Plasma neurofilament light chain level is not a biomarker of Charcot–Marie–Tooth disease progression: Results of 3-year follow-up study

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Abstract

**Background and purpose:** Charcot–Marie–Tooth disease (CMT) is a hereditary, slowly progressive neuropathy. Currently, there are no effective pharmacological treatments or sensitive disease activity biomarkers available. The aim of this study was to demonstrate the change in plasma neurofilament light chain (NFL) over time in a CMT cohort and analyse the association between CMT severity and NFL level.

**Methods:** Initially, 101 CMT patients and 64 controls were enrolled in the study. Repeated evaluation was performed in 73 patients and 28 controls at a 3-year interval. Disease severity assessment included clinical evaluation with CMT Neuropathy Score version 2 (CMTNSv2). Plasma NFL concentration was measured using the Simoa (single molecule array) NFL assay.

**Results:** Plasma NFL concentration was increased in the CMT group compared with controls \((p<0.001)\). Overall NFL level increased over the 3-year interval in both CMT \((p=0.012)\) and control \((p=0.001)\) groups. However, in 22 of 73 CMT patients and seven of 28 controls, the NFL level decreased from the baseline. Analysing the association between 3-year change in plasma NFL and disease severity (CMTNSv2), there was no correlation in the CMT group \((r=0.228, p=0.052)\) or different CMT subgroups.
INTRODUCTION

Charcot–Marie–Tooth disease (CMT) is the most common hereditary neuromuscular disorder, with estimated prevalence of 1 in 2500. It is a clinically and genetically heterogeneous group of disorders with the phenotype of chronic, slowly progressive neuropathy affecting both the motor and the sensory nerves [1, 2].

Currently, there are no effective pharmacological treatments for CMT available; however, promising treatments are reaching the stage of clinical translation. Biomarkers that could detect the effect of treatment on disease progression are crucial for successful clinical trials. Although several CMT-specific measures have been designed (CMT Neuropathy Score, CMT Functional Outcome Measure, CMT Health Index, CMT Paediatric Scale, nerve and muscle magnetic resonance imaging), evaluation of disease activity is still difficult due to the slow rate of disease progression [3–10].

Neurofilaments (NFs) are the major cytoskeletal proteins of neurons in both central and peripheral nervous systems. When neurons are damaged, NFs are released into the interstitial fluid and then diffuse into the cerebrospinal fluid and blood [11, 12]. In peripheral nervous system diseases associated with axonal injury or degeneration, the plasma concentration of NF light chain (NfL) increases, where it also correlates with disease severity [13, 14]. A recent study by Millere et al. [15] confirmed that the plasma NfL concentration is significantly higher in CMT patients than in controls and reflects the clinical severity of CMT. However, another study with longitudinal evaluation of a CMT cohort revealed no significant change in plasma NfL concentration over a 6-year interval [16]. Although the plasma concentration of NfL is a potential disease biomarker for CMT, knowledge about the suitability of NfL as a disease progression marker is limited and available from small patient cohort studies.

In this study, we demonstrate the change in plasma NfL over time in a previously published cohort of Latvian CMT patients [15]. Additionally, we evaluate the clinical disease progression and analyze the association between CMT severity and plasma NfL concentration in adult and paediatric CMT patients.

METHODS

Participant evaluation, blood sampling, and plasma NfL level measurement

A large previously published cohort of CMT patients [15] were repeatedly evaluated after 3 years using standardized tests for CMT patients: neurography, which was performed by a certified specialist according to the standard polyneuropathy protocol; and scoring of severity, which was performed in accordance with CMT Neuropathy Score version 2 (CMTNSv2) [17].

Blood sampling and storage were conducted following a strict standard operating procedure. Briefly, blood samples from patients and controls were taken in an outpatient setting by certified medical staff and processed within 1 h. Blood was collected into EDTA-containing tubes and centrifuged at 20°C at 2000 g for 10 min. Plasma was then aliquoted and stored at −20°C. As a control group, our study included healthy individuals without any known neurological diseases or neurological symptoms.

Plasma NfL level was measured in similar settings and the same NfL detection method in the same laboratory was used as described previously [15].

Statistical analysis

The normality of the continuous data was assessed with histograms, Q–Q plots, and the Shapiro–Wilk test. For normally distributed data, the t-test was used to compare means between groups, whereas the Mann–Whitney U-test was used for nonnormally distributed data. Discrete data were compared using Pearson chi-square test. Correlation between continuous data was assessed by Spearman correlation coefficient. All calculations were performed using SPSS.

