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Clinical trial

Cerebrospinal fluid neurofilament light chain predicts disease activity after the first demyelinating event suggestive of multiple sclerosis

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ABSTRACT

Background: The prediction of disease activity in patients with a first demyelinating event suggestive of multiple sclerosis (MS) is of high clinical relevance. Cerebrospinal fluid (CSF) neurofilament light chain (NfL) has shown to have prognostic value in MS patients. In this work, we measured CSF NfL in patients at the first demyelinating event in order to find a cut-off value able to discriminate patients who will have disease activity from those who will remain stable during the follow-up.

Methods: We included CSF samples collected within 30 days after the onset of the first demyelinating event from 32 patients followed-up for 3.8 ± 2.5 years. CSF NfL was measured with a newly developed in-house enzyme linked immunosorbent assay (ELISA).

Results: At the first demyelinating event, patients with subsequent disease activity had significantly higher baseline CSF NfL values compared to clinically and radiologically stable patients (median 812.5 pg/mL, range 205–2359 pg/mL vs 329.5 pg/mL, range 156–3492 pg/mL, $p = 0.002$). A CSF NfL cut-off value of 500 pg/mL significantly discriminated these two groups of patients with a 90% sensitivity and an 83.3% specificity.

Conclusion: Our results confirm that CSF NfL is a prognostic marker in the very early phases of MS. The validation of a cut-off value of 500 pg/mL could provide clinicians with a dichotomous variable that can simplify the prognostic assessment of patients at the first demyelinating event.

1. Introduction

In 85% of cases, multiple sclerosis (MS) presents at onset with an isolated central nervous system (CNS) demyelinating event, the clinically isolated syndrome (CIS) (Miller et al., 2012). CIS patients have an intrinsic high risk to subsequently develop MS, since about 2/3 of them will experience a second relapse or additional changes on follow-up magnetic resonance images (MRI) (Fisniku et al., 2008). For this reason, CIS is now included among the clinical phenotypes of MS (Lublin et al., 2014). Moreover, the two most recent revisions of diagnostic criteria have made it possible to define both dissemination in space and in time of demyelinating lesions soon after the first manifestation of the disease, thus facilitating an earlier diagnosis of MS (Polman et al., 2011; Thompson et al., 2018).

In the management of patients at the first demyelinating event, there is a compelling need for reliable tools that could help to identify those patients who will subsequently show disease activity. Indeed, these patients could benefit from an early and, consequently, more effective treatment (Comi et al., 2017).

Several studies, performed on large multicenter CIS populations, have shown that a combination of demographical, clinical, laboratory and MRI features is able to stratify the risk of subsequent conversion into MS (Kuhle et al., 2015; Spelman et al., 2016; Tintore et al., 2015). Among baseline characteristics of CIS patients, the presence of

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cerebrospinal fluid (CSF) oligoclonal bands (OCBs) is one of the factors associated with a higher risk of MS development (Tintore et al., 2015). Importantly, in the most recent update of diagnostic criteria for MS, CSF-specific OCBs have been re-entered as a diagnostic tool (Thompson et al., 2018), which gives the opportunity to analyze additional CSF biomarkers reflecting other early pathophysiological mechanisms underlying MS, such as axonal damage.

Neurofilament light chain (NfL) is a structural protein of axonal cytoplasm and it is highly expressed in large-calibre myelinated axons within the CNS (Zetterberg, 2016). NfL levels increase in the CSF as a consequence of axonal damage and their changes reflect ongoing disease activity in MS (Teunissen et al., 2009a). So far, studies on CSF NfL have been performed using the same commercially available enzyme-linked immunosorbent assay (ELISA) (Norgren et al., 2002). In the present study, we measured CSF NfL in a cohort of patients at the first demyelinating event by means of a newly developed ELISA (Gaetani et al., 2018). We retrospectively investigated the prognostic value of CSF NfL in defining the risk of subsequent disease activity, and we looked for a possible cut-off value able to discriminate patients who presented clinical and/or radiological signs of disease activity from those who were stable during the follow-up.

