Neurofilament light in CSF and serum is a sensitive marker for axonal white matter injury in MS

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ABSTRACT

Objective: In an ongoing, open-label, phase 1b study on the intrathecal administration of rituximab for progressive multiple sclerosis, an intraventricular catheter was inserted for drug delivery. The objective of this study was to characterize the limited white matter axonal injury evoked by catheter insertion by analyzing a panel of markers for tissue damage in CSF and serum.

Methods: Lumbar CSF and serum were collected before catheter insertion and at regular intervals during the follow-up period of 1 year. Levels of neurofilament light polypeptide (NF-L), glial fibrillary acidic protein, microtubule-associated protein tau, and S100 calcium binding protein B were measured in the CSF, and NF-L was also quantified in serum at each time point.

Results: One month after neurosurgical trauma, there was a distinct peak in NF-L concentration in both CSF and serum. In contrast, the biomarkers S100 calcium binding protein B, glial fibrillary acidic protein, and microtubule-associated protein tau did not show any significant changes. NF-L levels in both CSF and serum peaked at 1 month post surgery, returning to baseline after 6 to 9 months. A strong correlation was observed between the concentrations of NF-L in CSF and serum.

Conclusions: The NF-L level, in CSF and serum, appears to be both a sensitive and specific marker for white matter axonal injury. This makes NF-L a valuable tool with which to evaluate acute white matter axonal damage in a clinical setting. Serum analysis of NF-L may become a convenient way to follow white matter axonal damage longitudinally.

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GLOSSARY

EDSS = Expanded Disability Status Scale; **GFAP** = glial fibrillary acidic protein; **IT** = intrathecal; **MS** = multiple sclerosis; **NF-L** = neurofilament light polypeptide; **PMS** = progressive multiple sclerosis; **S100B** = S100 calcium binding protein B; **Tau** = microtubule-associated protein tau; **tTau** = total Tau.

Biomarkers of cell injury in the CNS are important tools to help evaluate neurologic disease processes. The presence of elevated levels of neurofilament light polypeptide (NF-L) in the CSF is an indicator of axonal damage in the CNS.[1] However, little is known about the dynamics of this biomarker following a damage-inducing event and how well elevated CSF NF-L levels are mirrored in the peripheral circulation.

We have initiated a phase 1b study to investigate the safety, feasibility, and efficacy of rituximab delivered intrathecally (IT) as a potential therapy in a group of patients with progressive multiple sclerosis (PMS) not responsive to other therapies. During this study, a ventricular catheter attached to an Ommaya reservoir was inserted surgically in order to secure safe delivery of the therapeutic antibody into the CSF compartment. In our preliminary analyses, we noted

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a distinct elevation of NF-L levels at the first CSF examination following the introduction of the ventricular catheter.

This study thus created a system similar to an experimental injury model, which enabled us to explore the dynamics of NF-L levels in CSF as a biomarker for white matter axonal injury. To further characterize the response to the surgical trauma, we also analyzed CSF levels of the astrocyte markers glial fibrillary acidic protein (GFAP) and S100 calcium binding protein B (S100B) and the neuronal marker microtubule-associated protein tau (Tau). In addition, NF-L levels in serum were quantified using a newly developed, sensitive method and compared with the corresponding values in CSF.

METHODS **Standard protocol approvals, registrations, and patient consents.** The intrathecal therapy–PMS trial (ClinicalTrials.gov identifier NCT01719159) is an open-label, interventional study primarily aimed at examining the feasibility and safety of IT administration of rituximab in PMS. Inclusion criteria were the diagnosis of PMS and failure to respond to or being ineligible for conventional therapies. The study was approved by the Regional Ethical Review Board of Umeå University (Dnr 08-157M). Informed consent from each patient was obtained before enrollment.

Patients. The study population comprised the first 12 patients included in the trial. At inclusion, the mean age was 47.3 years (range 31–60) and the mean disease duration was 15.3 years. The median Expanded Disability Status Scale (EDSS) score at inclusion was 6.5 (table 1).

