Serum and CSF neurofilament light chain levels in patients with acquired peripheral neuropathies

Running headline

Neurofilament light chain in peripheral neuropathies

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Abstract

Neurofilament light chain (NFL) levels reflect axonal damage in different inflammatory and neurodegenerative central nervous system conditions, in correlation with disease severity. Our aim was to determine the possible diagnostic and prognostic value of serum and cerebrospinal (CSF) NFL levels in subjects with different forms of acquired peripheral neuropathies (PN). Paired serum and CSF samples of 25 patients with acquired PN were analysed for NFL using an ultrasensitive technique (Quanterix, Simoa) and compared with a group of 25 age-matched healthy subjects. Demographic, clinical, CSF and neurophysiological data were reviewed. Cases with Guillain-Barré syndrome (N=5), multifocal motor neuropathy (N=3), chronic inflammatory demyelinating polyneuropathy (CIDP) and variants (N=12), anti-myelin-associated glycoprotein (MAG) neuropathy (N=3), both CIDP and anti-MAG neuropathy (N=1), and non-systemic vasculitic neuropathy (N=1) were studied. NFL levels were significantly ($p<0.001$) increased in patients with PN and were higher in the CSF (median 1407 pg/ml, range 140.2-12661) than in serum (median 31.52 pg/ml, range

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4.33-1178). A statistically significant correlation was observed between serum and CSF levels in cases with blood-nerve-barrier damage ($r=0.71$, $p<0.01$), and between serum NFL levels and disease activity at sampling ($r= 0.52$, $p<0.01$) and at last follow-up ($r= 0.53$, $p<0.01$) in all subjects. The increase of NFL values in both serum and CSF of patients with acquired PN and the significant correlation between serum NFL levels, disease severity and final outcome support the possible role of NFL as disease activity and prognostic biomarker also in peripheral nervous system disorders.

KEYWORDS

neurofilament, peripheral neuropathy, chronic inflammatory demyelinating polyneuropathy (CIDP), Guillain-Barré (GBS), myelin-associated glycoprotein (MAG)

1 INTRODUCTION

Neurofilament light (NFL) protein has been recently evaluated as a diagnostic and prognostic biomarker of several neurodegenerative and inflammatory central nervous system (CNS) conditions.[1-3] The high correlation between serum and cerebrospinal (CSF) NFL values with disease activity supports a key role of NFL in monitoring tissue damage and treatment effects.[4-6] Up to now, only two studies analysed serum NFL values in subjects with peripheral nervous system (PNS) diseases, and in particular in patients with vasculitic neuropathy[7] and inherited peripheral neuropathies (PN).[8] We studied cases with different acquired PN and compared serum/CSF NFL levels with clinical data, neurophysiological findings, and final outcome in order to investigate the utility of NFL as a diagnostic and prognostic biomarker in acquired PNS disorders.

2 METHODS

2.1 Study subjects

We studied 25 patients with well-defined acquired PN consecutively referred to our Neurology unit. Demographic and clinical data including time from onset, disease severity measured using the overall neuropathy limitations scale (ONLS) at sampling,[9] treatment, and clinical evaluation at last follow-up were collected (Table 1). The final diagnosis was defined according to 5 diagnostic categories: 1) Guillain-Barré syndrome (GBS), 2) chronic inflammatory demyelinating polyneuropathy (CIDP) and variants, 3) multifocal motor neuropathy (MMN), 4) non-systemic vasculitic neuropathy, and (5) anti-myelin-associated glycoprotein (MAG) neuropathy. Coexistence of CNS involvement was excluded in all cases. Samples from 25 age-matched healthy controls without a medical history of neurological diseases

were also included for comparison.

2.2 Neurophysiological study

All patients underwent nerve conduction studies to confirm and characterise PNS involvement. The main pattern (axonal, demyelinating, mixed axonal-demyelinating), the presence of conduction blocks and F waves were analysed in each case with a standard neurophysiological examination performed within 1 month of CSF/serum sampling. Slowing conduction velocity was considered suggestive of demyelination, while a reduction of potential amplitude was indicative of axonal loss.

2.3 Laboratory data

For each examined subject paired serum and CSF samples were obtained. Protein content, IgG index, and oligoclonal bands (OB) were analysed according to international guidelines.