2. Patients and methods

2.1. CSF sample collection and storage

We analyzed 32 CSF samples stored in the Biobank of the Section of Neurology, Department of Medicine, University of Perugia (Perugia, Italy). CSF was collected over a 10-year period (from 2006 to 2016), via lumbar puncture, in the same Institution, with the same standard operating procedures, and stored according to a protocol following international guidelines (Teunissen et al., 2009b). Specifically, in all patients lumbar punctures were performed in the morning between 8:00 and 11:00 and CSF was collected in sterile polypropylene tubes, centrifuged for 10 minutes at 2000 × g, divided into 0.5 mL aliquots and immediately frozen at −80 °C, pending analysis. CSF samples were collected within 30 days after the onset of the first demyelinating event.

2.2. CSF sample selection

We selected for this study CSF samples from patients satisfying, at the time of CSF collection, the following inclusion criteria: (i) a diagnosis of first demyelinating event suggestive of MS, including both CIS and possible MS (i.e. CIS with MRI evidence of dissemination in space but not in time or vice-versa) according to the 2010 revision of the McDonald criteria (Polman et al., 2011); (ii) a follow-up period lasting until the appearance of further disease activity or at least one year; (iii) age > 18 years.

2.3. Patients clinical and MRI assessment

At baseline, main demographic, clinical and neuroradiological characteristics of the patients were recorded. In all the patients, CSF routine analysis was performed, together with isoelectric focusing followed by immunofixation for IgG OCBs detection. Patients were followed-up clinically and radiologically according to the routine monitoring program and none of them underwent disease-modifying treatments before the appearance of subsequent clinical and/or MRI signs of disease activity. Disease activity was defined by the appearance, at any time during the follow-up, of one or more of the following: (i) a clinical relapse, (ii) new T2 lesions on a follow-up MRI scan, (iii) enlarging T2 lesions on a follow-up MRI scan, (iv) gadolinium-enhanced lesions on a follow-up MRI scan. Experienced neurologists diagnosed a relapse in presence of new neurological symptoms and signs suggestive of an acute inflammatory demyelinating event in the CNS, with duration of at least 24 h, in the absence of fever or infection (Polman et al., 2011). All the patients underwent contrast-enhanced brain and spinal cord MRI at the baseline and during the follow-up as part of the usual workup (3, 6 and 12 months after the onset, then yearly if asymptomatic). All images were acquired with a 1.5 Tesla magnet according to published guidelines (Filippi et al., 2013).

2.4. CSF NfL assessment

CSF NfL was measured in the Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry at the Sahlgrenska Academy, University of Gothenburg (Mölndal, Sweden), through a newly-developed in-house ELISA, as already described (Gaetani et al., 2018). All samples were analysed by board-certified laboratory technicians, all blinded to clinical data, by using one batch of reagents at a time.

2.5. Standard protocol approvals, registrations and patient consents

Patients gave informed consent to participate in the study. The study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethics committee.

3. Statistical analysis

Continuous variables are reported as mean ± standard deviations (median; range). For continuous variables, normal distribution was tested with the Shapiro–Wilk test. Group differences were assessed with an unpaired *t*-test for normally distributed variables or Mann–Whitney test for variables with a skewed distribution. Categorical variables are reported as numbers and percentages. Fisher exact test was performed to test for group differences. The accuracy of the diagnostic value of NfL was assessed by calculating the area under the curve (AUC) of the receiver operating characteristic (ROC) curve. The cut-off value of CSF NfL was calculated as the concentration that gave the maximum Youden's index (J = sensitivity + specificity – 1). All tests were 2-sided, and significance was set at $p < 0.05$. Statistical analyses were performed using R software, version 3.5.