Surgery. Under general anesthesia, a ventricular catheter was introduced into the right frontal horn through a 14-mm-diameter burr hole placed 2 cm to the right of the midline at the level of the coronal suture and connected to a subcutaneous Ommaya reservoir.

Clinical evaluations. Patients were evaluated clinically for possible side effects, relapses, and EDSS score at baseline and then at 1, 3, 6, 9, and 12 months post treatment. CSF was obtained through lumbar puncture, which was performed at each visit to follow immunologic measures and biomarkers. MRI was performed at baseline and at the 1-, 3-, 6-, and 12-month examinations.

During the study period, no relapses were recorded but new MRI lesions were observed in one patient. EDSS scores increased by 0.5 in 2 patients and were stable in the remainder.

Estimation of tissue involvement of the surgical trauma. The catheter canal was identified as a streak of no signal in T1-weighted sagittal MRI images, leading from the cortex to the frontal horn of the right lateral ventricle. The length of the canal was measured with in-program tools and the thickness of the cortex was measured in the same way, adjacent to image artifacts caused by the Ommaya reservoir. The percentage of white matter penetrated by the catheter was calculated. The volume of the affected white matter was estimated based on the known diameter of the ventricular catheter (3.1 mm).

Table 1	Baseline demographics of patients included in this study	
Age, y		
Mean (SD)	47.3 (9.6)	
Min-max	31–60	
Sex, n (%)		
Male	5 (42)	
Female	7 (58)	
Age at disease onset, y		
Mean (SD)	31.9 (10.4)	
Min-max	15–46	
Disease duration, y		
Mean (SD)	15.3 (5.5)	
Min-max	6–23	
EDSS score at inclusion		
Median (IQR)	6.5 (6.0–7.0)	
Min-max	4–7.5	
CSF mononuclear cell count		
Mean (SD)	2.3 (2.6)	
Min-max	0–8	

Abbreviations: EDSS = Expanded Disability Status Scale; IQR = interquartile range; max = maximum; min = minimum.

Biomarker analysis. The concentration of NF-L in CSF was measured using an ELISA (NF-light assay; UmanDiagnostics AB, Umeå, Sweden) according to the ELISA kit instructions.[2] The manufacturer of the kit states the assay's lower limit of quantification as 32 ng/L and mean intra- and interassay coefficient of variation as 4% and 6%, respectively.

The concentration of GFAP in CSF was measured using an in-house ELISA based on polyclonal antibodies.[3] The assay has a lower limit of quantification of 70 ng/L and intra- and interassay coefficients of variation of 4% and 8%, respectively.

CSF total Tau (tTau) concentration was determined using a sandwich ELISA (Innotest hTAU-Ag; Innogenetics, Ghent, Belgium) specifically developed to measure all Tau isoforms irrespective of phosphorylation status.[4]

S100B concentration was determined by an electrochemiluminescence immunoassay using a modular system and the S100B reagent kit (Elecsys S100; Roche Diagnostics, Penzberg, Germany).[5]

Serum NF-L concentration was determined using the NF-light assay (UmanDiagnostics AB), transferred to the Simoa platform using a Homebrew Kit (Quanterix Corp., Boston, MA). The lower limit of quantification, determined by the blank mean signal ±10 SD, was 1.95 ng/L. All samples were measured in duplicate and were well above the lower limit of quantification. Analyses were performed by a board-certified laboratory technician using a single batch of reagents with intra- and interassay coefficients of variation below 10% and 15%, respectively. The method is described in detail elsewhere.[6]

Statistical analysis. Statistical analyses were performed in IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY). The p values were calculated using Wilcoxon signed rank test for related samples.

| Table 2	Estimation of tissue composition and volume involved by the intraventricular catheter insertion		
	Gray matter	White matter	Total volume
No.	12	12	12
Minimum	17.4	224.2	245.3
Maximum	26.4	295.1	319.3
Mean (% of total volume)	23.6 (8.4)	256.6 (91.6)	280.1 (100)
SD	2.7	23.0	24.5

Volumes are represented in cubic millimeters.