2.4 NFL analysis

Paired CSF and serum samples were centrifuged at room temperature, aliquoted in polypropylene tubes within 1 hour of collection, and then stored at -80°C. The concentration of NFL protein was determined in duplicates by investigators blinded to clinical data using a HD-1 immunoassay analyzer, Quanterix SimoaTM, which runs ultra-sensitive paramagnetic bead-based enzyme-linked immunosorbent assays.

2.5 Statistical analyses

A rank based non-parametric test (Mann-Whitney test) was used to differentiate PN and controls. Pearson correlation analysis was applied to test the correlation between clinical and CSF/serum data. Statistical analysis was performed using PRISM 6 (GraphPad Software analysis).

3 RESULTS

The cohort included patients with a final diagnosis of GBS (N=5), MMN (N=3), CIDP and variants (N=12), anti-MAG neuropathy (N=3), and non-systemic vasculitic neuropathy (N=1). In one case CIDP and anti-MAG neuropathy coexisted. The median time interval between the initial symptoms and sampling was 12 months (range 0-168), and 9 subjects received previous therapies (4 with steroids, 2 with intravenous immunoglobulins, and 3 with multiple drugs). The median ONLS at sampling was 4 (range 0-12), and did not change at the last follow-up (Table 1). Abnormal CSF suggestive for possible blood-nerve barrier (BNB) damage (defined by an increase of protein content, an IgG index >

0.7, or the presence of oligoclonal bands) was noted in 12 cases. Neurophysiological examination revealed a demyelinating pattern in 11 subjects, an axonal one in 5, and a mixed pattern in 8 cases (Table 1). Serum NFL levels were significantly ($p<0.001$) increased in the PN group (median 31.52 pg/ml, range 4.33-1178) compared to healthy controls (median 6.91 pg/ml, range 2.67-12.78), and were not significantly different according to the final diagnosis (Fig. 1 A and B, respectively). NFL values were higher (fold change 44.6) in the CSF (median 1407 pg/ml, range 140.2-12661) than in serum. A significant correlation ($r= 0.71$, $p <0.01$) between NFL levels in the CSF and serum was observed only in subjects with possible signs of BNB damage (Supplemental figure). The association between NFL values, sex, and age was not statistically significant. A significant correlation was noted between serum NFL levels and ONLS at sampling ($r= 0.52$, $p<0.01$) and at last follow-up ($r= 0.53$, $p<0.01$), Fig. 1 C and D, respectively, while no association was noted between ONLS and CSF NFL values. Highest serum NFL levels were noted in a young child with a severe GBS who had not significantly increased CSF values and showed no relevant improvement at the last follow-up. No significant differences of NFL values were noted according to neurophysiological characteristics (axonal/demyelinating pattern, presence of conduction blocks, prolonged F wave).

4 DISCUSSION

Although we studied only a small patient population, we here provide preliminary data on the increase of serum NFL protein levels in patients with different forms of acquired PN, extending previously reported findings on inherited PN and vasculitic neuropathies.[7,8] According to our data, NFL levels are increased also in the CSF of patients with different acquired PN, a finding previously noted only in subjects with CNS disorders [2-4,6] and GBS in a single report.[10] These data suggest that serum and CSF NFL levels could reflect an ongoing axonal damage in both CNS and PNS conditions. PNS involvement, reflected by NFL levels, does not depend on the main PN pattern, since no differences were noted according to neurophysiological findings. Our observations confirm previous data on inherited PN, were no differences of plasma NFL levels were noted among patients with axonal and demyelinating forms of Charcot-Marie-Tooth disease. These findings seem to reflect the prominent axonal loss in PN disorders, as previously suggested in inherited conditions.[8] Moreover, in our cohort, different final diagnoses did not influence serum/CSF NFL values, suggesting the possible use of this biomarker in PNS disorders, independently on the clinical subtypes. Interestingly, we noticed a correlation between CSF and serum NFL levels only in subjects with a possible BNB damage, supporting the idea that disrupted BNB could early contribute in the inside-out transport of inflammatory mediators and biomarkers of neurodegeneration. Biomarkers that enable the identification of BNB dysfunction are not well established, and only pathological examinations or radiological studies seem reliable methods to assess BNB derangements. However, we assume that an increase of protein content/IgG index or the presence of oligoclonal bands might be indicative of BNB

