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Extended Abstract

Salivary ^{1}H-NMR Metabolomics in Primary Sjögren Syndrome. Preliminary Results of a Pilot Case-Control Study †

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Primary Sjögren Syndrome (pSS) is a multisystem autoimmune disease which mainly involves exocrine glands, such as salivary and lacrimal. Pathogenesis is not completely understood even if is distinguished by lymphocyte glands tissue infiltration, which leads to anatomical modification and hypofunction. Age at diagnosis is typically between 3rd and 5th decade. European incidence *per* year is 3 to 11 cases on 100.000 subjects, with a female-male ratio of 10:1 and a 0.01% e 0.72% prevalence.

Diagnostic criteria are resumed in the 2016 ACR/EULAR Consensus of Classification Criteria for pSS, and are based on the sum of weighted scores applied to 5 items: anti-SSA/Ro antibody positivity, focus score of ≥1 foci/4 mm^2, abnormal ocular staining, a Schirmer's test result of ≤5 mm/5 min and an unstimulated salivary flow rate (SFR) of ≤0.1 mL/minute. The prognosis of pSS is favorable with a patient's life expectancy comparable with general population. Quality of life is reduced by the diverse manifestations of the disease. Cardiovascular disease, infections, solid tumors, and lymphoma are the main causes of death [1].

The aim of this pilot study is to compare the salivary metabolome of pSS and healthy controls (HC). Cases were selected from a cohort of pSS patients; age and sex matched HC were recruited from a cohort of volunteers. Strict inclusion and exclusion criteria were applied, in order to select the most homogeneous study population. To all recruited patients was asked to not eat, drink, smoke and use oral hygiene products one hour before saliva collecting. Whole unstimulated saliva was collected into a sterile Eppendorf. SFR was contextually evaluated. All samples were immediately frozen into liquid nitrogen and stored into −80 °C refrigerator until analysis.

7 pSS and 6 HC female patients were recruited. All samples were centrifuged at 15,000 rpm/10 min, 4 °C. 100 μL of buffer were added to 500 μL of supernatant; the solution was inserted in the

NMR tube of a Bruker Avance III HD 600 MHz spectrometer for the ^{1}H-NMR analysis. Mono-dimensional and bi-dimensional measurements were performed using different pulse sequences such as Carr-Purcell-Meiboom-Gill (cpmg) and different correlation sequence such as COrrelation SpectroscopY (COSY) and Heteronuclear Single Quantum Coherence (HSQC) (Figure 1). Software aided data analysis were performed.

33 metabolites were detected. An high metabolite variability was observed. Normalized spectral matrix multivariate statistical analysis (PCA and PLS-DA) returned interesting results, describing significant differences of metabolites expressions between groups (Figure 1).

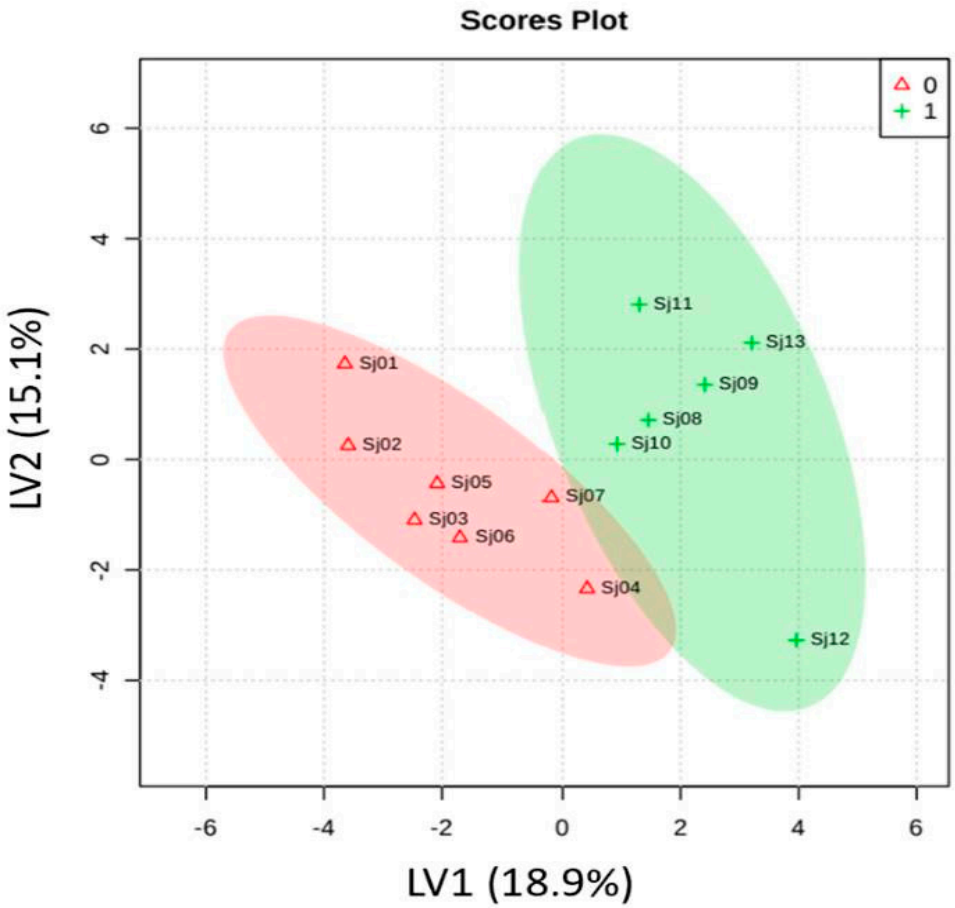

Figure 1. Partial Least Squares Discriminant Analysis (PLS-DA). Chemo-metric differences between groups emerged (SS vs. HC); algorithm supervised analysis showed separation of variables' clusters (metabolites).

Conflicts of Interest: The authors declare no conflict of interest.

Reference

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