Metabolic Profiling for the Identification of Distinct Signatures in Active Rheumatoid Arthritis Urine Samples

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Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder that affects joints. It is characterized by persistent synovitis, joint destruction, systemic inflammation, and disability. High-throughput analysis technologies can generate comprehensive profiles, allowing for the identification of more accurate diagnostic biomarkers that contribute to the improvement of disease outcome. The purpose of this study is to describe the urine metabolomic profile of active RA patients and discover signature molecules with predictive and prognostic capacity.

Urine samples from 148 RA patients were collected at General Hospital of Athens "Laiko". A control population of 14 age-matched individuals, exhibiting similar prevalence of cardiovascular disease, were also recruited. RA patients were classified based on the disease activity score, DAS28, and those with a cutoff value >5.1 (High; n=18) were used in the analysis. ^{1}H NMR spectroscopy was employed for global metabolic profiling and sample pH was adjusted at 7.4 in a TSP-containing buffer. After *noesy* spectra acquisition, the assigned peaks were aligned and binned (0.0005 ppm) using *icoshift* (Matlab). Following the exclusion of H_2O peaks, data normalization was performed using the Probabilistic Quotient Normalization (PQN), with reference to Control samples. Multivariate (OPLS-DA) and Univariate (t-test) methods were used to compare High Disease Activity RA and Controls. Therapy-related metabolites were excluded from the analysis, in order to study underlying pathogenic mechanisms.

Peak assignment of the aligned *noesy* spectra resulted in the identification of 62 known and 14 unknown metabolites. Supervised analysis was carried out to evaluate perturbations in the metabolic profile of High DAS28 RA. The urine metabolome of patients with High activity demonstrated elevated levels of acetate, trimethylamine N-oxide (TMAO), acetone and 3-indoxylsulfate and relatively lower levels of creatinine, creatine phosphate, citrate, glycine, valine, glucose, 3-hydroxyisovalerate and guanidoacetate, compared to Controls. Univariate analysis confirmed the statistical significance and the important role of these small molecules in active RA.

In conclusion, these data indicate that the metabolic profile in urine of active RA patients shows significant perturbations. The distinct metabolic signature is likely attributed to changes in specific pathways, including glycine, serine and threonine metabolism, glycoxylate and dicarboxylate metabolism and glycolysis/ gluconeogenesis. Future efforts will focus on the identification of activity-related profiles, as well as markers for therapy response.