Journal of Neuroimmunology 299 (2016) 112–117

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Glial and neuronal markers in cerebrospinal fluid in different types of multiple sclerosis

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ARTICLE INFO

Article history:
Received 12 February 2016
Received in revised form 17 July 2016
Accepted 4 August 2016
Available online xxxx

ABSTRACT

In the present study, CSF concentrations of NFL, t-tau, p-tau, GFAP, S-100B, YKL-40, MCP-1, α-sAPP, β-sAPP, and Aβ38, Aβ40, Aβ42 were measured in 324 MS patients to test whether a correlation among the biomarkers exists and whether the profile of CSF biomarkers varies among the different types of MS. The CSF concentrations of NFL were significantly higher in RRMS while CSF concentrations of GFAP were higher in PPMS. CSF concentrations of NFL correlated with YKL-40 in CIS patients while CSF concentrations of GFAP correlated with YKL-40 in RRMS patients.

1. Introduction

Multiple sclerosis (MS) is classically described as a demyelinating disease of the central nervous system (CNS) due to the histological findings of white matter lesions in brains. However, there is evidence that not only myelin and oligodendrocytes are implied in MS pathogenesis, but also neuronal damage and astroglial activation (Eng and Ghirnikar,1994; Bonneh-Barkay et al., 2010; Trapp et al., 1998; Filippi et al., 2003). Clinically, MS is considered a chronic autoimmune condition. Even though, the involvement of the immune system has been thoroughly described in MS, there is also evidence that diffuse neurodegenerative processes takes part in the MS pathogenesis from the early stages of the disease (Frischer et al., 2009). Probably, the predominance of autoimmune activation at the onset of the disease explains the inflammatory course of the relapsing-remitting forms of MS. While the predominance of neuronal and glial degeneration would be associated with progressive forms of MS. Ideally, knowing the underlying histopathological process in each MS patient could lead to an individual treatment for a specific target. Notwithstanding, it is not feasible to obtain brain biopsies. Thus, MS patients are classified by their clinical course as clinically isolated syndrome (CIS), relapsing-remitting MS (RRMS), secondary progressive MS (SPMS) and primary progressive MS (PPMS), and they are treated according to the international standards.

Cerebrospinal fluid (CSF) is the closest body fluid to brain tissue (Blennow et al., 2010). Therefore, CSF from a diagnostic lumbar puncture could reflect the features of brain damage at MS onset and could differentiate the different patterns of MS.

The purpose of the present study was to investigate glial and neuronal biomarkers in CSF samples from patients with different types of MS and to test whether a correlation among the biomarkers exists and whether the profile of CSF biomarkers varies among the different types of MS. Hence, we analysed biomarkers related to axonal damage (neurofilament light protein: NFL) (Trapp et al., 1998), neuronal injury (total-tau: t-tau and tau phosphorylated at threonine 181: p-tau) (Binder et al., 1985), glial activation (human chitinase 3-like 1 protein: YKL-40 and monocyte chemoattractant protein: MCP-1) (Bonneh-Barkay et al., 2010; Van Der Voorn et al., 1999), astrocytic damage (glial fibrillary acidic protein: GFAP and S-100B protein: S-100B) (Eng and Ghirnikar, 1994; Donato, 2001), and amyloid metabolism (α-cleaved soluble amyloid-precursor protein: α-sAPP; β-cleaved soluble amyloid-precursor protein: β-sAPP; 38,

Abbreviations: Aβ38, Aβ40, Aβ42, 38, 40 and 42 amino acid long fragments of amyloid β; α-sAPP, α-cleaved soluble amyloid-precursor protein; β-sAPP, β-cleaved soluble amyloid-precursor protein; CIS, Clinically isolated syndrome; CNS, Central nervous system; CSF, Cerebrospinal fluid; EDMUS, European database for multiple sclerosis; EDSS, Expanded disability status scale; GFAP, glial fibrillary acidic protein; IDIBELL, Bellvitge Biomedical Research Institute; MCP-1, monocyte chemoattractant protein; MS, multiple sclerosis; NFL, neurofilament light; OPLS-DA, orthogonal projection to latent structure discriminant analysis; p-tau, tau phosphorylated at threonine 181; PPMS, primary progressive multiple sclerosis; RRMS, relapsing remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; S-100B, S-100B protein; t-tau, total tau protein; YKL-40, human chitinase 3-like 1 protein.

