**AB0127**

ANTIGENIC ASSESSMENT FOR THE j2GPIox-PF4 COMPLEX IN A MONOCENTRIC COHORT OF PATIENTS WITH APS, THROMBOSIS DURING SARS-COV-2 INFECTION AND VITT

**Keywords:** Autoantibodies, Vaccination/Immunization, Anti-phospholipid syndrome

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**Background:** Platelet factor 4 (PF4) is a protein with a pro-clotting function expressed by activated platelets with a high affinity for anionic glycosaminoglycans present on the platelet surface. It has been shown that the positively charged surface of PF4 tetramer interacts with the negatively charged regions of j2glycoprotein 1 (j2GP) domains, stabilising the link between the antigen and the phospholipid surface, thus increasing the possibility of binding with the respective antibodies. In particular, a tetramer of PF4 selectively binds to j2GP stimulating the dimerization of the same, which is crucial in platelet activation and therefore in thrombotic manifestations of antiphospholipid syndrome (aPS). j2GP may be a common denominator in syndromes such as aPS and heparin-induced thrombocytopenia (HIT), which share similar clinical manifestations as thrombocytopenia and thrombosis. Other syndromes which share the same clinical and laboratory features of HIT despite not having previously received heparin, appear to be associated with the presence of anti-PF4 antibodies. Such pathologies could only be explained by HIT antibodies with heparin-independent platelet-activating properties. One of these could be vaccine-induced immune thrombotic thrombocytopenia (VITT) post-somministration of ChAdOx1 nCoV-19 vaccine. Recent studies have shown structural similarities between heparin and j2GP, which may be responsible for thrombotic events in those infected with SARS-Cov-2 and VITT, who never had heparin. In particular, oxidised-j2GP (j2GPox) may mimic heparin by structural analogy and link to PF4. Considering the structural similarities between heparin and j2GP, and demonstrating the immunogenicity of the hypothesised complex in APS, the alternative molecule could be represented by j2GPox itself, thus explaining the thrombotic events following vaccination in subjects who have never received heparin.

**Objectives:** The aim of the study is to test the potential immunogenicity of the j2GPox-PF4 complex and the presence of antibodies against this complex in patients with aPS, thrombosis during infection with SARS-Cov-2 or VITT.

**Methods:** 34 patients with proven diagnosis of APS, 17 patients with thrombosis related to infection SARS CoV-2 and 3 patients with VITT were enrolled. Only one aPS patient received heparin prior to testing. Antibodies to the j2GPox-PF4 complex were detected by homogeneous immunoassay (Luminex) based on recombinant molecules. The content of the analytes was compared between SS patients and those who were not treated with heparin in the cohort.

**Results:** Anti-j2GPox-PF4 antibodies were detected in 11 of 34 aPS patients (32%) and all VITT patients (100%), while none of covid-19 patients tested positive. In saliva samples, we found significantly increased values of ICAM1, IL-6, and IL-19 in saliva, which correlated with the odds of the patients meeting SS classification criteria from 2002 and/or 2016 who were diagnosed with SS by a rheumatologist and 3 patients who did not meet SS classification criteria who were not finally diagnosed. Serum samples were collected and centrifuged at 1800g, divided into aliquots and stored at -80°C. Saliva samples were cold collected to prevent degradation of proteins, centrifuged at 1800g at 4°C and stored at -80°C. A semiquantitative analysis of protein expression was performed using the “Proteome Profiler Human XL Cytokine Array Kit” arrays from R&D system that detects 105 proteins containing interleukins, chemokines, inflammatory factors and other soluble proteins. Analytical optical densities were quantified using Image J software. The content of the analytes was compared between SS patients and non-SS patients using an unpaired Student’s t test and a measure of the odds of an analyte happening in one group compared to the odds of the same analyte happening in another group (OR: odds ratio) is shown. Statistical analysis were performed using Microsoft Excel software. P values ≤0.05 were defined as significant and values of 0.1-0.05 were considered as borderline.

**Results:** We found increased serum levels of CD14 (OR:1.21; P=0.033), EGF (OR:1.18; P=0.050), IP-10 (OR:1.23; P=0.091) in patients diagnosed with SS who met SS classification criteria, compared with patients who did not meet SS classification criteria who were not diagnosed, although only in the case of CD14 and EGF, statistical significance was reached. In saliva samples, we found significantly increased values of ICAM1 (OR:1.67; P=0.03), IL-6 (OR:1.18; P=0.05), IL-12 (OR:1.23; P=0.042) and IL-19 (OR:1.51; P=0.039) in patient who did not meet SS classification criteria, compared with patients who did.

**Conclusion:** Sjögren’s syndrome patients presented significantly higher levels of CD14 and EGF in serum, as well as significantly decreased levels of ICAM1, IL-6 and IL-19 in saliva, when compared to patients who did not meet SS classification criteria. It is necessary to confirm these results in a larger cohort of patients.