RESULTS **Brain tissue injury caused by catheter insertion.** The average volume occupied by catheters was 280 mm³ (95% confidence interval: 266–294). Of this volume, 91.6% consisted of white matter and 8.4% of gray matter (table 2).

Release of NF-L in CSF and serum in response to brain catheter insertion. At the 1-month follow-up after the

insertion of the ventricular catheter, we noted a distinct elevation in NF-L concentration in CSF for all patients compared with the baseline values taken before the operation. NF-L levels had returned to baseline after 6 months for the majority of patients. By 12 months, NF-L levels had returned to baseline values or below for all patients (figure 1A). We also investigated the corresponding presence of NF-L in serum using a novel and highly sensitive assay, which revealed an identical pattern of NF-L concentrations, albeit at lower levels, also in serum (figure 1B).

The median (interquartile range) NF-L concentration in CSF increased from 409 ng/L (362–680) at baseline to 2,460 ng/L (1,365–4,501) at 1-month follow-up ($p = 0.005$). At 3-month follow-up, the value had declined to 1,037 ng/L (636–1,463, $p = 0.007$), and at 6-month follow-up, it had further declined to 636 ng/L (443–1,080, $p = 0.041$) (figure 1C). In serum, the median (interquartile range) NF-L concentration increased from 17 ng/L (14–23) at baseline to 51

Figure 1   Time curves for NF-L release in CSF and serum
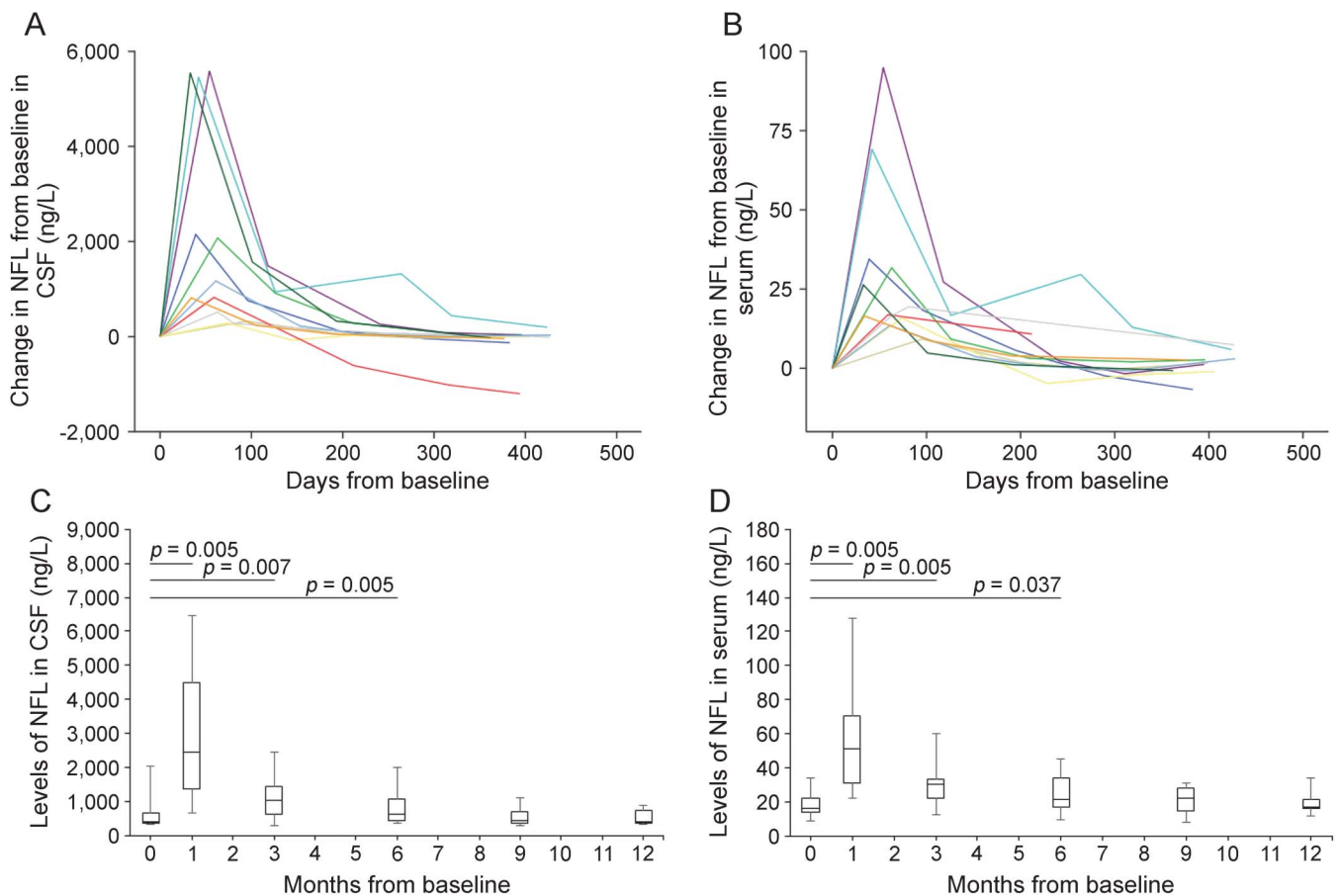
Values in individual patients are shown in the upper panels (A and B) and as aggregated data for the whole group in the lower panels (C and D). The change from baseline is plotted for each individual (n = 12) at the top panels (A and B) while the lower panels (C and D) display the actual values at the different time points as boxplots indicating medians, interquartile range, and range. The time indicators for the boxplots are approximate since the intervals between the lumbar punctures were not exactly the depicted months on the x-axis. The CSF and serum sampling occurred within ±1 week for the 1-month sampling occasion and ±2 weeks for the other occasions. The left panels (A and C) show values from CSF while the right panels (B and D) show values from serum. The p values for statistically significant changes relative to baseline are indicated. NF-L = neurofilament light polypeptide.