4. Results

4.1. Characteristics of the patients

The selected CSF samples were from 32 consecutive patients (mean age 38.3 ± 11.5 years, F/M = 1.7) followed-up for a mean time of 3.8 ± 2.5 years. The details of demographic, clinical and neuroradiological characteristics of the patients are reported in Table 1. During the follow-up, 20 patients (62.5%) presented disease activity, with a mean time to the appearance of clinical/MRI signs of disease activity of 1.4 ± 1.1 years. The remaining 12 patients (37.5%) were stable during the follow-up period. The two groups resulted to be homogeneous as far as it concerns the main clinical and MRI features (Table 1).

4.2. CSF NfL

The median CSF NfL concentration was 631 pg/mL (range 156–3492) in the entire cohort. Patients with subsequent disease activity had significantly higher CSF NfL concentrations (median 812.5 pg/mL, range 205–2359) as compared to stable patients (median 329.5 pg/mL, range 156–3492) ($p = 0.002$) (Fig. 1A). In the ROC analysis, NfL was found to have a high diagnostic accuracy in distinguishing these two groups of patients, with a specificity of 91% and a sensitivity of 90% (AUC = 0.89, 95% CI 0.76–1.00) (Fig. 1B). Finally, a CSF NfL cut-off value of 500 pg/mL was found to significantly discriminate the two groups of patients at the first demyelinating event with a sensitivity of 90% and a specificity of 83.3%.

Table 1
Demographic, clinical, MRI and CSF features of the study groups. .

	Study cohort	Patients with subsequent disease activity	Patients without subsequent disease activity	Difference between groups
Demographic characteristics				
N	32	20	12	/
Age (yrs); mean ± SD (median; range)	38.3 ± 11.5 (38; 21–65)	35.6 ± 10.6 (36; 21–50)	42.9 ± 11.9 (42.5; 26–65)	$p = 0.623$
Female/male ratio	1.7	1.9	1.4	$p > 0.999$
Characteristics of patients at the first demyelinating event				
Optic neuritis; n (%)	7 (21.9)	5 (25)	2 (16.7)	$p = 0.970$
Haemispheric syndrome; n (%)	6 (18.8)	3 (15)	3 (25)	
Brainstem/cerebellar syndrome; n (%)	4 (12.5)	2 (10)	2 (16.7)	
Partial myelitis; n (%)	15 (46.9)	10 (50)	5 (41.7)	
EDSS; mean ± SD (median; range)	1.8 ± 0.8 (2; 1–4)	1.7 ± 0.6 (1.5; 1–3)	2.1 ± 1 (2; 1–4)	$p = 0.085$
MRI characteristics				
T2 lesions; mean ± SD (median; range)	6 ± 4.8 (5; 1–18)	7 ± 5.6 (6; 1–18)	4.3 ± 2.1 (4.5; 1–8)	$p = 0.127$
0–3 T2 lesions; n (%)	11 (34.4)	8 (40)	3 (25)	$p = 0.062$
4–9 T2 lesions; n (%)	16 (50)	7 (35)	9 (75)	
> 9 T2 lesions; n (%)	5 (15.6)	5 (25)	0 (0)	
Patients with Gd+ lesions; n (%)	20 (62.5)	13 (65)	7 (58.3)	$p > 0.999$
CSF characteristics				
OCB+ patients; n (%)	19 (59.4)	13 (65)	6 (50)	$p = 0.642$
NfL pg/mL; mean ± SD (median; range)	791.2 ± 687.1 (631; 156–3492)	892.3 ± 496.2 (812.5; 205–2359)	622.6 ± 924.9 (329.5; 156–3492)	$\mathbf{p = 0.002}$
Follow-up characteristics				
Time (yrs) to disease activity during follow-up; mean ± SD (median; range)	/	1.4 ± 1.1 (1; 0.3–3.5)	/	/

Legend. Yrs: years. **EDSS:** Expanded Disability Status Scale. **MRI:** magnetic resonance imaging. **Gd+:** gadolinium-enhancing lesions. **CSF:** cerebrospinal fluid. **OCB+:** patients with CSF IgG oligoclonal bands. **NfL:** neurofilament light chain.

