Original Research Paper

Factors contributing to CSF NfL reduction over time in those starting treatment for multiple sclerosis: an observational study

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ABBREVIATIONS

BMI Body Mass Index

CI Confidence Interval

CSF Cerebrospinal fluid

DMT Disease-modifying Treatment

EDSS Expanded Disability Status Scale

IQR Interquartile Range

JC John Cunningham

NfL Neurofilament light chain

PMS Progressive Multiple Sclerosis

SPMS Secondary Progressive Multiple Sclerosis

RRMS Relapsing-remitting Multiple Sclerosis

SD Standard deviation

T1 Timepoint 1

T2 Timepoint 2

ABSTRACT

Background: In multiple sclerosis (MS) neurofilament light chain (NfL) is a marker of neuronal damage secondary to inflammation and neurodegeneration. NfL levels drop after commencement of disease-modifying treatment, especially the highly active ones. However, the factors that influence this drop are unknown.

Objective: To examine the patient and treatment-related factors that influence CSF NfL before and after starting treatment.

Methods: Eligible patients across two centres with two CSF NfL measurements, clinical and MRI data were included as part of an observational cohort study.

Results: Data were available in 61 patients, of which 40 were untreated at the first CSF sampling (T1) and treated at the second (T2; mean T1-T2: 19 months). CSF NfL reduction correlated with age (beta=1.24 95%CI(1.07,1.43); R^2=0.17; p=0.005), Expanded Disability Status Scale (EDSS) (beta=1.12 95%CI(1.00,1.25); R^2=0.21; p=0.05) and the type of MS (beta=0.63 95%CI(0.43, 0.92); R^2=0.12; p=0.018; reference=relapsing MS). The treatment effect on a baseline NfL of 702 pg/mL was 451 pg/ml 95%CI(374,509) in a 30-year-old versus 228 pg/ml 95%CI(63,350) in a 60-year-old. There was no association in CSF NfL reduction with BMI, disease duration or sex. In cladribine- and alemtuzumab-treated patients, the CSF NfL T2/T1 ratio did not correlate with lymphocyte depletion rate at 23 weeks.

Conclusions: In this observational study, we found that factors reflecting early disease stage, including a younger age, lower disability and relapsing MS were associated with treatment response in CSF NfL. Other factors were not found to be related, including lymphopaenia in highly-active treatments.

Keywords: Multiple sclerosis, Biomarkers, Treatment response

INTRODUCTION

In multiple sclerosis (MS), disease-modifying treatments (DMTs) predominantly target inflammation in the central nervous system. Accordingly, there is a great need for treatments that impact on the degenerative component of the disease reflected by the rate of brain volume loss and accrual of disability.[1–3] Neurodegeneration can be quantified by structural cellular proteins such as neurofilament light (NfL) which are released in the cerebrospinal fluid (CSF) and blood.[4] In MS, there is abundant evidence that NfL levels reflect ongoing inflammatory-driven neuroaxonal damage (e.g., relapses or MRI disease activity) and that sNfL levels predict disease activity over the next few year.[5]

In longitudinal cohorts, elevated baseline NfL correlates with Expanded Disability Status Scale (EDSS) progression, greater activity in brain lesions and increased brain volume loss at follow-up.[6–8] Importantly, DMTs are able to reduce NfL levels and show promise as secondary outcome measures in clinical trials.[7,9,10] Highly effective DMTs are better at inducing larger NfL reductions compared to so-called platform DMTs.[6,7,11] However, NfL levels are also influenced by age, body mass index (BMI), blood volume, kidney function and cardiovascular risk profile.[12–15]

In healthy controls, there is a consistently positive association between CSF NfL and age but also with sex where men display higher levels.[15,16] However, the influence of these factors on treatment response is largely unknown. Similarly, we do not yet know what exactly defines treatment response and/or efficacy. However, we often use lymphocyte depletion as a marker of treatment response in immune reconstitution therapies.[17] In our observational

cohort of MS patients with repeat CSF NfL measurements, we aimed to establish whether baseline CSF NfL levels were influenced by patient characteristics and whether CSF NfL reductions following treatment were associated with the baseline patient characteristics. Lastly, we evaluated whether lymphocyte depletion after immune reconstitution therapies influenced drop in CSF NfL levels (i.e. treatment response).

