISEASES (IMIDS)

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Background: Immune-Mediated Inflammatory Diseases (IMIDs) are a group of conditions/diseases characterized by common pathways leading to inflammation, which may result from a dysregulation of the normal immune response. The activity of these diseases is usually measured by the assessment of clinical symptoms.

Objectives: To apply a three-stage proteomic strategy to find plasma biomarkers useful to monitor disease activity in patients three different IMIDs: rheumatoid arthritis (RA), psoriatic arthritis (PsA) and systemic lupus erythematosus (SLE).

Methods: A three-stage proteomic strategy was conducted: **1) Discovery stage:** a random selection of 80 plasma samples from patients with different RA activities according to the DAS28, which were classified as low activity (LA, n=40) and high activity (HA, n=40). The analysis was conducted by shotgun mass spectrometry (MS) applying an iTRAQ 8-plex strategy, by nanoLC-MS/MS using three different MS instruments. **2) Verification stage:** a targeted proteomics strategy was used to verify the candidate biomarkers from the screening, using the same 80 samples of this first stage. This analysis was performed by Multiple Reaction Monitoring (MRM) with the aid of synthetic heavy-labelled peptides, by nanoLC-MS/MS in an ESI-QTRAP 5500. **3) Validation stage:** ELISA experiments were performed to confirm the previous results on an independent set of plasma samples from patients with RA (n=170), PsA (n=80) and SLE (n=80).

Results: **1) Discovery stage:** The MS analysis led to the identification of 186 proteins. A panel of 9 proteins was identified as candidate biomarkers for disease activity monitoring in RA. Most of these proteins are related with the pathogenic process and the effects caused by this type of disease (inflammation and immune disorder in joints). **2) Verification stage:** four from the 9 proteins (SAA1, AACT, HPT, A1AG1) were found to be quantitatively altered between patients with extreme disease activities ($p<0.05$). All these proteins were up-regulated in patients with HA. **3) Validation stage:** as shown in the top row of the Figure, serum amyloid 1 (SAA1), alpha-1-antichymotrypsin (AACT) and haptoglobin (HPT) are higher in the three IMIDs compared to healthy controls, being the highest in RA. Regarding their levels in patients with different disease activities, they are all increased in the plasma of HA individuals compared to LA (bottom row). Interestingly, the levels of these 3 proteins are associated with the activity of PsA: HPT ($p<0.0001$), SAA ($p=0.0209$) and AACT ($p=0.0418$), showing a potential utility for the molecular monitoring of this disease.

Conclusion: Using a three-stage proteomic strategy, we have identified HPT, SAA1 and AACT as protein biomarker candidates for monitoring PsA activity.

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Figure 1. Results obtained in the validation stage of the three biomarker candidates identified by proteomics. ****, $p < 0.0001$; ***, $p < 0.0005$; **, $p < 0.01$; *, $p < 0.05$.

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THU0219

THE INVOLVEMENT OF MITOCHONDRIAL ACTIVATION VIA GLUTAMINOLYSIS IN HUMAN B CELL DIFFERENTIATION AND ITS RELEVANCE TO THE PATHOGENESIS OF SLE

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Background: B cells play a crucial role in Systemic Lupus Erythematosus (SLE). Recently, "Immunometabolism" attract much attention. Glucose and glutamine are important nutrition for energy production such as ATP in various cells. It has been reported that aerobic glycolysis, glutaminolysis and mitochondrial functions enhanced in cancer cells. However, the involvement of metabolic reprogramming in plasmablast differentiation and its relevance to the pathogenesis of SLE remained elusive.

Objectives: We first investigated the abnormality of mitochondria in B cells from patients with SLE by flow cytometry. Next, B cells were isolated from healthy donors (HDs) and metabolic reprogramming were assessed in vitro.

Methods: First, peripheral blood mononuclear cells (PBMCs) were obtained from age-matched 31 HDs and 29 patients with SLE. The mitochondrial membrane potential was measured with DiOC6 by flow cytometry. In addition, CD19⁺ cells were isolated from HDs and stimulated with CpG (TLR9 ligand) and IFN- α . Change of aerobic glycolysis, glutaminolysis and mitochondrial functions were assessed in the absence of glucose or glutamine and in the addition of metformine, which is known as AMPK activator, in vitro.