5. Discussion

The main finding of our study is that CSF NfL, as measured with a new ELISA soon after the first demyelinating event, is able to predict future inflammatory disease activity. Indeed, patients with subsequent relapses or new/enhancing MRI lesions at the follow-up had, at baseline, significantly higher concentrations of CSF NfL than clinically and MRI stable patients. CSF NfL was able to discriminate with a high accuracy between these two groups of patients.

Previously, CSF NfL has been repeatedly tested in CIS patients as a predictor of conversion to MS with conflicting results. Indeed, while some authors have found that higher CSF NfL concentrations are associated with a higher risk of subsequent MS development (Arrambide et al., 2016; Martínez et al., 2015; Modvig et al., 2016; Teunissen et al., 2009a), others have not replicated these results (Avsar et al., 2012; Fialová et al., 2013; Khalil et al., 2013). Our findings are in line with studies showing a prognostic value for CSF NfL in the earliest phases of MS and strengthen evidence on NfL as a disease-severity marker, by confirming previous results with a different assay.

Of interest, the patient with the highest CSF NfL concentration in our cohort (3492 pg/mL), did not show subsequent disease activity during the follow-up. This patient (male, 32 years-old at CSF sampling), presented at onset with a cervical myelitis and with a high lesion load in the spinal cord. Patients with spinal relapses have been shown to have higher CSF NfL compared to patients with brain relapses (Kuhle et al., 2013), and this could at least partially justify such a high concentration of NfL in the CSF. Unfortunately, the patient was lost to follow-up after 14 months. It is conceivable that this short follow-up time might have influenced the possibility to detect further signs of disease activity.

In our cohort, when setting a cut-off value of 500 pg/mL, CSF NfL was able to identify patients with subsequent disease activity with high sensitivity (90%) and specificity (83.3%). Two other studies have previously tested a cut-off for CSF NfL in early MS patients (Arrambide et al., 2016; Hakansson et al., 2017). In these studies, cut-off values were set at 450 pg/mL (with a sensitivity of 93% and a specificity of 62%) (Hakansson et al., 2017), and at 900 pg/mL (in this case, sensitivity and specificity were not reported) (Arrambide et al., 2016). The cut-off value we have found is close to the first one and, as compared to

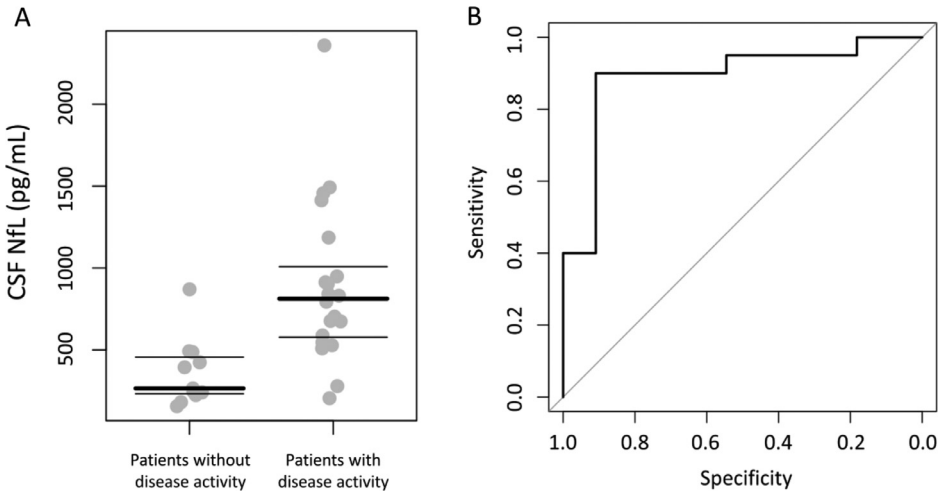

Fig. 1. A: CSF NfL values (pg/mL) at the first demyelinating event in clinically/MRI stable patients and in patients with subsequent clinical/MRI disease activity; $p = 0.002$. In the group of patients without disease activity, one dot is not depicted in the figure (NfL value = 3492 pg/mL). **B:** Diagnostic value of CSF NfL in discriminating between the two groups of patients (AUC = 0.89, 95% CI 0.76–1.00).

