Discriminating multiple-sclerosis patient groups : a principal-component analysis of CSF metabolite profiles

N. W. Lutz[1], A. Viola[1], I. Malikova[1,2], S. Confort-Gouny[1], J-P. Ranjeva[1], J. Pelletier[1,2], and P. J. Cozzone[1]

[1]Centre de Résonance Magnétique Biologique et Médicale (CRMBM), UMR CNRS n°6612, Faculté de Médecine de Marseille, Université de la Méditerranée, Marseille, France, [2]Service de Neurologie, Hôpital de la Timone, Faculté de Médecine de Marseille, Université de la Méditerranée, Marseille, France

Introduction

Over the past 15 years, ^{1}H MRS analysis of metabolite levels in cerebrospinal fluid (CSF) has been employed to characterize different manifestations of multiple sclerosis (MS) [1-4]. As with other non-metabolic diseases of the central nervous system, MS effects on the biochemical composition of CSF are subtle [2]. Therefore, concentration ranges of virtually all metabolites quantified by ^{1}H MRS overlap considerably between different MS groups and controls [2,4]. Consequently, distinguishing patient groups on the basis of individual metabolite levels is not feasible [4]. We investigated the possibility to discriminate between patient groups by using a large number of metabolites combined (principal component analysis, PCA). PCA is an unsupervised technique for examining relationships among several quantitative variables, and is increasingly used in comprehensive analysis of metabolic processes (metabolomics). PCA was applied to three particularly homogeneous populations, consisting of untreated clinically isolated-syndrome (CIS) patients with or without inflammatory plaques, and control subjects.

Methods

CSF samples were analyzed for a total of 33 MS patients diagnosed with CIS, i.e. the first MS episode, and 10 controls (lumbar-puncture protocol as described previously [2]). Twenty-one of the MS patients investigated showed contrast-enhanced (inflammatory, active) brain plaques at MRI using gadolinium-DTPA as a contrast agent. CSF samples were concentrated by lyophilization, and prepared in D_2O for ^{1}H MRS analysis as described elsewhere [4]. ^{1}H MR spectra were acquired at pH 7.0 and 28° C on an AVANCE 400 spectrometer (Bruker, Wissembourg) using TR=15 s, a 90°-pulse with water suppression, and 64K data points. Spectra were referenced using trimethylsilyl tetradeuteropropionate (TSP-d_4) as an internal standard, and evaluated with Bruker's deconvolution software MDCON (Topspin 1.3). The metabolite concentrations obtained were used for PCA, using the JMP 6.0.3 statistics software (SAS, Cary, NC, USA).

Results and Discussion

PCA generated 28 principal components, corresponding to the number of quantified metabolites. A three-dimensional data plot based on the first three principal components (P1, P2 and P3) was produced. Figures 1 and 2 show two-dimensional projections of this plot, for two different projection angles (see orientation of axes P1-P3). Green, black and red dots represent data from controls, CIS without and with inflammatory plaques, respectively. Some data clustering is observed for the groups with inflammatory plaques (Fig. 1) and controls (Fig. 2). However, clusters are not well separated. Overlap is most pronounced for the CIS group without inflammatory plaques.

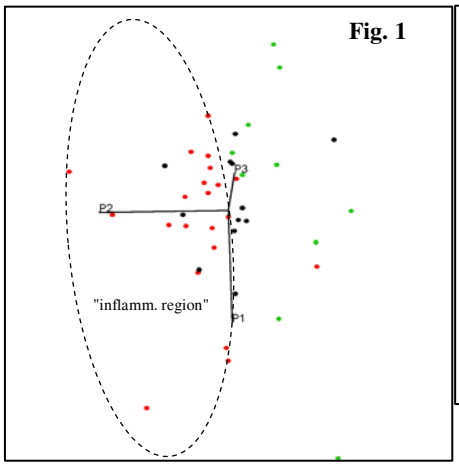

Fig. 1

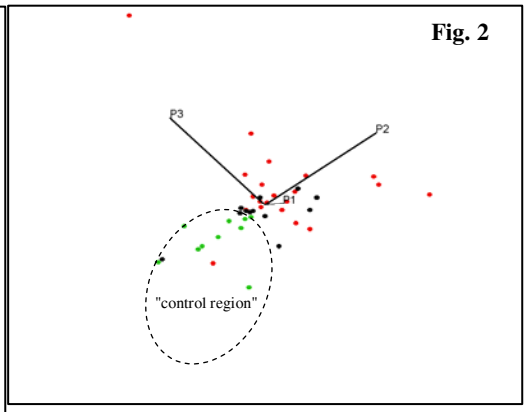

Fig. 2

Principal-component eigenvectors for metabolite levels that have been shown to present significant inter-group differences in Mann-Whitney *U* tests [4] were calculated for P1-P3, along with cumulative percent of variation. Values given in Table 1 are based on correlation rather than covariance matrices to give equal weighting to metabolite levels of differing variability (overall, concentrations varied by three orders of magnitude!). Eigenvectors represent the contribution of each metabolite to each principal component. Based on eigenvectors of the correlation matrix, all metabolites in question, except for acetate, contributed similarly to P1 (Table 1), while eigenvectors of the covariance matrix had intrinsically very low values for metabolites other than the most concentrated compounds (data not shown). However, it should be noted that also a number of metabolite levels that had *not* shown statistically significant inter-group differences, were characterized by relatively high eigenvector values of the correlation matrix (not shown), and that P1, P2 and P3 combined only explained 47.3% of the variance (vs. 96.1% for the covariance matrix).

Table 1 PCA - eigenvectors and cumulative percent of variation

	P1	P2	P3
eigenvectors			
lactate	0.29	0.12	0.15
β-hydroxyisobutyrate	0.24	0.01	0.02
β-glucose	0.24	0.21	-0.02
glutamine	0.24	0.14	-0.11
acetate	0.06	-0.45	0.15
creatinine	0.16	0.09	0.14
fructose	0.20	0.17	-0.02
cumul. percent	28.2	38.4	47.3

Overall, PCA suggests the existence of metabolic profiles characteristic of CIS with and, to a much lesser extent, without inflammatory plaques. Nevertheless, unambiguous patient classification for diagnostic purposes cannot be expected due to poor separation of groups.

References

1. Koschorek F et al. (1993) *Neurosurg Rev* 16: 307-315. 2. Nicoli F et al. (1996) *C R Acad Sci Paris* 319: 623-631. 3. Aasly J et al. (1997) *Acta Neurol Scand* 95: 9-12. 4. NW Lutz et al. (2006) *Proc Intl Soc Magn Reson Med*, 14th Scientific Meeting, Seattle, WA, USA, 1986.