Standard protocol approval and patient consent

The study was approved by the Central Medical Ethics Committee of Latvia (No. 3/18-03-21). Written informed consent was obtained from all participants in the study. The data supporting the findings of this study are available on request from the corresponding author. They are not publicly available due to privacy/ethical restrictions.

RESULTS

Plasma NfL concentration and disease severity assessment

Initially, 96 CMT patients and 60 healthy subjects were recruited in this study [15]. An additional five patients and four healthy controls were enrolled (Table 1). The patient group was subdivided according to the genetic findings. There was no significant difference in sex (chi-squared, $\chi^2 = 2.017, p = 0.156$) or age (independent samples t-test,
t(162.847) = −1.264, p = 0.208) between CMT and control groups, or between controls and CMT subgroups (one-way analysis of variance, F = 0.648, p = 0.754).

As previously demonstrated in a study by Millere et al. [15], plasma NfL concentration was increased in the CMT group (median = 12.5 pg/mL, interquartile range [IQR] = 7.5 pg/mL) compared with controls (median = 5.2 pg/mL, IQR = 3.0 pg/mL; Mann-Whitney U-test, U = 749,000, p < 0.001). NfL concentrations measured in the study groups are shown in Figure 1.

There was no difference in disease severity measured by CMTNSv2 across CMT subgroups (Kruskal-Wallis H-test, H = 8.633, p = 0.195). Assessing the association between NfL level and CMTNSv2, there was a weak significant correlation in the overall CMT group (Spearman correlation, r = 0.284, p = 0.004; Figure 2) and a very strong significant correlation in the HINT1 subgroup (Spearman correlation, r = 0.986, p < 0.001; Table 1).

### Three-year follow-up results

Repeated evaluation and blood sample testing were performed in 73 patients and 28 controls at a 3-year interval (Table 2). There was no significant difference in age between the control group and CMT group (independent samples t-test, t(69.012) = −0.081, p = 0.945) or between CMT groups (Kruskal-Wallis H-test, H = 6.524, p = 0.480).

Plasma NfL concentration in the CMT group (median = 14.6 pg/mL, IQR = 7.5 pg/mL) was higher than in the control group (median = 5.8 pg/mL, IQR = 3.8 pg/mL) in the follow-up testing (Mann-Whitney U-test, U = 193,500, p < 0.001). NfL level increased over the 3-year interval in both the CMT group (median change = 1.6 pg/mL, IQR = 4.4 pg/mL) and control group (median change = 0.6 pg/mL, IQR = 1.1 pg/mL; paired-samples t-test, t(72) = −5.673, p < 0.001) for the CMT patients; Wilcoxon signed ranks test, Z = −3.325, p = 0.001 for the controls: Table 2).

There were seven control subjects and 22 CMT patients whose plasma NfL concentration decreased over the 3-year period. We found that plasma NfL levels decreased for 11 CMT1A patients, four CMTX1 patients, one CMT2A patient, one CMT2F patient, two patients of other genetic subtypes, and three CMT patients of unknown pathogenic cause.

In the 3-year follow-up evaluation, CMTNSv2 was higher than at baseline (median change = 1.0, IQR = 3.0, Wilcoxon signed ranks tests Z = −5.673, p < 0.001). None of the patients had a decrease in CMTNSv2 during the 3-year period.

Analysing the association between 3-year change in plasma NfL concentration and disease severity (CMTNSv2), there was no significant correlation in the CMT group (Spearman correlation, r = 0.228, p = 0.052) or the different CMT subgroups (Figure 3).

### Paediatric CMT patients

In addition, 19 of 101 CMT patients were children (<18 years of age) and we analysed them separately (CMT1A = 7, CMTX1 = 2,
**DISCUSSION**

In this study, we present longitudinal data of plasma NfL concentration in different CMT subgroups. In addition, we evaluate disease progression and its association with the change in NfL levels in adult and paediatric patients.

NfL is a nonspecific measure; it is documented in healthy subjects and in various neurological disorders, and it increases with age [18–20]. More importantly, blood NfL levels can also be influenced by other diseases including change of body mass index, diabetes, and hypertension [21]. However, several studies have shown a higher plasma NfL concentration in CMT patients compared with matched controls. To replicate these findings, we assessed our patients and controls in similar settings and used the same NfL detection methods. Consequently, in our study, plasma NfL levels were increased in CMT patients compared with controls; however, the difference in concentration between patients and controls reached statistical significance only in CMT1A, CMTX1, and CMT2A subgroups and additionally in the subgroup with CMT due to biallelic HINT1 variants on follow-up evaluation, reflecting variable levels in NfL concentration between subtypes. Although we detected the difference in NfL concentration between CMT patients and controls, we cannot rule out the influence of other potential interfering factors such as comorbidities. Future studies are needed to provide deeper understanding of the NfL release mechanisms in disease.
### TABLE 2
Three-year follow-up data of plasma NfL concentration and CMTNSv2 in CMT.

