In the quest of robust biomarkers for inflammatory bowel disease phenotypes using GC×GC-HRTOFMS

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We developed, validated, and implemented a strict QA/QC system for a GC×GC-HRTOFMS method for the metabolic profiling of human serum. We were able to highlight sets of biomarkers capable to discriminate between various inflammation phenotypes representative of inflammatory bowel diseases.

During this study, two of the main challenges of untargeted metabolomics were especially considered.

First, the issue of data handling –large datasets and low number of samples compared to variables- was considered by the definition of a workflow of data preprocessing and processing, including the creation of a study template, the rigorous selection of good chromatographic quality features, and the multiplication of statistic techniques to be combined before test validation. In practice, 94 injections were made over 4 weeks, consisting of 70 study samples along with 16 QC samples and 8 reinjections due to QC system rejection. The chromatogram template included 524 verified features that were then reduced to less than two hundreds after selection of the ones having an analytical variation under 30%, based on the QC samples. This resulted in the finding of robust biomarkers that positively discriminated between the different phenotypes of inflammation, including high and low inflammation, remission, and healthy statutes.

Second, the identification of unknown compounds was enhanced by using state-of-the-art high-resolution (HR) time-of-flight mass spectrometry and allowed to name and characterize putative biomarkers with higher degree of confidence. This is a mandatory step for integration of the results obtained in biological pathways interpretation, as well their possible use in at the clinical level.

In conclusion, this study, through the use of optimized and fully controlled GC×GC and high resolution mass spectrometry, highlighted inflammation biomarkers able to discriminate between and better understand different phenotypes of inflammatory bowel diseases.