MATERIAL AND METHODS

Study design and participants

This was an observational cohort study based at Barts Health NHS Trust and University Hospitals Plymouth, United Kingdom. Samples obtained from people with relapsing or progressive MS above the age of 18 with two independent CSF NfL measurements were selected for inclusion into the study (cfr. results section for rationale behind repeat LPs). Samples were collected between February 2016 and September 2020. The study was approved by the London City and East Research Ethics Committee (RE C ref: 20/LO/0023). Signed informed consents were obtained from all participants.

Demographics, disease and treatment characteristics

Demographic data, disease and treatment characteristics were obtained from the individuals' electronic medical records, including age, sex, type of MS (RRMS or PMS), DMT status, disease duration, EDSS, BMI and comorbidities (arterial hypertension, hyperlipidaemia, diabetes,

epilepsy, stroke, recent head trauma, kidney disease and other neurological disorders). When incomplete records were available for comorbidities the individual was contacted by telephone. Disease duration was defined as the number of years from the first episode of focal neurologic dysfunction suggestive of demyelination. In order to determine the lymphocyte depletion ratio after treatment with alemtuzumab and cladribine, we used the total lymphocyte count (cells/L) of the blood sample taken closest to 20 weeks after the first round of treatment compared to a baseline value.

CSF NfL measurements

CSF samples were obtained by lumbar puncture and collected in polypropylene tubes. CSF samples were centrifuged at 2500 rpm for 10 minutes and aliquoted and stored at −80°C until use. CSF NfL measurements were measured in the neuroimmunology laboratory at the Blizard Institute, Queen Mary University of London, London, United Kingdom, using the commercially available and validated solid-phase sandwich ELISA from UmanDiagnostics (Umeå, Sweden).[18] The test used two highly specific noncompeting monoclonal antibodies: an NfL-capturing antibody coated to the solid phase of a strip plate and a tracer antibody conjugated to horseradish peroxidase for the detection of captured NfL protein. CSF NfL measurements (pg/mL) were calculated using a standard curve according to the manufacturer's instructions.[19] The detection limit of the ELISA was 33 pg/mL. Intra- and interassay coefficients of variation were below 10%. All NfL analyses were performed in duplicate. CSF NfL measurements were categorized as normal or elevated according to age-related reference values defined by the manufacturer.[19,20]

Statistical analysis

We described the median CSF NfL values and interquartile range (IQR) at timepoint 1 (T1), and timepoint 2 (T2) within three groups of patients: untreated at T1/treated at T2, treated at T1/treated at T2 and untreated at T1/untreated at T2. The small size of the patient groups precluded formal statistical testing. In order to determine whether there were differences in baseline CSF NfL according to a range of patient characteristics, we used the Wilcoxon test (sex, type of MS, presence/absence of comorbidities) or Spearman correlation test (age, EDSS, disease duration, BMI). To assess associations between patient characteristics and change in CSF NfL over time in individuals untreated at T1 and treated at T2, we performed a series of univariable linear regression analyses. The log of CSF NfL at T2 was used as outcome and modelled in function of the log of CSF NfL at T1 in combination with single patient characteristics (age, disease duration, sex, type of MS, BMI and EDSS). In order to illustrate the results, predicted values of CSF NfL at T2 were found using the regression of T2 CSF Nfl on age and median T1 CSF NfL (702 pg/mL). We calculated the Spearman correlation between the log of the ratio of T2 to T1 CSF NfL and lymphocyte depletion ratio after treatment with cladribine and alemtuzumab. Point estimates and confidence intervals were exponentiated (e^x) when reported in the manuscript. We applied a nominal significance threshold (p=0.05), and all tests were two-sided. All analyses were performed using the statistical package R v3.6.1.