<table>
<thead>
<tr>
<th>Study participants</th>
<th>Number of patients</th>
<th>Mean age, years (SD)</th>
<th>Median baseline NfL, pg/mL (IQR)</th>
<th>Median follow-up NfL, pg/mL (IQR)</th>
<th>Median change in NfL, pg/mL (IQR)</th>
<th>Median baseline CMTNSv2 (IQR)</th>
<th>Median follow-up CMTNSv2 (IQR)</th>
<th>Median change in CMTNSv2 (IQR)</th>
<th>Changes in CMTNSv2/changes in NfL Spearman correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>All CMT patients</td>
<td>73</td>
<td>38.0 (±16.4)</td>
<td>12.6 (6.8)</td>
<td>14.6 (7.5)</td>
<td>1.6 (4.4)</td>
<td>11.0 (9.0)</td>
<td>13.0 (8.0)</td>
<td>1.0 (3.0)</td>
<td>0.225, p = 0.054</td>
</tr>
<tr>
<td>CMT1A (PMP22 dup)</td>
<td>37</td>
<td>36.2 (±16.5)</td>
<td>12.5 (6.7)</td>
<td>14.8 (7.5)</td>
<td>2.3 (4.1)</td>
<td>11.0 (7.0)</td>
<td>13.0 (6.0)</td>
<td>1.0 (3.0)</td>
<td>0.302, p = 0.069</td>
</tr>
<tr>
<td>CMTX1 (GJB1)</td>
<td>12</td>
<td>37.9 (±19.2)</td>
<td>16.0 (6.2)</td>
<td>16.4 (15.2)</td>
<td>1.3 (11.6)</td>
<td>17.0 (19.0)</td>
<td>21.0 (16.0)</td>
<td>2.0 (4.8)</td>
<td>~0.29, p = 0.929</td>
</tr>
<tr>
<td>CMT2A (MFN2)</td>
<td>4</td>
<td>28.2 (±14.9)</td>
<td>15.7 (9.0)</td>
<td>14.4 (10.0)</td>
<td>-1.0 (6.3)</td>
<td>6.5 (15.5)</td>
<td>6.5 (15.0)</td>
<td>0.0 (0.0)</td>
<td>NA</td>
</tr>
<tr>
<td>CMT2F (HSBP1)</td>
<td>1</td>
<td>65.0 (NA)</td>
<td>22.2 (NA)</td>
<td>21.1 (NA)</td>
<td>-1.1 (NA)</td>
<td>16.0 (NA)</td>
<td>16.0 (NA)</td>
<td>0.0 (NA)</td>
<td>NA</td>
</tr>
<tr>
<td>NMAN (HINT1)</td>
<td>5</td>
<td>42.6 (±23.7)</td>
<td>12.9 (7.2)</td>
<td>15.8 (5.8)</td>
<td>4.6 (2.3)</td>
<td>14.0 (10.0)</td>
<td>14.0 (10.0)</td>
<td>2.0 (3.0)</td>
<td>0.527, p = 0.362</td>
</tr>
<tr>
<td>CMT2N (AARS1)</td>
<td>2</td>
<td>46.5 (±13.4)</td>
<td>3.1 (NA)</td>
<td>4.3 (NA)</td>
<td>1.2 (NA)</td>
<td>9.5 (NA)</td>
<td>10.5 (NA)</td>
<td>1.0 (NA)</td>
<td>NA</td>
</tr>
<tr>
<td>CMT1E (PMP22 SNV)</td>
<td>1</td>
<td>32.0 (NA)</td>
<td>6.9 (NA)</td>
<td>10.9 (NA)</td>
<td>4.0 (NA)</td>
<td>21.0 (NA)</td>
<td>27.0 (NA)</td>
<td>6.0 (NA)</td>
<td>NA</td>
</tr>
<tr>
<td>HMNSC (BSCL2)</td>
<td>1</td>
<td>45.0 (NA)</td>
<td>11.5 (NA)</td>
<td>9.2 (NA)</td>
<td>-2.3 (NA)</td>
<td>2.0 (NA)</td>
<td>6.0 (NA)</td>
<td>4.0 (NA)</td>
<td>NA</td>
</tr>
<tr>
<td>CMT21 (MPZ)</td>
<td>1</td>
<td>63.0 (NA)</td>
<td>35.4 (NA)</td>
<td>60.0 (NA)</td>
<td>24.6 (NA)</td>
<td>20.0 (NA)</td>
<td>23.0 (NA)</td>
<td>3.0 (NA)</td>
<td>NA</td>
</tr>
<tr>
<td>CMT2Z (MORC2)</td>