ng/L (31–71) at 1-month follow-up ($p = 0.005$), declined to 30 ng/L (22–34) at 3-month follow-up ($p = 0.005$), and then to 21 ng/L (17–34) at 6-month follow-up ($p = 0.037$) (figure 1D).

There was a strong correlation between NF-L concentrations in CSF and serum ($R = 0.775$). NF-L in CSF explained 59.4% ($R^2 = 0.601$, adjusted $R^2 = 0.594$) of NF-L in serum looking at all sampling occasions for all patients (figure 2). In an analysis of individual patients, the correlation between CSF and serum was generally stronger with an $R^2 > 0.9$ in 9 of 12 patients (figure e-1 at Neurology.org/nn).

Release of Tau, S100B, and GFAP in response to brain catheter insertion. A heterogeneous response to the insertion of the ventricular catheter was registered for GFAP, S100B, and tTau. There were no significant differences between the time points for CSF sampling for any of these markers (figure 3).

DISCUSSION In this study, we show that a discrete injury to a brain region comprised predominantly of white matter causes a robust increase in NF-L levels in both CSF (5-fold over baseline) and serum (3-fold over baseline) with a predictable time course. Increases were still evident after 3 months, but values had generally returned to baseline at 6 months. A strong correlation was found between CSF and serum NF-L levels, which indicates an ongoing process of NF-L release into the CSF that is in equilibrium with serum. A similar pattern was not observed for GFAP, S-100B, or tTau, indicating that the

damage to gray matter and astrocytes was insufficient to generate a homogeneous response.

