CSF Neurofilament Light Chain Levels in Primary Progressive MS: Signs of Axonal Neurodegeneration

Marc Pawlitzki1,2*, Stefanie Schreiber1,3, Daniel Bittner1,3, Julia Kreipe4, Frank Leypoldt1, Klemens Rupprecht5, Roxana O. Carare6, Sven G. Meuth2, Stefan Vielhaber1,3 and Peter Körtvélyessy1,3,5,7*

1 Department of Neurology, Otto-von-Guericke University, Magdeburg, Germany, 2 Department of Neurology with Institute of Translational Neurology, University Hospital of Muenster, Münster, Germany, 3 German Center for Neurodegenerative Diseases, (DZNE), Magdeburg, Germany, 4 Neuroimmunology, Institute of Clinical Chemistry, University Hospital Schleswig-Holstein, Kiel, Germany, 5 Department of Neurology, Charité-Universitätsmedizin Berlin, Berlin, Germany, 6 Faculty of Medicine, University of Southampton, Southampton, United Kingdom, 7 German Center for Neurodegenerative Diseases (DZNE), Berlin, Germany

Objectives: Elevated neurofilament light chain (NFL) levels within the cerebrospinal fluid (CSF) are a biomarker representing axonal neurodegeneration in rapid progressive neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). It is unclear to what extent the levels of NFL increase in the CSF (CSF-NFL) in a chronic neuroinflammatory process with axonal neurodegeneration, as found in primary progressive multiple sclerosis (PPMS).

Methods: We used a multicenter approach to statistically compare CSF-NFL levels between PPMS patients (n = 50), ALS patients (n = 50), and healthy controls (n = 50). Clinical findings, including disease duration, expanded disability status scale (EDSS), electrophysiological recordings such as visual evoked potentials or spinal and cerebral MRI, and previously administered treatment were selected as experimental parameters retrospectively.

Results: Median [range] CSF-NFL concentrations in PPMS patients were significantly higher than in the controls [1724 (799–4275) pg/ml vs. 1202 (612–2934) pg/ml, \( p = 0.015 \)], and significantly lower compared to ALS patients [1724 (799–4275) pg/ml vs. 10238 (2610–35138) pg/ml, \( p < 0.001 \)]. There was no correlation between CSF-NFL and disease duration (\( p = 0.5 \)), EDSS (\( p = 0.2 \)) or treatment (\( p = 0.3 \)).

Conclusion: We conclude that CSF-NFL may mirror the proposed slow axonal degeneration in PPMS, but does not reflect the disease severity.

Keywords: multiple sclerosis and neuroimmunology, neurofilament light chain (NFL), amyotrophic lateral sclerosis, primary progressive multiple sclerosis, cerebrospinal fluid (CSF)

INTRODUCTION

The pattern underlying the concentrations of neurofilament light chain (NFL) in the cerebrospinal fluid (CSF), referred to as CSF-NFL, in diseases with slow progressive axonal degeneration, including primary progressive multiple sclerosis (PPMS), are not clear. Aggressive axonal injury after acute inflammatory events in patients with multiple sclerosis (MS), and rapidly progressive
neurodegenerative diseases with a predominant affection of the central motor system such as amyotrophic lateral sclerosis (ALS), result in a clear pathological increase of the CSF-NFL levels (1–3). In contrast, studies of PPMS display a wide spectrum of CSF-NFL concentrations, including cases with low (<500 pg/ml) (4, 5) or not detectable CSF-NFL concentrations (6), and cases with high CSF-NFL levels (>10,000 pg/ml) (7) comparable with the documented ranges in relapsing remitting MS disease courses (7–9), painting a heterogeneous picture (10, 11). Reasons may be the small sample sizes selected for these PPMS studies (6, 8) or the inclusion of patients with partially acute inflammatory disease activity and only occasional motor impairment, thus not the typical long-standing chronic disease progression (12).

Here we conducted a cross-sectional multicenter study measuring the CSF levels of the stable protein NFL (1) in patients suffering from mainly long-standing PPMS without acute inflammatory disease activity, and compared them to the CSF levels in ALS patients and disease controls.