**REFERENCES:**


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**AB0128**

DIFFERENTIAL EXPRESSION OF PROTEINS IN PATIENTS WITH SUSPECTED SJÖGREN’S SYNDROME

**Keywords:** Biomarkers, Cytokines and chemokines, Sjögren syndrome

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**Background:** Sjögren’s Syndrome (SS) is a chronic systemic autoimmune disease characterized by involvement of the exocrine glands in addition to a wide spectrum of systemic manifestations. At present, conventional biomarkers do not respond to unresolved needs regarding diagnosis, stratification of phenotypes and appropriate effective therapies.1 SS is an orphan disease and new biomarkers could constitute potential future therapeutic targets.2

**Objectives:** The purpose of this study was to carry out an exploratory analysis in six serum and saliva samples from suspected SS patients to identify protein biomarkers to distinguish SS patients who were diagnosed with SS from those who were not.

**Methods:** We selected six patients from a cohort of 199 consecutive patients attended the rheumatology department for suspected SS: 3 patients who met SS classification criteria from 2002 and/or 2016 who were diagnosed with SS by a rheumatologist and 3 patients who did not meet SS classification criteria who were not finally diagnosed. Serum samples were collected and centrifuged at 1800g, divided into aliquots and stored at -80°C. Saliva samples were cold collected to prevent degradation of proteins, centrifuged at 1800g at 4°C and stored at -80°C. A semiquantitative analysis of protein expression was performed using the “Proteome Profiler Human XL Cytokine Array Kit” arrays from R&D system that detects 105 proteins containing interleukins, chemokines, inflammatory factors and other soluble proteins. Analytical optical densities were quantified using Image J software. The content of the analytes was compared between SS patients and non-SS patients using an unpaired Student’s t test and a measure of the odds of an analyte happening in one group compared to the odds of the same analyte happening in another group (OR: odds ratio) is shown. Statistical analysis were performed using Microsoft Excel software. P values ≤0.05 were defined as significant and values of 0.1-0.05 were considered as borderline.

**Results:** We found increased serum levels of CD14 (OR:1.21; P=0.033), EGF (OR:1.18; P=0.050), IP-10 (OR:1.23; P=0.091) in patients diagnosed with SS who met SS classification criteria, compared with patients who did not meet SS classification criteria who were not diagnosed, although only in the case of CD14 and EGF, statistical significance was reached. In saliva samples, we found significantly increased values of ICAM1 (OR:1.67; P=0.03), IL-6 (OR:1.18; P=0.05), IL-12 (OR:1.23; P=0.042) and IL-19 (OR:1.51; P=0.039) in patient who did not meet SS classification criteria, compared with patients who did.

**Conclusion:** Sjögren’s syndrome patients presented significantly higher levels of CD14 and EGF in serum, as well as significantly decreased levels of ICAM1, IL-6 and IL-19 in saliva, when compared to patients who did not meet SS classification criteria. It is necessary to confirm these results in a larger cohort of patients.

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Background: Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease with diverse pathogenesis. Excessive apoptosis and impaired clearance of apoptotic cells are the primary drivers of interferon I (IFN) signaling pathways. Upregulation of the long noncoding RNA Growth arrest-specific 5 (GAS5) was found to be associated with apoptosis [1]. Oncological studies discovered that GAS5 is regulated by IFN signaling pathways and acts as a positive feedback loop on IFN response [2,3]. In a mouse model, GAS5 was linked to SLE susceptibility [4]. However, the exact mechanism is still unknown. Furthermore, GAS5 has been proposed to act by interfering with glucocorticoid action, with a key role in regulating glucocorticoid resistance and sensitivity [5].

Objectives: To evaluate the expression levels of GAS5 in serum of SLE patients in comparison with healthy controls, assess their relation to SLE Disease Activity Index 2000 (SLEDAI-2K) and Systemic Lupus International Collaborating Clinics/American College of Rheumatology Disease Activity Index (SDI), and explore their association with each of the 3 IFN stimulatory genes (MX1, IFI44L, IFIT1) as well as IFN signature to elucidate how GAS5 shares in the pathogenesis of SLE.

Methods: 30 adult SLE patients and 20 age and sex-matched healthy controls were enrolled. SLE disease activity and damage were assessed by SLEDAI-2K and SDI respectively. The expression levels of GAS5 as well as 3 IFN stimulatory genes (MX1, IFI44L, IFIT1) were measured by quantitative real-time PCR. IFN signature score was calculated as described in a previous study [6].

Results: GAS5 expression levels were insignificantly upregulated in SLE patients compared to control (p-value 0.11). At cut-off value 13-fold change, GAS5 could discriminate SLE from control (AUC 0.63) with high specificity 100 % and modest sensitivity 63%. Their expression levels were not associated with SLEDAI-2K or SDI scores. GAS5 levels were positively correlated with the cumulative dose of steroids in comparison with healthy controls, assess their relation to SLE Disease Activity Index 2000 (SLEDAI-2K) and Systemic Lupus International Collaborating Clinics/American College of Rheumatology Disease Activity Index (SDI), and explore their association with each of the 3 IFN stimulatory genes (MX1, IFI44L, IFIT1) as well as IFN signature to elucidate how GAS5 shares in the pathogenesis of SLE.

Conclusion: GAS5 may be considered a diagnostic biomarker for SLE, but it is not a reliable biomarker for disease activity or damage. GAS5 is suggested to be incorporated into the IFN signaling pathway in SLE raising possible contribution in SLE pathogenesis.

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