Data availability

Data included in this analysis will be shared following request from any qualified investigator to the corresponding author.

RESULTS

Patient characteristics

Demographic and clinical characteristics of the 61 patients included in the study can be found in Table 1. The mean interval between T1 and T2 was 19 months (SD ±8). At T1, 38 (62%) were female, the mean age was 45 years (SD ± 13), 23 (44%) had relapsing MS and 52 (85.2%) were not on a DMT, whilst 12 (19.7%) remained untreated at T2. In people untreated at T1 and treated at T2 (n=40), the reason to refer for a second LP was to determine treatment efficacy (n=31), clinical deterioration despite treatment (n=6), and to check John-Cunningham virus status (n=3). The median EDSS score was 4 (IQR 2-6), whilst the mean disease duration was 7 years (SD ±7). In these 40 patients, the mean time between the first LP and initiating treatment was 5 (SD ±3) months and 16 (SD ±7) between the initiation of treatment and the second LP (Table 1).

Association between baseline CSF NfL levels and patient characteristics

Associations between baseline CSF levels and baseline characteristics were weak in magnitude. There was a trend for association between baseline CSF NfL levels and male sex with marginally higher values (Table 2) but not with age (Figure S1A), the type of MS, disease

duration, EDSS or BMI (Table 2). The majority of the patients reported no comorbidities (n=46) and there was no difference in CSF NfL between patients with or without comorbidities (Table 2, Table S1).

The effect of treatment initiation and individual patient characteristics on CSF NfL levels

Reductions in CSF NfL levels between the two sampling timepoints were apparent in untreated patients at T1 and those that had started treatment at T2 (n=40) (Figure 1, Table 3). The influence of patient characteristics on the change in CSF NfL in these 40 patients that were untreated at T1 and treated at T2 can be found in Table 4 and Figure 2A.

Looking at this in more dept, T2 CSF NfL was found to be associated with age at T1. For every 10-year increase in age, the second CSF NfL measurement increased by 24% (beta=1.24 95% CI(1.07, 1.43); R^2=0.17; p=0.005), conditional on the CSF NfL measurement at T1. For illustrative purposes, we have plotted the median CSF NfL value at T1 (702 pg/mL) in our untreated/treated cohort and the CSF NfL changes among the different ages in a regression model (Figure 2B). For an individual with a median T1 NfL value, the predicted CSF NfL drop at second measurement varied from 451 pg/mL 95%CI(374, 509) in a 30-year-old to 228 95%CI(63, 350) in a 60-year-old.

With regards to the association between CSF NfL reduction and baseline EDSS, for every one point increase in the EDSS scale at baseline, there was an associated increase in CSF NfL by 15% (beta=1.15 95%CI(1.06, 1.25); R^2=0.21; p=0.002) at T2 (Table 4, Figure 2A). Also, having

relapsing MS as a diagnosis was associated with a 37% reduction in CSF NfL at T2 compared to progressive MS (beta=0.63 95% CI(0.43, 0.92); R^2=0.12; p=0.018) (Table 4, Figure 2A).

A similar relationship was not observed with sex (beta=1.06 95% CI(0.69, 1.44); R^2=0.02; p=0.78), BMI (beta=1.00 95%CI(0.96, 1.04); R^2=0; p=0.955), or disease duration (beta=1.27 95%CI(0.93, 1.75); R^2=0.03; p = 0.132) (Table 3, Figure 2A). We included a a subanalysis in cladribine treated individuals in Supplementary Data (Table S2). Lymphocyte depletion ratio at 23 weeks (SD ± 6.8) after the first round of treatment in those treated with immunosuppressive therapies such as alemtuzumab and cladribine (n=37), also did not correlate with CSF NfL ratio T2/T1 (r=0.007 95%CI(-0.33, 0.32); p=0.97) (Figure S1B).