These observations were made in a phase 1b trial of IT-administered rituximab as a potential therapy in a group of patients with PMS who were not responsive to other therapies. The study design included frequent CSF sampling through lumbar punctures for an extended follow-up period, enabling us to follow the time courses of tissue injury biomarkers. The distinct peak in NF-L 1 month post surgery was an unexpected finding that prompted us to investigate whether NF-L release was a specific response to the neurosurgical procedure. A set of biomarkers was chosen to probe both the extent and the kinetics of the evoked damage. However, NF-L was the only marker that displayed a consistent pattern with levels showing an increase in the CSF of all patients at 1-month follow-up, albeit over a large range (<300 to >5,000 ng/L increase over baseline; figure 1A). The surgical trauma was the only logical explanation for the observed NF-L increase. Previous studies have shown that NF-L release in the CSF correlates with focal inflammatory activity in multiple sclerosis (MS).[7,8] In this study, only one patient experienced new T2 lesions and no relapses occurred during the observation period, thus MS inflammatory activity cannot explain the NF-L peak at the 1-month time point. A possible factor that may contribute to the variation in the magnitude of the response, apart from the degree of axonal damage, is the amount of immediate contact with the subarachnoid space of the individual catheter tracts.

The average induced lesion volume corresponded to only approximately 0.3 cm³ of brain tissue, 92% of which was composed of white matter. This is equal to the size of a spherical lesion with a diameter of 8 mm and corresponds to approximately 0.25% of an average brain volume of 1,200 cm³. The fact that we observed a distinct and significant increase in the levels of both CSF and serum NF-L following such a small lesion shows that NF-L is a very sensitive marker for CNS injury. The lack of significant or consistent responses from the other markers tested indicates that NF-L, which is an abundant component of nerve fiber tracts, also appears to be specific for white matter damage. It also agrees well with the observation that CSF NF-L is a sensitive marker for focal inflammatory white matter lesions in MS.[7–9]

Time curves of NF-L from individual patients showed almost identical profiles in both serum and CSF, but with much lower levels present in serum. Of note, one patient also showed a second peak at 9 months that was mirrored precisely in CSF and serum. Although we were not able to identify the reason for this second peak, it provided further support that NF-L in serum is in a continuous equilibrium with the CSF (figure 1, A and B). Furthermore, at the

Figure 2 Correlation between concentrations of NF-L in serum and CSF
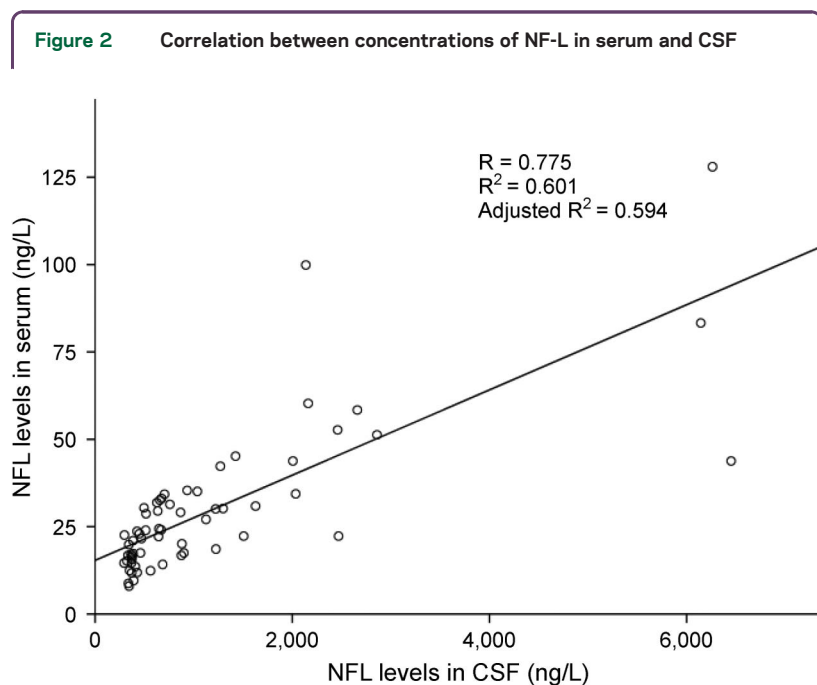
The data indicate that approximately 60% of the concentration in serum may be explained by the concentration in CSF. NF-L = neurofilament light polypeptide.