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http://dx.doi.org/10.1016/j.jneuroim.2016.08.004
0165-5728/

40 and 42 amino acid long fragments of amyloid β: Aβ38, Aβ40, Aβ42) (Gehrmann et al., 1995; Ferguson et al., 1997).

2. Materials and methods

2.1. Patients and clinical assessments

The present observational study was approved by the ethics committee of Bellvitge University Hospital, L'Hospitalet de Llobregat, Spain and informed consent was obtained from all patients. Samples were obtained from the Bellvitge Biomedical Research Institute (IDIBELL), the CSF biobank MS Unit collection. Samples were matched with clinical data from patients recruited and prospectively followed at the MS Unit, Bellvitge University Hospital. All clinical data were entered into the European Database for Multiple Sclerosis (EDMUS) (Confavreux et al., 1992). Patients were diagnosed according to the Poser and McDonald criteria as appropriate and were classified as having CIS, RRMS, SPMS or PPMS according to the disease course when the lumbar puncture was performed (Poser et al., 1983; McDonald et al., 2001; Polman et al., 2005). The cohort of CIS and RRMS patients collective in the present study was the same as was evaluated in the previous published study (Mañé-Martínez et al., 2015). For the inclusion into the relapsing group, the first signs of relapse had to start within one month of sampling. The neurological deficits were scored with the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983).

2.2. CSF sampling and biochemical analyses

CSF samples were collected in 109 CIS patients, 192 RRMS patients, 6 SPMS patients and 17 PPMS patients by lumbar puncture into polypropylene tubes, centrifuged at 2200 x g for 10 min, aliquoted into 1 mL cryo tubes that were stored at −80 °C pending analyses. Concentrations of CSF biomarkers were analysed in blind fashion at the Clinical Neurochemistry Laboratory, Institute of Neuroscience and Physiology, University of Gothenburg, using enzyme-linked immunosorbent assays as described (Mañé-Martínez et al., 2015).

2.3. Statistics

Continuous variables were described by their mean and standard deviation or median and interquartile range, depending on their distribution, and categorical variables by numbers and percentages. Differences between groups were analysed with Kruskal-Wallis test followed by pairwise post hoc comparisons using the Mann-Whitney *U* test, p values ≤ 0.05 were considered significant. Spearman's test was used to analyse correlations between demographics and CSF biomarker concentrations, p values ≤ 0.05 were considered significant. Correlations among different biomarkers were analysed using age-adjusted Spearman's test and p values were adjusted using Bonferroni (Holms) correction. Univariate statistical analyses were prepared using Statistical Package for the Social Sciences 20.0 (SPSS Inc, Chicago, IL). Multivariate analysis was performed to find differences between the relapsing and remitting phases, using orthogonal projection to latent structure discriminant analysis (OPLS-DA) implemented in the software SIMCA-P+ v. 12 (Umetrics, Umea, Sweden) (Andreasson et al., 2012). The OPLS-DA algorithm finds the projection direction, score vector, that gives the largest covariance between the variables and the pre-defined classes (i.e. relapsing and remitting phases) and that maximizes the separation between the classes. The variables that are found to have an influence on the projection (VIP: Variable importance on the projection plots) and that contribute to discriminate between the classes are summarized in the VIP plot. The higher the VIP bar, the more influential is the variable on the model. The VIP plot also gives a 95% confidence interval (CI) for the contribution of each variable, and a large inaccuracy (Hjalmarsson et al., 2014).

3. Results

Demographics and clinical characteristics of MS patients are shown in Table 1.