DISCUSSION

In cross-sectional cohorts, NfL levels in blood and CSF have been noted to increase with age in both healthy individuals and MS patients,[6,15,21,22] and correlated with higher EDSS levels and progressive MS.[23,24] In the present study, we evaluated some key factors that might influence CSF NfL dynamics longitudinally in a cohort of MS patients.

In untreated MS patients commencing treatment after their baseline CSF assessment, we found that the greatest CSF NfL reductions occurred in those starting treatment at younger age with lower disability levels and a relapsing-remitting disease phenotype. A younger age, low EDSS score and relapsing-remitting MS disease phenotype are all inter-related i.e. summative their relative contribution, but on the whole reflect an earlier disease stage. We

did not, however, find an association with disease duration. However, “true” disease duration can be unclear which may, in part, explain the lack of observed association in our study.

Age, EDSS and the type of MS explained separately between 12% and 21% reduction in CSF NfL levels. These findings are underscored by the fact that we found no strong association between age, disability level or type of MS and CSF NfL level at baseline in our cohort. A rise in CSF NfL values with age is a characteristic that has only been observed within healthy controls but not in MS as it is most likely confounded by disease activity.[16]

As age, EDSS and type of MS reflect similar disease processes, we did not take them forward into a multivariate regression analysis in order to detect individual contributions. Interestingly, in a meta-analysis of CSF NfL literature, investigators reported a 3.3% yearly increase in CSF NfL in healthy controls with age which corresponds to approximately 33% over 10 years.[16] We observed that for every 10-year increase in age, the second CSF NfL measurement increased by 24%. However, it is difficult to postulate whether this observation is the result of mere age-related neurodegeneration or whether other (inflammatory) processes are equally involved.

It is possible that the association with earlier disease stage reflects the diminishing inflammatory penumbra from early to advanced disease,[25,26] leading to a less pronounced effect of DMTs on CSF NfL levels. Alternatively, it could reflect the diminishing number of axons over time with disease.[27,28] The latter is supported by pathology studies demonstrating axonal loss of about 60%, and up to >90% synaptic loss in more advanced stages of the disease,[27,29] supporting the need for early treatment of MS patients.[30] However, this does

not imply that treating with DMTs at a later disease course will not be effective as reflected by the relative success of siponimod in secondary progressive MS.[31]

Whilst age, EDSS and type of MS explained just under a quarter of the reduction in CSF NfL following treatment, the majority of the variance remained unexplained by the variables we studied in this cohort. To our knowledge this is the first exploration of factors influencing treatment response in terms of CSF NfL values in MS. It is likely that this unaccounted for variation may be related to the underlying pathology of MS, lesion location and volume, neuronal plasticity, as well as the influence of genetic factors on treatment effect. A larger cohort analysis with more in-depth data collection, incorporating MRI markers of disease activity including T2 lesion load and brain volume will hopefully tease out some of these additional contributing factors.

Beyond age and gender, many of the factors that we have looked at, for instance BMI and co-morbidities were factors identified in blood NfL studies,[12–15] and studies focused on CSF NfL variation are still needed. We also did not find an association between CSF NfL reductions and lymphocyte depletion from immunosuppressive treatments such as cladribine or alemtuzumab. Although it is tempting to hypothesize that the level of immune cell depletion might reflect treatment efficacy, the evidence for this hypothesis is limited and also not supported by our findings.[17] Qualitative changes in immune phenotype and regulatory networks may be more important than total lymphocyte reductions.[17,32] Following alemtuzumab and cladribine, it has been shown that the depletion of memory B cells is substantial, longstanding and potentially more reflective of their mode of action.[33,34]

The roles of BMI as a risk factor for increased MS disease activity and reduced treatment effect have made them an important considerations in our study.[35,36] However, we did not find an association between BMI and both absolute CSF NfL levels or reductions in CSF NfL after treatment initiation. A cross-sectional study of 2,586 MS patients analyzing plasma NfL levels observed only a small reduction 0.02 pg/mL for every unit of BMI.[12] Our CSF cohort was likely not powered to capture such small effects. In addition, the effect of BMI is felt to be related to overall blood volume in blood NfL which may not be relevant in the CSF.