Figure 3   Time curves for GFAP, S100B, and tTau release in CSF
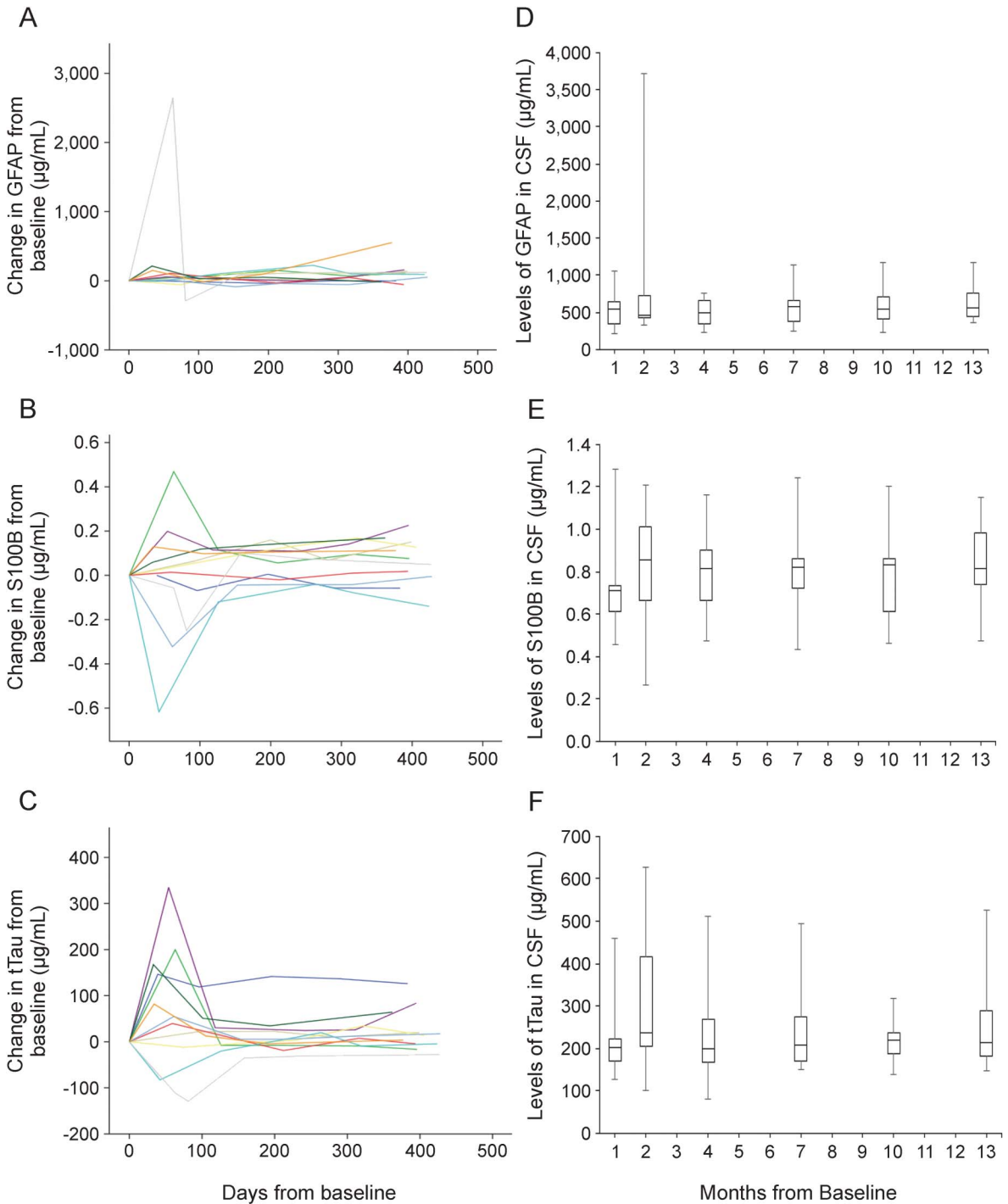
Values for individual patients (A–C) and aggregated data for the whole group (D–F) are shown. The change from baseline is plotted for each individual (n = 12) (A–C) while the actual values at the different time points are displayed as boxplots indicating medians, interquartile range, and range (D–F). The time indicators for the boxplots are approximate since the intervals between the lumbar punctures were not exactly the depicted months on the x-axis. The CSF sampling occurred within ±1 week for the 1-month sampling occasion and ±2 weeks for the other occasions. GFAP = glial fibrillary acidic protein; S100B = S100 calcium binding protein B; tTau = total Tau.