Results: We first examined the abnormality of mitochondria in B cells from patients with SLE using DiOC6 as a marker of depolarization-activated mitochondrial membrane. Baseline characteristics of SLE were males: females=1:28, age 40.2 years, disease duration 132.2 months, SLEDAI 6.4 and BILAG 6.2 at the timing of admission due to exacerbation of SLE. The percentage of IgD⁺CD27⁺ memory B cells were higher than those of HDs, while the percentage of IgM memory B cells were lower than that of HDs. The percentage of CD24⁺DiOC6⁺ cells in IgD⁺CD27⁺ memory B cells from patients with SLE were higher than that of HDs. These results indicate that B cells from patients with SLE have the abnormality of differentiation and mitochondrial functions. Next, we assessed the change of aerobic glycolysis, mitochondrial functions and glutaminolysis in the process of plasmablast differentiation in vitro. Stimulation with CpG and IFN- α , 1) enhanced aerobic glycolysis, which was assessed by lactate production. 2) increased the area of cytoplasm including many expanded mitochondrial cristae with slightly wider and loosely organized intermembrane space in electric microscopy, accompanied with ROS production and DiOC6 up-regulation, 3) induced CD27^{hi}CD38^{hi} plasmablasts differentiation and immunoglobulin production. Lactate production was decreased in the absence of both glucose or glutamine. ROS production and DiOC6 expression were decreased in the absence of glutamine, leading to inhibition of plasmablasts differentiation and immunoglobulin production. On the other hand, this tendency was not shown in the absence of glucose. Next, we evaluated oxygen

consumption rate (OCR). OCR was also suppressed in the absence of glutamine. Metformin, abrogated glutamine uptake, resulting in suppression of ROS production, DiOC6 expression, plasmablast differentiation and immunoglobulin production.

Conclusion: These results suggest that mitochondrial activation via glutaminolysis may play an important role in the differentiation from IgD⁺CD27⁺ double negative B cells to plasmablasts and production of immunoglobulins in patients with SLE.

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THU0220

PEPTIDYLARGININE DEIMINASE 4 DEFICIENCY AMELIORATED A MURINE MODEL OF LUPUS BY REDUCING NEUTROPHIL MIGRATION TO THE KIDNEY

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Background: Peptidylarginine deiminase 4 (PADI4) is reported to play several biological roles in neutrophils, including neutrophil extracellular trap formation, apoptosis, and epigenetic regulation. Neutrophils could play pivotal roles in the pathogenesis of SLE.

Objectives: We set to determine the pathological role of neutrophils and PADI4 in SLE using *Padi4* knock out (KO) mice.

Methods: A murine model of imiquimod (IMQ)-induced lupus was analyzed. A TLR7 agonist, IMQ, was administered topically to the ear skin of B6 wild-type (WT) and *Padi4* KO mice. Proteinuria, spleen weight, serum anti-dsDNA level, frequencies of spleen and renal immune cells, and the histopathological findings of ear skin and kidney, were assessed. Neutrophil migration and adhesion were evaluated by adoptive transfer experiments *in vivo*, and adherence assays *in vitro*.

Results: Compared with IMQ-treated WT (WT-IMQ) mice, *Padi4* KO-IMQ mice showed decreased spleen weight. Moreover, proteinuria, and dermatitis were not exacerbated in the *Padi4* KO-IMQ mice. There was a positive correlation between the frequency of kidney neutrophils and the degree of proteinuria, while significant decreases in kidney neutrophils were noted in the *Padi4* KO-IMQ mice. Notably, the elevation of serum anti-dsDNA levels, or the degree of immune complex deposition in the kidney, showed no significant difference between the WT-IMQ and *Padi4* KO-IMQ mice. In the adoptive transfer experiment, there was a significant decrease in the transferred *Padi4* KO neutrophil infiltrations in the kidney compared with those of WT neutrophils. The frequency of adhesive neutrophils toward ICAM-1 was significantly decreased in the *Padi4* KO neutrophils primed by the TLR7 agonist.

Conclusion: Nephritis was ameliorated in *Padi4* KO-IMQ mice. Our study shed light on the importance of neutrophils in the pathogenesis of SLE, and the suppression of neutrophil function by inhibition of PADI4 will be a unique therapeutic strategy for SLE.

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