The main limitations of our study were its observational nature, our inability to account for unknown factors that might influence CSF NfL dynamics and the fact that people were self-selected by virtue of having two NfL measurements. The extent to which our results can be generalized to the typical population commencing DMT is unknown. Although our CSF cohort is of a limited size, our findings point to potential early determinants in the longitudinal evolution of CSF NfL in real-life clinical settings and these data are currently scant. However, LPs are relatively invasive precluding large-scale validation studies with repeated sampling.

CONCLUSIONS

In conclusion, a younger age, lower disability levels and relapsing-remitting MS were associated with greater reductions in CSF NfL in MS patients starting treatment. These factors typify an earlier disease stage, and were independent of other patient and treatment-related factors. The loss of sensitivity of NfL reduction over the course of the disease may reflect the loss of CNS tissue with time leading to a reduction in NfL release. The relative contribution of

each patient characteristic in influencing CSF NfL dynamics following treatment needs to be explored in future CSF studies.

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REFERENCES

1. Giovannoni G, Turner B, Gnanapavan S, et al. Is it time to target no evident disease activity (NEDA) in multiple sclerosis? *Mult Scler Relat Disord* 2015; 4: 329–333.
2. Giovannoni G. Disease-modifying treatments for early and advanced multiple sclerosis: a new treatment paradigm. *Curr Opin Neurol* 2018; 31: 233–243.
3. Hauser SL, Cree BAC. Treatment of Multiple Sclerosis: A Review. *Am J Med* 2020; 133: 1380-1390.e2.
4. Khalil M, Teunissen CE, Otto M, et al. Neurofilaments as biomarkers in neurological disorders. *Nat Rev Neurol* 2018; 14: 577–589.
5. Bittner S, Oh J, Havrdová EK, et al. The potential of serum neurofilament as biomarker for multiple sclerosis. *Brain* 2021; 1–29.
6. Cantó E, Barro C, Zhao C, et al. Association Between Serum Neurofilament Light Chain Levels and Long-term Disease Course Among Patients With Multiple Sclerosis Followed up for 12 Years. *JAMA Neurol* 2019; 76: 1359–1366.
7. Kuhle J, Kropshofer H, Haering DA, et al. Blood neurofilament light chain as a biomarker of MS disease activity and treatment response. *Neurology* 2019; 92: e1007–e1015.
8. Kuhle J, Plavina T, Barro C, et al. Neurofilament light levels are associated with long-term outcomes in multiple sclerosis. *Mult Scler* 2020; 26: 1691–1699.
9. Hauser SL, Bar-Or A, Cohen JA, et al. Ofatumumab versus Teriflunomide in Multiple Sclerosis. *N Engl J Med* 2020; 383: 546–557.
10. Cross A, Bennett J, von Büdingen HC, et al. Ocrelizumab treatment reduced levels of neurofilament light chain and numbers of B cells in the cerebrospinal fluid of patients with relapsing multiple sclerosis in the OBOE study (S56.008). *Neurology* 2019; 92:

S56.008.