3.1. CSF biomarker concentrations and demographics

The correlation between age at lumbar puncture and CSF biomarker concentrations was evaluated in a total of 324 patients. A positive correlation was found between age and GFAP, YKL-40, S-100B, p-tau, α-sAPP and β-sAPP, whereas a negative one was found for NFL (Spearman's test correlation index: NFL: −0.21, $p < 0.0001$; GFAP: 0.24, $p < 0.0001$; YKL-40: 0.16, $p = 0.004$; S-100B: 0.14, $p = 0.01$; p-tau: 0.15, $p = 0.007$; α-sAPP: 0.16, $p = 0.004$; β-sAPP: 0.15, $p = 0.005$); no significant correlation was found for MCP-1, t-tau, Aβ38, Aβ40 and Aβ42. Similar correlations were found in the subgroup of CIS and RRMS patients ($n = 301$) (Spearman's test correlation index: NFL: −0.16, $p = 0.003$; GFAP: 0.21, $p < 0.0001$; YKL-40: 0.14, $p = 0.02$; S-100B: 0.15, $p = 0.01$; p-tau: 0.14, $p = 0.02$; α-sAPP: 0.12, $p = 0.04$; β-sAPP: 0.12, $p = 0.03$). There were no significant correlations between biomarker concentrations and the duration of the disease at LP time. In relation to gender, significantly higher CSF concentrations of MCP-1, YKL-40, GFAP, p-tau, α-sAPP, β-sAPP, Aβ38, Aβ40, Aβ42 were found in males vs females (Table 2). The subgroup of relapsing-remitting patients ($n = 301$) showed similar data except for YKL-40 (males: 105 ng/mL (80–165); females 94 ng/mL (67–146), $p = 0.04$) and GFAP (no significant differences in CSF concentrations between males and females), while no differences were found in the PPMS subgroup. There were no significant correlations between the EDSS at the time of the lumbar puncture and CSF biomarker concentrations except for YKL-40 (CI: 0.17, $p = 0.002$);

Table 1
Demographics and clinical characteristics of MS patients at baseline.

	CIS ($n = 109$)	RRMS ($n = 192$)	SPMS ($n = 6$)	PPMS ($n = 17$)	Total ($n = 324$)
Gender, female, n (%)	75 (68%)	121 (63%)	4 (67%)	7 (41%)	207 (64%)
Age at MS onset, y, mean (SD)[a]	31.1 (9.8)	28.8 (8.8)	33.6 (13.9)	45.1 (11.3)	30.5 (10.1)
Age at LP, y, mean (SD)[b]	32 (9.8)	34.8 (8.9)	43.5 (10)	44.2 (23.5)	34.5 (10.8)
Disease duration at LP, y, mean (SD)[c]	0.3 (0.5)	5.5 (6.2)	9.4 (7.1)	4.6 (3.1)	3.8 (5.5)
EDSS at LP, median (IQR)[d]	2 (0–2.0)	2 (1.0–2.5)	4.5 (4.0–6.0)	3 (2.0–3.5)	2 (1.0–2.5)
Patients under treatment before LP, n	0	7	4	0	11

Abbreviations: CIS = clinically isolated syndrome; EDSS = Expanded Disability Status Scale; IQR = interquartile range; LP = lumbar puncture; No = n = number of cases; PPMS = primary progressive multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; SD = standard deviation; SPMS = secondary progressive multiple sclerosis; y = years.

[a] At MS onset, CIS and RRMS patients were younger compared to PPMS ($p < 0.0001$ for both comparisons).

[b] At LP time, CIS patients were younger compared to RRMS ($p = 0.001$), SPMS ($p = 0.01$) and PPMS ($p < 0.0001$). RRMS patients were younger compared to SPMS ($p = 0.03$) and PPMS ($p < 0.0001$).

[c] Disease duration at lumbar puncture was shorter in CIS patients compared to RRMS, SPMS and PPMS patients ($p < 0.0001$ for all comparisons).

[d] EDSS at LP was lower in CIS vs RRMS ($p = 0.004$), SPMS ($p < 0.0001$) and PPMS ($p < 0.0001$). EDSS at LP was lower in RRMS vs SPMS ($p < 0.0001$) and PPMS ($p = 0.001$). EDSS was lower in PPMS vs SPMS ($p = 0.01$).