**MATERIALS AND METHODS**

**Patients, Controls, and Clinical Assessment**

Our study was approved by the local ethics committees in Magdeburg (No. 07/17) and Kiel (D525/16) and a general commitment of the Charité Berlin Clinic for external analyzies of retrospective data in line with an external German ethics vote. We included CSF samples from (i) \( n = 50 \) patients with clinically definite PPMS according to the McDonald criteria 2010 (13) recruited at the Departments of Neurology at the Otto-von-Guericke University Magdeburg, the Charité Berlin and the University Hospital Schleswig-Holstein, Kiel, Germany; (ii) \( n = 50 \) patients suffering from probable and definite ALS according to the revised El Escorial criteria (14) recruited at the Department of Neurology in Magdeburg; and (iii) \( n = 50 \) healthy controls (HC) recruited in Magdeburg, comprising cases with non-specific complaints who underwent lumbar puncture (LP) during a routine diagnostic examination conducted to rule out any neurological condition. None of the controls suffered from a neurological disorder (neuroinflammatory or neuromuscular), in particular not from MS, peripheral polyneuropathies, muscle or motor neuron disease, nor did they display any specific abnormalities during the neurological examination (15, 16). In addition to the clinical classification, patients included in the control group also fulfilled the following laboratory criteria defining a non-inflammatory CSF: <5 cells/μl CSF, <2 mM lactate in the CSF, no disruption of the blood/CSF barrier (defined by the albumin CSF/serum quotient), no oligoclonal bands (OCB) in the CSF, an no intrathecal immunoglobulin (IgG, IgA, or IgM synthesis (17). All patients were retrospectively recruited between 2012 and 2017.

Clinical scoring [Expanded disability status scale (EDSS)] (18) was performed in close timely proximity to the LP. Electrophysiological measurements [(visual evoked potentials (VEP)] and cerebral as well as spinal magnetic resonance images were available for the patients from points in time close, but not identical to the performance of LP.

EDSS, T2-weighted MRI conducted close to the LP, and CSF levels were selected as parameters to verify the PPMS diagnosis, which was based on the clinical progression over the course of 1 year. These parameters were observed together with the existence of cerebral or spinal cord T2-intensive lesions or oligoclonal bands (OCB) (13), and resulted in the following overall constellation: either patients showed evidence for (a) a dissemination in space (DIS) in the brain due to the existence of at least one T2-intensive lesion found in at least one cerebral area characteristic for MS [periventricular, juxtaglomerular, or infratentorial; in 50 (100%) of the patients], or (b) a DIS in the spinal cord due to the existence of at least two T2-intensive lesions [34 (68%)], or (c) CSF OCB [42 (84%)]. Moreover, neither did any of the PPMS patients present an enhanced T2 lesion load compared to previous diagnostic MRI scans, nor could any Gadolinium-enhanced cerebral or spinal cord T1-weighted MRI lesions be detected. Thus, the absence of MRI progression might mirror a mostly non-inflammatory phenotype of PPMS patients in line with previous investigations (19, 20).

VEP evaluations were documented to describe axonal integrity loss in PPMS according to previously defined diagnostic criteria of PPMS (13). Latencies of the P100 exceeding 2.5 SDs from normative data were considered as abnormal VEPs. Twenty PPMS patients revealed abnormal VEPs in both eyes, 11 patients showed abnormal VEPs in one eye only, and 13 patients had normal VEPs. For the remaining 6 patients, no data were available.

Disease duration was defined as the time in months between symptom onset and the LP.

**CSF Measures**

For all PPMS patients, the LP was performed at each respective University, while for all ALS subjects and the healthy controls, the LP was conducted in Magdeburg. At each center, CSF cells were counted immediately after the LP and total protein, albumin quotient (Q_{alb}) and oligoclonal bands (for PPMS patients only) were measured. Every sample was stored at \(-80^\circ\text{C}\) and shipped on dry ice for CSF-NFL measurement. CSF-NFL levels were determined in Magdeburg using commercially available ELISA kits (UmanDiagnostics NF-light®, Umeå, Sweden, catalog number 10-7001 CE). Intraassay coefficient of variance is 7.4% and interassay coefficient of variance is 6% (21). Every measurement is performed together with a blank and a commercial positive and negative control provided by the manufacturer. Samples were measured in serial procedures and not in batches.

**Statistical Analysis**

Statistical analysis was conducted using SPSS 21 (IBM). Groups were compared with respect to categorical (using a \( \chi^2 \)-test) and continuous variables (using a \( t \)-test or Mann-Whitney U-test or a Kruskal–Wallis one-way analysis of variance (ANOVA) applying pairwise Dunn-Bonferroni post-hoc testing).
Spearman’s rank correlations were performed between CSF-NFL and age, between CSF-NFL and further CSF measures (cell count, protein, Q\(_{\text{alb}}\)), as well as between CSF-NFL and clinical scores (EDSS). P-values < 0.05 were deemed to be statistically significant.