11. Delcoigne B, Manouchehrinia A, Barro C, et al. Blood neurofilament light levels segregate treatment effects in multiple sclerosis. *Neurology* 2020; 94: e1201–e1212.
12. Manouchehrinia A, Piehl F, Hillert J, et al. Confounding effect of blood volume and body mass index on blood neurofilament light chain levels. *Ann Clin Transl Neurol* 2020; 7: 139–143.
13. Akamine S, Marutani N, Kanayama D, et al. Renal function is associated with blood neurofilament light chain level in older adults. *Sci Rep* 2020; 10: 1–7.
14. Korley FK, Goldstick J, Mastali M, et al. Serum NfL (Neurofilament Light Chain) Levels and Incident Stroke in Adults with Diabetes Mellitus. *Stroke* 2019; 50: 1669–1675.
15. Khalil M, Pirpamer L, Hofer E, et al. Serum neurofilament light levels in normal aging and their association with morphologic brain changes. *Nat Commun* 2020; 11: 1–9.
16. Bridel C, Van Wieringen WN, Zetterberg H, et al. Diagnostic Value of Cerebrospinal Fluid Neurofilament Light Protein in Neurology: A Systematic Review and Meta-analysis. *JAMA Neurol* 2019; 76: 1035–1048.
17. Lünemann JD, Ruck T, Muraro PA, et al. Immune reconstitution therapies: concepts for durable remission in multiple sclerosis. *Nat Rev Neurol* 2019; 29: 2–5.
18. Petzold A, Altintas A, Andreoni L, et al. Neurofilament ELISA validation. *J Immunol Methods* 2010; 352: 23–31.
19. IBL International. *NF-light ® (Neurofilament light) ELISA Instructions for Use*, https://www.ibl-international.com/media/catalog/product/U/D/UD51001_IFU_EU_en_NF-light_ELISA_v2019-02.pdf (2015, accessed 18 January 2021).
20. Reyes S, Smets I, Holden D, et al. CSF neurofilament light chain testing as an aid to

determine treatment strategies in MS. *Neurol Neuroimmunol neuroinflammation* 2020; 7: e880.

21. Vågberg M, Norgren N, Dring A, et al. Levels and age dependency of neurofilament light and Glial Fibrillary Acidic Protein in healthy individuals and their relation to the brain parenchymal fraction. *PLoS One* 2015; 10: 1–8.
22. Bhan A, Jacobsen C, Myhr KM, et al. Neurofilaments and 10-year follow-up in multiple sclerosis. *Mult Scler J* 2018; 24: 1301–1307.
23. Novakova L, Zetterberg H, Sundström P, et al. Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. *Neurology* 2017; 89: 2230–2237.
24. Thebault S, Abdoli M, Fereshtehnejad SM, et al. Serum neurofilament light chain predicts long term clinical outcomes in multiple sclerosis. *Sci Rep* 2020; 10: 1–11.
25. Leray E, Yaouanq J, Le Page E, et al. Evidence for a two-stage disability progression in multiple sclerosis. *Brain* 2010; 133: 1900–1913.
26. Confavreux C, Vukusic S, Moreau T, et al. Relapses and Progression of Disability in Multiple Sclerosis. *N Engl J Med* 2000; 343: 1430–1438.
27. Petrova N, Carassiti D, Altmann DR, et al. Axonal loss in the multiple sclerosis spinal cord revisited. *Brain Pathol* 2018; 28: 334–348.
28. Ferguson B, Matyszak MK, Esiri MM, et al. Axonal damage in acute multiple sclerosis lesions. *Brain* 1997; 120 (Pt 3: 393–9.
29. Petrova N, Nutma E, Carassiti D, et al. Synaptic Loss in Multiple Sclerosis Spinal Cord. *Ann Neurol* 2020; 88: 619–625.
30. Vaughn CB, Jakimovski D, Kavak KS, et al. Epidemiology and treatment of multiple sclerosis in elderly populations. *Nat Rev Neurol* 2019; 15: 329–342.
31. Kappos L, Bar-Or A, Cree BAC, et al. Siponimod versus placebo in secondary

progressive multiple sclerosis (EXPAND): a double-blind, randomised, phase 3 study. *Lancet* 2018; 391: 1263–1273.

32. Baker D, Marta M, Pryce G, et al. Memory B Cells are Major Targets for Effective Immunotherapy in Relapsing Multiple Sclerosis. *EBioMedicine* 2017; 16: 41–50.