this latter, has a slightly lower sensitivity but a higher specificity. The differences in the cut-off values and in its accuracy may rely on several aspects, including the different assays used to measure NfL as well as the different tested populations. Moreover, in one of these studies (Hakansson et al., 2017), disease activity was defined also by the presence of sustained disability worsening, a parameter that was not included in our study. In another recent investigation on CSF NfL in CIS patients (Martínez et al., 2015), the authors found a mean NfL value of 1770 pg/mL in patients converting to MS, which is higher to that found in our cohort (mean NfL value of 892.3 pg/mL). This discrepancy can be justified by the different assays used, the different times of CSF sampling, as well as the differences of the two populations in terms of disease activity. In fact, while a mean CSF NfL concentration of 1770 pg/mL has been reported in a group of CIS patients who had a second relapse within a year from the diagnosis, thus converting to clinically defined MS (Martínez et al., 2015), we found a mean CSF NfL value of 892.3 pg/mL in a population of patients at the first demyelinating event who presented, throughout the entire follow-up – and not only in the first year – relapses or even just MRI signs of disease activity.

Our results suggest that CSF NfL, as a measure of the degree of ongoing axonal damage, can accurately identify early MS patients who will further show evidence of disease activity. CSF NfL makes it possible to measure the final event of different pathophysiological processes that take place during MS, and it should be considered as a biomarker able to summarize the ongoing pathology of this disease. Of interest, CSF NfL has been shown to correlate with T2 lesion load and gadolinium-enhanced lesions, thus suggesting that the prognostic value of this biomarker could be dependent on other well-defined prognostic factors (Gaetani et al., 2018; Teunissen et al., 2009a). One limit of our study relies on the relatively small sample size, which did not allow us considering multivariable analyses. Therefore, we did not test whether or not CSF NfL correlates with other prognostic markers and if it could be considered an independent prognostic factor. For example, in our cohort, patients with subsequent disease activity had a non-significantly higher number of T2 lesions on baseline MRI, compared to clinically stable patients. This suggests that in a larger cohort, the prognostic value of NfL should be corrected at least for the total number of demyelinating lesions on brain and spinal cord MRI. Nevertheless, despite these limitations, the measurement of CSF NfL could represent a reliable and easily-quantifiable measure of the individual risk of early MS patients to have subsequent disease activity.

The identification of a reproducible CSF NfL cut-off value may provide clinicians with a dichotomous variable able to simplify the management of patients after a first demyelinating event. Further studies performed on different and larger populations should retest a CSF NfL cut-off value of around 450–500 pg/mL in order to confirm and refine our findings. Given the promising results for this marker, a reference material should be produced for external calibration of NfL assays to allow for the establishment of assay-independent cut-off values.

Declaration of Competing Interest

LGa participated on advisory boards for and received speaker or writing honoraria and funding for travelling from Almirall, Biogen, Biogen-Idec, Genzyme, Mylan, Novartis, Roche, Teva. AM received travel grants from Teva and Sanofi Genzyme to attend national conferences. PC received/receive research support from Bayer Schering, Biogen-Dompé, Boehringer Ingelheim, Eisai, Lundbeck, Merck-Serono, Novartis, Sanofi-Aventis, Sigma-Tau, and UCB Pharma. KB has served as a consultant or at advisory boards for Alzheon, BioArctic, Biogen, Eli Lilly, Fujirebio Europe, IBL International, Pfizer, and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. HZ has served at advisory boards for Eli Lilly, Roche Diagnostics and Pharmasum Therapeutics and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. MDF participated on advisory boards for and received speaker or writing honoraria and funding for travelling from Bayer, Biogen Idec, Genzyme, Merck, Novartis, Roche and Teva. PE, LGe AB, LP and PS report no conflict of interest.

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