RESULTS

Cohorts

The demographics, clinical and CSF data of the cohorts are shown in Table 1. There were no age or sex (p = 0.2) related differences between PPMS, ALS and HC, whereas the values for the CSF cell count, protein and Q\(_{\text{alb}}\) varied between the diagnostic groups Table 1. Mean [SD] disease duration was significantly longer in PPMS [86 (952) months] compared to ALS [15 (17) months, p < 0.001]. For PPMS, disability severity was high (median EDSS 6) at the time of the LP. The PPMS patients recruited from the three different University centers did not differ with respect to age, sex, CSF protein, oligoclonal band positivity, median EDSS or disease duration. There were, however, inter-center differences regarding the prevalence of spinal cord lesions (p = 0.017), and the values for CSF cell count and Q\(_{\text{alb}}\). Several PPMS patients, n = 11 (28%), received immunomodulatory and immunosuppressive therapy (“treatment attempt”), comprising Rituximab (n = 2), and intravenous (n = 3) or intrathecal (n = 6) methylprednisolone. The Berlin cohort contained significantly more treated patients than the Magdeburg and Kiel cohorts (p = 0.001) Table 1.

CSF-NFL

When considering the whole sample, we found small- to medium-effect size correlations between CSF-NFL and age (rho = 0.2, p = 0.03), Q\(_{\text{alb}}\) (rho = 0.3, p < 0.001), and CSF protein (rho = 0.4, p < 0.001), while there was no correlation with sex (Z = −0.4, p = 0.7) or CSF cell count (rho = 0.01, p = 1.0).

There was a significant group effect of CSF-NFL levels when comparing PPMS to ALS (p < 0.001), and post-hoc analysis revealed lower median [range] levels for PPMS compared to ALS [1724 (799–4275) pg/ml vs. 10238 (2610–35138) pg/ml, p < 0.001], ALS compared to HC [1202 (612–2934) pg/ml, p < 0.001] and PPMS compared to HC (p = 0.015) Figure 1. In PPMS, CSF-NFL concentrations did not differ between treated or untreated patients (z = −1.0, p = 0.3) Figure 2, between patients with completely normal VEPs or only one eye with pathological VEPs (rho = −1.9, p = 0.05), and also not between study centers (p = 0.5); CSF-NFL concentrations were not related to disease duration (rho = −0.9, p = 0.5) or EDSS (rho = 0.17, p = 0.2) Figure 2.

DISCUSSION

We investigated CSF-NFL levels in clinically definite PPMS patients suffering from a mainly long-standing disease without inflammatory disease activity. To our knowledge this is the largest cohort of patients investigated to date addressing the question...
whether CSF-NFL levels can mirror disease associated axonal neurodegeneration and its value as a clinical biomarker in PPMS patients. Our analysis reveals that PPMS diagnosis relates to significantly different levels of CSF-NFL than the levels found for the other two groups, and affirms a moderate PPMS CSF-NFL significantly different levels of CSF-NFL than the levels found for patients. Our analysis reveals that PPMS diagnosis relates to different levels of CSF-NFL than the levels found for the other two groups, and affirms a moderate PPMS CSF-NFL level situated in-between values for HC and ALS patients.

Since several studies have shown contradicting results of lower (1) or higher CSF-NFL levels (22, 23) in PPMS compared to relapsing remitting MS, the degree of chronic axonal loss in the absence of acute inflammatory activity remains unclear. In line with previous results, we found significantly elevated CSF-NFL values in PPMS compared to HC (1, 6, 22, 24). The previously published studies comprised smaller PPMS samples, or included patients displaying inflammatory disease activity and short-lasting disease symptoms leading us to question whether a common PPMS disease course was observed. We addressed the shortcomings of former studies by taking a considerably large PPMS cohort into account, characterized by a primary chronic progressive disease course mainly with long-lasting symptoms.

The subjects included in our PPMS cohort are likely to suffer from corticospinal tract (CST) demyelination, typically found in long-standing PPMS (12). This is supported by the high median EDSS, reflecting manifest motor impairment, and the considerably great prevalence of spinal cord lesions in our PPMS cohort. Table 1 NFL is a cytoskeleton protein, that is highly abundant in large-caliber myelinated axons found abundantly in the CST (25–27). A former study using diffusion tensor imaging (DTI) has shown that an increase in CSF-NFL reflects CST degeneration in ALS (28). We thus assume that CSF-NFL elevation in PPMS particularly mirrors chronic CST degeneration, in addition to cortical thinning (29).