33. Baker D, Herrod SS, Alvarez-Gonzalez C, et al. Both cladribine and alemtuzumab may effect MS via B-cell depletion. *Neurol Neuroimmunol NeuroInflammation* 2017; 4: 1–10.

34. Baker D, Pryce G, Herrod SS, et al. Potential mechanisms of action related to the efficacy and safety of cladribine. *Mult Scler Relat Disord* 2019; 30: 176–186.

35. Huppke B, Ellenberger D, Hummel H, et al. Association of Obesity with Multiple Sclerosis Risk and Response to First-line Disease Modifying Drugs in Children. *JAMA Neurol* 2019; 76: 1157–1165.

36. Kvistad SS, Myhr KM, Holmøy T, et al. Body mass index influence interferon-beta treatment response in multiple sclerosis. *J Neuroimmunol* 2015; 288: 92–97.

TABLES

Table 1: Characteristics of the study cohort (n=61)

The basic characteristics of the complete cohort are described. Categorical variables were described as frequency and percentages. Continuous and ordinal variables by mean or median and standard deviation or interquartile range (IQR), respectively. IQR was defined as the difference between the 25^{th} and 75^{th} percentile. (Abbreviations: MS = multiple sclerosis, EDSS = Expanded Disability Status Scale, CSF NfL = cerebrospinal fluid neurofilament light chain, SD = Standard Deviation, IQR = Interquartile range, BMI = body mass index, SC = subcutaneous).

	CSF NfL measurement at:	
	Timepoint 1	Timepoint 2
Patients, n	61	61
Sex, n (%)		
Female	38 (62.3)	
Male	23 (37.7)	
Type of MS, n (%)		
Relapsing	27 (44.3)	
Progressive	34 (55.7)	
Age (years), mean (±SD)	45 (± 13)	47 (±13)
Disease duration (years), mean (±SD)	7 (±7)	9 (±7)
EDSS, median (IQR)	4 (2-6)	5 (3-7)
BMI, mean (±SD)	26.3 (± 5.3)	/
Comorbidities, n (%)		/
Present	15 (24.6)	
Absent	46 (75.4)	
Time to baseline (months), mean (±SD)	/	19 (± 8)
Timing between first and second LP (months), mean (±SD)	21 (±8)	
Timing between first LP and start DMT (months), mean (±SD)	5 (±3)	
Duration on DMT by second LP (months), mean (±SD)	16 (±7)	
CSF NfL, n (%)		
Normal	30 (49.2)	48 (78.7)
Elevated	31 (50.8)	13 (21.3)
Treatment, n (%)		

None	52 (85.2)	12 (19.7)
Dimethyl fumarate	2 (3.3)	3 (4.9)
Fingolimod	1 (1.6)	1 (1.6)
Natalizumab	3 (4.9)	5 (8.2)
Alemtuzumab	2 (3.3)	9 (14.8)
SC cladribine (Off-Label)	1 (1.6)	31 (50.8)

Table 2: Statistical associations of CSF NfL at baseline with patient characteristics (n=61)

The baseline characteristics were sex, type of MS, age, disease duration, EDSS, BMI and the presence of comorbidities. Interquartile range was defined as the difference between the 25th and 75th percentile. (Abbreviations: MS = multiple sclerosis, EDSS = Expanded Disability Status Scale, CSF NfL = cerebrospinal fluid neurofilament light chain, BMI = body mass index, IQR = Interquartile range, CI = confidence interval).