in patients with peripheral neuropathy, in accordance with previous reports.[10] We also demonstrate that serum NFL levels and not CSF ones correlate with disease activity at sampling, and might predict subsequent progression of disability. These data suggest that serum NFL levels might be more reliable than CSF ones for monitoring disease activity and tissue damage in PNS conditions. Differences in the mechanisms of release and/or clearance of NFL into blood and CSF in CNS and PNS diseases and the predominant expression of NFL in large-caliber myelinated axons and in the postsynaptic terminal might partially explain the lack of association between clinical findings and CSF NFL levels in our cohort. The heterogeneous population included in our study and the short follow-up available in patients with acute neuropathies might also explain this discrepancy with the previous single observation that CSF NFL in the acute stage of GBS seems to predict long-term outcome.[11] In conclusion, we present initial data suggesting that serum NFL levels might reflect neuroinflammation-mediated axonal injury in acquired PN, independently of the final diagnosis and pathogenesis. The main limitation of our study is the small sample size. Future extensive and prospective studies with larger cohorts of patients with PN and repeated determinations in individual cases combined with better analysis of inflammatory profiling are mandatory. These data might confirm the role of serum NFL levels as predictors of disease progression and treatment response also in PNS conditions.

CONFLICTS OF INTERESTS S.F. received a support for congress attendance by Shire. The other authors declare that they have no conflict of interests.

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TABLE LEGEND

TABLE 1.

Demographic, clinical, and neurophysiological data of analysed patients.

Abbreviations: ONLS: overall neuropathy limitations scale; NFL: neurofilament light chain; GBS: Guillain-Barré syndrome; MMN: multifocal motor neuropathy; CIDP: chronic inflammatory demyelinating polyneuropathy; MAG: myelin-associated glycoprotein.