We conducted a direct CSF-NFL comparison between PPMS and ALS, with both diseases sharing clinical signs resulting from the degeneration of the long cerebral and spinal cord tracts in the central motor system (10). These commonalities could be reflected by significantly elevated CSF-NFL concentrations for both PPMS and ALS patients compared to healthy controls. However, ALS patients have a shorter disease duration than PPMS patients, with ALS being characterized by a more rapid and aggressive neurodegenerative disease course relating to a median survival time of 36 months (30), compared to 14–33 years in PPMS (31). The corresponding neuroaxonal damage and depletion in ALS is presumed to take place in a more accelerated manner, potentially explaining the CSF-NFL group differences between PPMS and ALS (10). The predominant demyelination of axons with still partially intact cytoskeleton and neurons in PPMS could also explain the CSF-NFL group differences between PPMS and ALS (10). Moreover, in PPMS, a stronger CSF-NFL level increase was documented at the disease onset, suggesting a greater progression of neuroaxonal degeneration in early and inflammatory active disease stages compared to the long-standing disease pattern (22, 32).

Moreover, when considering our previous work on CSF-NFL, PPMS patients presented significantly lower levels not only compared to ALS patients but also compared to patients with a slow neurodegenerative disease like frontotemporal dementia (FTD) (21), emphasizing the differences between primary neurodegeneration (slow: FTD, rapid: ALS) and inflammatory driven neuro-axonal injury (PPMS).

In line with recent PPMS studies we did not find any relationship between the clinical impact, as measured using the EDSS, and CSF-NFL (1, 7, 8). The concentrations of CSF-NFL are considered to reflect more cumulative underlying pathological processes that are not captured by disability scores at any given time, especially since slow progressive PPMS rarely presents a disease course with acute clinical worsening or dynamic changes in the EDSS score (33). Accordingly, those studies showing a—modest—correlation between increased CSF-NFL levels and clinical function mainly included patients at initial disease stages with acute clinical exacerbations (6, 24), or patients with relapsing remitting MS (24). The lack of relationship between NFL and VEP could be explained by the fact, that the abnormal VEP describes a localized, circumscribed axonal injury far away from lumbar CSF, which might not trigger the release of sufficient amounts of NFL in contrast to spinal injury (27, 34).

The PPMS patients recruited at the three different Universities differed with respect to (i) CSF cell count and album quotient, which were greater in the Kiel cohort, and (ii) treatment frequency, which was highest in the Berlin cohort. These findings lead us to assume that the Kiel patients display more (clinically silent) inflammatory activity (without corresponding relapses and MRI contrast-enhancing lesions), and we could...
also deduce that the Berlin University regularly applies off-label drugs in accordance with positive case series reported by the Berlin group (19, 35). However, the above parameters did not affect the PPMS patients’ CSF-NFL levels, which did not differ between (i) the centers or (ii) treated and untreated cases.

Limitations of the present study include the absence of MRI measurements obtained exactly at the same point in time as the LP was performed. We therefore cannot completely exclude the presence of Gd-enhancing lesions. Moreover, longitudinal studies are needed to evaluate if NFL is a suitable biomarker to predict and adjudicate disability progression in PPMS. In addition, future studies should address the questions (i) if CSF-NFL could also aid to differentiate potential PPMS disease mimics, e.g., hereditary spastic paraplegia, and (ii) if composite measurement of DTI CST involvement, viewed together with CSF-NFL levels, may provide a better correlate with the overall functional state of PPMS patients. A further point of interest would be to measure the contribution of the inflammatory vs. neurodegenerative components influencing the CSF-NFL levels, through contrasting PPMS patients with acute inflammatory (MRI-) disease activity with those patients in progressive disease stages and the possible relations to therapeutic approaches.

In our current study we were able to demonstrate that CSF-NFL levels can clearly discriminate between slowly progressive neuroinflammatory (PPMS) and more rapid neurodegenerative (ALS) processes, but do not correlate with assessments of PPMS severity on a clinical disease scale. We conclude, that CSF-NFL mirrors the inflammation driven neurodegenerative aspect in MS and thus, CSF-NFL might not be an ideal biomarker in PPMS.

ETHICS STATEMENT

We confirm that we have read the Journal’s position on issues involved in ethical Publication and affirm that this report is consistent with those guidelines.

AUTHOR CONTRIBUTIONS

MP has access to all the data and takes responsibility for the data, accuracy of the data analysis, and interpretation of the data and drafting the manuscript for intellectual content. SS, DB, JK, FL, KR, SM, SV Design and conceptualization of the study; revising the manuscript for intellectual content and language improvement. PK Design and conceptualization of the study; revising the manuscript for intellectual content, Study supervision.

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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