Demographic variable	Wilcoxon-test median CSF NfL at T1 (pg/mL) (IQR), P-value	Spearman rank Correlation rho (95%CI), P-value
Sex		
Female	565 (300-939); 0.07	/
Male	792 (552-1328); 0.07	/
Type of MS		
Relapsing	641 (383-992); 0.67	/
Progressive	711 (412-1059); 0.67	/
Age	/	0.20 (-0.43,0.06); 0.12
Disease duration	/	-0.12 (-0.39, 0.13); 0.35
EDSS	/	0.18 (-0.08, 0.43); 0.16
BMI	/	-0.05 (-0.30, 0.19); 0.68
Comorbidities		
Present	492 (306-827); 0.23	/
Absent	688 (434-1070); 0.23	/

Table 3: Median CSF NfL (pg/mL) values at different sampling timepoints according to treatment status (n=61)

Median CSF NfL in the different treatment cohorts is presented. Interquartile range is defined as the difference between the 25th and 75th percentile. (CSF NfL = cerebrospinal fluid neurofilament light, IQR = interquartile range)

CSF NfL (pg/mL), median (IQR)	**Timepoint 1**	**Timepoint 2**
Untreated/Treated	702 (466-1067)	329 (208-512)
Untreated/Untreated	621 (438-783)	500 (388-812)
Treated/Treated	340 (239-1089)	373 (318-488)

Table 4: Results of the regression analysis log(CSF NfL at T2) in function of log(CSF NfL at T1) and patient characteristics (n=40)

Table summarizing results of a univariable regression analysis with log(CSF NfL at T2) as the outcome in function of log(CSF NfL T1) and different patient characteristics (age, sex, disease duration, BMI, EDSS, type of MS). Analysis was performed in individuals untreated at T1 and treated at T2 (n=40). Point estimates and confidence intervals were exponentiated (e^x). (T1 = Timepoint 1, T2 = timepoint 2, CI = confidence interval, BMI = body mass index, ref = reference, EDSS = Expanded disability Status Scale, MS = multiple sclerosis).

	Univariable analysis		
	Point estimate (95% CI)	P-value	Adjusted R-square
Analysis in patients untreated at T1 and treated at T2 (n=40)			
Age	1.24 (1.07, 1.43)	0.005	0.17
Sex (ref=female)	1.06 (0.69, 1.64)	0.780	-0.02
Disease duration	1.27 (0.93, 1.75)	0.132	0.03
BMI	1.00 (0.96, 1.04)	0.955	0.00
EDSS	1.15 (1.06, 1.25)	0.002	0.21
Type of MS (ref=relapsing)	0.63 (0.43, 0.92)	0.018	0.12

FIGURES

Figure 1: Longitudinal evolution of CSF NfL (n=61)

Spaghetti plots of CSF NfL values at first (T1) and second (T2) lumbar puncture in our full cohort (n=61). Patients are subdivided into three groups: untreated at T1 and treated at T2 (red, n=40), untreated at T1 and T2 (blue, n=12) and treated at T1 and T2 (green, n=9). Median values are represented by thicker lines in each respective group at T1 and T2. The red cross marks a patient with an outlier CSF NfL value at T1 of 10,000 pg/mL whose data point was omitted from the graph for illustration purposes. (Abbreviations: CSF NfL = cerebrospinal fluid neurofilament light, T1 = Timepoint 1, T2, Timepoint 2)

Figure 2: Regression analysis and predictions based on log(CSF NfL at T2) as a function of log(CSF NfL T1) and individual patient characteristics (n=40)

A) A forest plot visualising point estimates and confidence intervals of the univariable regression analysis with log(CSF NfL at T2) as the outcome modelled as a function of log(CSF NfL at T1) and individual patient characteristics. B) Spaghetti plot visualising predictions of CSF NfL at T2 based on age and the median CSF NfL value at T1 (702 pg/mL) in the untreated/treated group for the regression model described in A. Light grey lines represent the raw CSF NfL data for people untreated at T1 and treated at T2 (n=40). CSF NfL values of more than 1000 pg/mL were omitted from this plot for visualisation purposes. (T1 = timepoint 1, T2 = timepoint 2, CI = confidence interval, MS = multiple sclerosis, BMI = body mass index, EDSS = Expanded disability Status Scale, ref = reference).