<td>1</td>
<td>46.0 (NA)</td>
<td>14.9 (NA)</td>
<td>15.3 (NA)</td>
<td>0.4 (NA)</td>
<td>7.0 (NA)</td>
<td>11.0 (NA)</td>
<td>4.0 (NA)</td>
<td>NA</td>
</tr>
<tr>
<td>SMALED2A (BICD2)</td>
<td>1</td>
<td>43.0 (NA)</td>
<td>21.4 (NA)</td>
<td>7.4 (NA)</td>
<td>-14.0 (NA)</td>
<td>17.0 (NA)</td>
<td>17.0 (NA)</td>
<td>0.0 (NA)</td>
<td>NA</td>
</tr>
<tr>
<td>CMT with unknown monogenic cause</td>
<td>7</td>
<td>36.6 (±12.5)</td>
<td>10.0 (5.6)</td>
<td>7.8 (7.5)</td>
<td>-1.0 (2.36)</td>
<td>8.0 (8.0)</td>
<td>9.0 (11.0)</td>
<td>1.0 (1.0)</td>
<td>0.270, p = 0.588</td>
</tr>
<tr>
<td>Control group</td>
<td>28</td>
<td>37.8 (±11.5)</td>
<td>5.2 (3.3)</td>
<td>5.8 (3.8)</td>
<td>0.6 (1.1)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: CMT, Charcot–Marie–Tooth disease; CMTNSv2, CMT Neuropathy Score version 2; dup, duplication; HMNSC, hereditary motor neuronopathy type 5C; IQR, interquartile range; NA, not applicable; NfL, neurofilament light chain; NMAN, neuromyotonia and axonal neuropathy; SMALED2A, spinal muscular atrophy with lower extremity predominance type 2A.
A recently published study by Rossor et al. [16] showed paired data of NfL concentration after a 6-year interval in 27 CMT patients. No significant change in plasma NfL for patients with CMT was detected. Sandelius et al. [11] published longitudinal data of nine CMT patients and 13 controls 1 year after baseline, when no significant changes in plasma NfL levels were detected. In our study, we were able to collect paired blood samples from 73 patients and 28 controls. We obtained follow-up data only after 3 years from the baseline data due to COVID-19 pandemic restrictions. Additionally, CMT is a slowly progressing disease, and a longer period between evaluations potentially may show changes in biomarker levels. NfL level increased significantly from baseline after the 3-year interval in both the CMT group and the control group. Interestingly, the NfL concentration decreased in 22 patients (with CMT1A, CMT2A, CMTX1, CMT2F, hereditary motor neuronopathy type 5C, and spinal muscular atrophy with lower extremity predominance type 2A subtypes and three with unidentified monogenic cause) and in seven controls. The reasons for this are not clear, but a reduction of plasma NfL in patients with CMT1 over time has been reported before [18]. Therefore, it is important to note that there is a significant variation between CMT types in the change in NfL concentration over time.

