Metabolites from Cerebrospinal fluid (CSF): Multiplesclerosis (MS) pattern-recognition applying ^{1}H NMR and statistical methods

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Metabonomics is increasingly being used to investigate complex body fluid composition applying several spectroscopic methodologies. Among these, high-resolution ^{1}H NMR spectroscopy coupled with pattern recognition is a method of great success due to its ability to quantify a large range of metabolites simultaneously without preconceived ideas of the biomarkers associated with pathology. The use of multivariate techniques such as PLS-Discriminant Analysis on a complex data provides a statistical tool for discriminating between spectra from different classes of samples, thus reducing the large numbers of spectral features to key metabolic perturbations.

In this study, 37 cerebrospinal fluid (CSF) samples (25 from patients with multiple sclerosis [MS]_and 12 from disease controls - idiopathic polyneuropathy and meningitis) were examined by ^{1}H NMR spectroscopy and the data analyzed by multivariate statistics. The study was approved by our local Ethics Committee and all individuals gave informed consent. All CSF samples were collected for clinical diagnostic purposes, and a small portion of the sample was kept for ^{1}H-NMR analyses. All ^{1}H NMR spectra were acquired at 499,9 MHz using a INOVA 500 spectrometer (Varian) and a 5 mm triple resonance inverse probe. Spectra were recorded at 298 K and represented the sum of 64 transient acquired over 64 K data points with a spectral width of 10 kHz. FID were transformed using 1 degree of zero filling and 0.5 Hz exponential multiplication. The reference was 2.5 mM (TPS) at =0.0 was added (aqueous solution 100 l, 2.5 mM) to the CSF sample (500 L). All spectra were treated prior to the multivariate statistics and pattern recognition by adjusting the TPS peak for possible shift and to the same height. Each speactum was basline corrected using a linear fit and the final data set was autoscaled. The ^{1}H NMR spectra demonstrated resonance arising from acetate, alanine, β-hydroxybutyrate, citrate, formate, glucose, glutamine, glutamate, myo-inositol, isobutyrate, lactate, succinate, tyrosine and valine. PLS-DA demonstrated that CSF from MS and disease control patients were different with increased, glutamine, glutamate, -hydroxybutirate and acetoacetate in patients with MS (Figure 1a and b). Scores plot (Figure 1c) discriminates the group with MS from the disease control group. Leave-one-out crossvalidation indicated only one misclassification.

The increase in glutamine might be related to aminoacids degradation, while -hydroxybutirate and acetylacetate are ketonic bodies which are an alternative energetic route when glucose availability is low or inefficient.

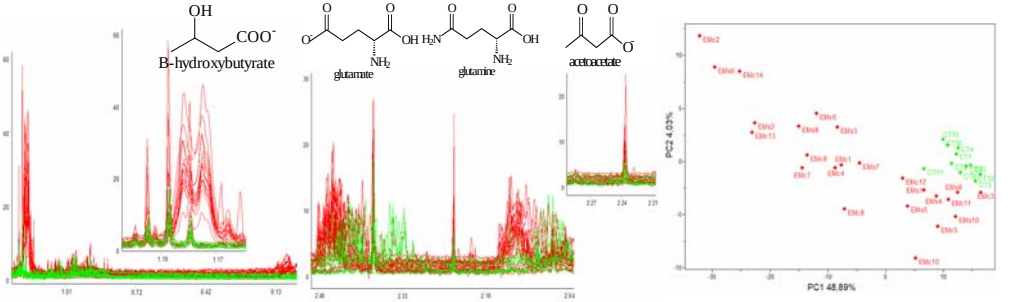

Figure 1. Spectral profiles demonstrated that CSF from MS and disease control patients were different with increased **a**. -hydroxybutirate and b. glutamine, glutamate, and acetoacetate in patients with MS c. Scores plot (PLS-DA) discriminates the group with MS from the disease control group.

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