FIGURE LEGEND

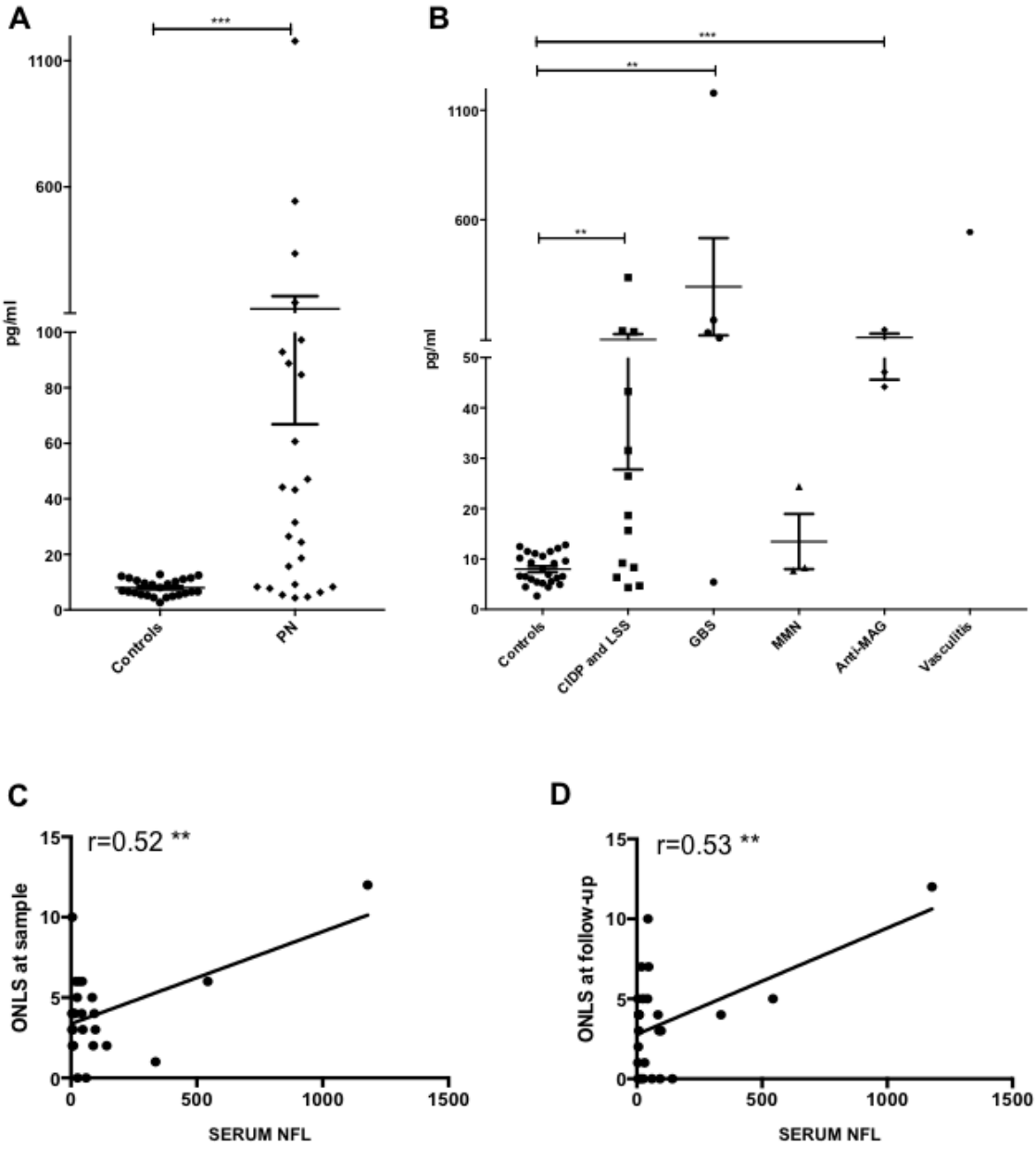

FIGURE 1.

NFL levels in the analysed cohort in correlation with the final diagnosis and ONLS.

A. Serum NFL levels were significantly increased in patients with PN in comparison with healthy controls ($p < 0.001$).

B. Significant difference of NFL values was observed between patients with CIDP and variants, GBS, and anti-MAG associated neuropathy in comparison with healthy controls. However, no significant difference was noted among patients with PN according to the final diagnosis.

C-D. A significant correlation between serum NFL values and clinical disability at sampling (C) and at last follow-up (D) was also noted. NFL values were expressed in pg/ml.

** $p<0.01$ *** $p< 0.001$

Abbreviations: PN: peripheral neuropathies; CIDP: chronic inflammatory demyelinating polyneuropathy; LSS: Lewis Sumner syndrome; GBS: Guillain-Barré syndrome; MMN: multifocal motor neuropathy; MAG: myelin-associated glycoprotein. ONLS: overall neuropathy limitations scale; NFL: neurofilament light chain.

SUPPLEMENTAL FIGURE

Correlation between serum and CSF NFL levels in patients with PN according to possible BNB damage.

A. No significant correlation was observed between serum and CSF NFL values in the whole cohort of PN cases. **B-C.** A significant association ($p<0.01$) between serum and CSF NFL levels was detected only in subjects with BNB damage.

Abbreviations: CSF: cerebrospinal fluid; BNB: blood-nerve-barrier.

Table 1. Demographic, clinical, and neurophysiological data of analysed patients.

Number of analysed cases	25
Age at recruitment, median (range), years	53 (3-86)
Female, n (%)	11 (44%)
Time from onset	
Acute neuropathies, median (range), months	0 (0-3)
Chronic neuropathies, median (range), months	36 (0-168)
Neurophysiological pattern, n (%)	
Axonal	5 (20%)
Demyelinating	11 (44%)
Mixed	8 (32%)
Negative	1 (4%)
Presence of conduction blocks	6 (24%)
Prolonged F wave	22 (88%)
ONLS at sample, median (range)	4 (0-12)
Serum NFL value, median (range), pg/ml	31.52 (4.33-1178)
Acute neuropathies, median (range), pg/ml	84.72 (5.41-1178.37)
Chronic neuropathies, median (range), pg/ml	25.42 (4.33-543.67)
CSF NFL value, median (range), pg/ml	1407 (140.2-12661)
Acute neuropathies, median (range), pg/ml	1357.2 (140.17-2474.69)
Chronic neuropathies, median (range), pg/ml	1436.45 (288.23-12660.61)
Final diagnosis, n (%)	
GBS	5 (20%)
MMN	3 (12%)
CIDP and variants	12 (48%)
Anti-MAG neuropathy	3 (12%)
CIDP + anti-MAG neuropathy	1 (4%)
Non-systemic vasculitic neuropathy	1 (4%)
Follow-up, median (range), months	
Acute neuropathies, median (range), months	4 (1-14)
Chronic neuropathies, median (range), months	78 (2-228)
ONLS at last follow-up, median (range)	4 (0-12)

Abbreviations: ONLS: overall neuropathy limitations scale; NFL: neurofilament light chain; GBS: Guillain-Barré syndrome; MMN: multifocal motor neuropathy; CIDP: chronic inflammatory demyelinating polyneuropathy; MAG: myelin-associated glycoprotein.