Due to CMT heterogeneity and the slow rate of disease progression, sensitive clinical outcome measures and biomarkers are difficult to develop. Recently, an association between CMT disease severity (CMTNS) and plasma NfL concentration has been described [11, 15, 16]. In contrast to the previously reported modest correlations between CMTNSv2 and NfL levels [11, 15], our study revealed no significant correlation between these measures. Furthermore, similarly to data published by Rossor et al. [16], plotting the 3-year change in plasma NfL against the 3-year change in CMTNSv2 presented no significant correlation (Spearman correlation, \( r = 0.228 \), \( p = 0.052 \)).

All previously published cohorts where NfL concentration was evaluated in CMT patients had a mean age > 18 years. We hypothesized that detection of increased NfL that reflects the rate of axonal degeneration is better in younger patients at an earlier stage of disease. Therefore, we analysed a paediatric cohort of 19 CMT patients and evaluated 11 children after a 3-year period separately.

### TABLE 3 Three-year follow-up data of plasma NfL concentration and CMTNSv2 in CMT paediatric patients.

<table>
<thead>
<tr>
<th>Study participants</th>
<th>Number of patients</th>
<th>Mean age, years (SD)</th>
<th>Median baseline NfL pg/mL (IQR)</th>
<th>Median follow-up NfL pg/mL (IQR)</th>
<th>Median change in NfL pg/mL (IQR)</th>
<th>Median baseline CMTNSv2 (IQR)</th>
<th>Median follow-up CMTNSv2 (IQR)</th>
<th>Median change in CMTNSv2 (IQR)</th>
<th>Changes in CMTNSv2/changes in NfL Spearman correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>All CMT paediatric patients</td>
<td>11</td>
<td>13.0 (4.0)</td>
<td>13.1 (10.2)</td>
<td>11.6 (7.0)</td>
<td>11.1 (4.4)</td>
<td>6.0 (9.0)</td>
<td>7.0 (9.0)</td>
<td>0.0 (0.0)</td>
<td>0.256, ( p = 0.447 )</td>
</tr>
<tr>
<td>CMT1A (PMP22 dup)</td>
<td>6</td>
<td>12.3 (4.7)</td>
<td>12.3 (9.7)</td>
<td>10.9 (2.8)</td>
<td>0.7 (8.0)</td>
<td>10.0 (5.0)</td>
<td>10.0 (4.0)</td>
<td>0.0 (0.8)</td>
<td>0.393, ( p = 0.441 )</td>
</tr>
<tr>
<td>CMTX1 (GJB1)</td>
<td>2</td>
<td>13.0 (5.7)</td>
<td>27.5 (NA)</td>
<td>33.2 (NA)</td>
<td>5.7 (NA)</td>
<td>3.5 (NA)</td>
<td>3.5 (NA)</td>
<td>0.0 (NA)</td>
<td>NA</td>
</tr>
<tr>
<td>CMT2A (MFN2)</td>
<td>1</td>
<td>13.0 (NA)</td>
<td>2.7 (NA)</td>
<td>2.9 (NA)</td>
<td>0.2 (NA)</td>
<td>2.0 (NA)</td>
<td>2.0 (NA)</td>
<td>0.0 (NA)</td>
<td>NA</td>
</tr>
<tr>
<td>NMAN (HINT1)</td>
<td>1</td>
<td>13.0 (NA)</td>
<td>7.1 (NA)</td>
<td>11.8 (NA)</td>
<td>4.7 (NA)</td>
<td>2.0 (NA)</td>
<td>6.0 (NA)</td>
<td>4.0 (NA)</td>
<td>NA</td>
</tr>
<tr>
<td>CMT with unknown monogenic cause</td>
<td>1</td>
<td>17.0 (NA)</td>
<td>13.1 (NA)</td>
<td>14.1 (NA)</td>
<td>1.0 (NA)</td>
<td>2.0 (NA)</td>
<td>2.0 (NA)</td>
<td>0.0 (NA)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: CMT, Charcot-Marie-Tooth disease; CMTNSv2, CMT Neuropathy Score version 2; dup, duplication; IQR, interquartile range; NA, not applicable; NfL, neurofilament light chain; NMAN, neuromyotonia and axonal neuropathy.
NfL concentrations decreased from the baseline after the 3-year interval. In our study, we used CMTNSv2 as a measure of disability for all patients including children. At present, the CMT Paediatric Scale is used in paediatric patients; however, both scales are evaluating the same underlying construct and can be used for disability assessment in children [22]. After the analysis, there was no correlation between change in NfL and disease progression. In addition, it is important to note that no age-matched controls were established for the children cohort. Therefore, more studies with paediatric CMT patients should be performed to evaluate NfL as a potentially suitable prognostic biomarker in the paediatric CMT population.

In conclusion, our study data provide additional information about plasma NfL level as a biomarker in CMT. We have assessed changes in NfL level over time in adults and paediatric patients and evaluated capability of NfL for monitoring disease progression. Although we detected increased NfL concentrations in patients with CMT compared with controls, the change in NfL concentration at different time points may vary according to subtype and patient age. Furthermore, we have shown in the pilot data that there is no association between change in plasma NfL levels over time and disease progression. Consequently, NfL level does not reflect disease severity and rate of progression. To conclude, our study and previously published data show that NfL as a CMT progression biomarker has limited potential.

**AUTHOR CONTRIBUTIONS**

Signe Setlere: Data curation; writing – original draft; investigation; methodology. Arta Grosmane: Data curation; writing – original draft; investigation; methodology. Natalja Kurjane: Methodology; investigation. Linda Gailite: Investigation; methodology. Dmitrijs Rots: Investigation; methodology. Dmitrijs Rots: Investigation; methodology. Henrik Zetterberg: Supervision; funding acquisition; methodology. Viktorija Kenina: Conceptualization; funding acquisition; project administration; data curation; supervision; methodology; investigation.

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**CONFLICT OF INTEREST STATEMENT**

H.Z. has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Passage Bio, Pionente Therapeutics, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures at symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg, which is a part of the GU Ventures Incubator Program (outside submitted work).

**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**REFERENCES**