individual patient level, the correlation between NF-L in CSF and serum reached an R^2 value of >0.9 in 9 of 12 patients (figure e-1). These observations suggest that the source of NF-L in serum is CSF rather than effusion directly from the site of injury since one would expect more variation between values in serum and CSF if individual routes contributed to NF-L appearance to the 2 compartments.

Previous studies have shown the potential for serum NF-L to be an indicator of MS risk in clinically

isolated syndrome[10] and was recently reported to correlate with CSF levels and MRI measures in patients with MS.[11] Our study provides further evidence for a strong correlation between NF-L in serum and CSF and represents an important advancement since the analytical sensitivity of our serum assay is 25-fold higher than previously reported.[6] This facilitates the quantification of serum NF-L in neurologically normal controls, as well as in individuals with mild impairment,[12] which was not possible before.[11] Hence, serum NF-L may provide an easier and less invasive alternative to the determination of NF-L in CSF and gives essentially the same information.

Although we believe that our data reliably reflect NF-L release to CSF and serum in conjunction with small local, mainly white matter injury reliably, the data need to be interpreted with caution. We have only examined a small number of patients with PMS. Although the injury made by catheter insertion followed a standardized procedure, allowing variation of the response to a uniform lesion to be measured with accuracy, the inflicted injury was artificial in nature. Hence, it is perhaps not straightforward to extrapolate these findings directly to other clinical situations. Repeated and paired sampling in both CSF and serum is unique and has enabled us in this study to follow the dynamics of NF-L in both compartments simultaneously. However, one drawback regarding our ability to study the dynamics of NF-L over time was constrained by the relatively long period from the damage-inducing event to the first sampling occasion 1 month afterward. Thus, we cannot determine when the peak of NF-L occurs after a white matter lesion. From the strong correlation between serum and CSF levels of NF-L, this question may be resolved in future studies utilizing frequent blood sampling in relation to known white matter lesion–inducing events.

In this study, we report that NF-L in CSF appears to be a sensitive and specific marker for white matter axonal damage in the CNS. The correlation between NF-L levels in serum and CSF shows promise for serum NF-L as a valuable biomarker to follow the individual dynamics of a CNS damage-inducing event closely.

AUTHOR CONTRIBUTIONS

Joakim Bergman was partly responsible for patient management and sample collection, was responsible for data analyses, and participated in writing the first draft and revisions of the manuscript. Ann Dring was partly responsible for data analyses and manuscript revisions. Henrik Zetterberg was responsible for codevelopment of the serum NF-L assay, and was responsible for data analyses and manuscript revisions. Kaj Blennow was responsible for codevelopment of the serum NF-L assay, interpretation of the results, and revisions of the manuscript. Niklas Norgren was responsible for codevelopment of the serum NF-L assay, interpretation of the results, and revisions of the manuscript. Jonathan Gilthorpe participated in data analyses and manuscript revisions. Tommy Bergenheim participated in conceptualization and design of the study, was responsible for all neurosurgical procedures in the study, and participated in manuscript revisions. Anders Svenningsson had the main responsibility for the conceptualization and design of the study, the main responsibility for patient management, and participated in writing the first draft and further revisions of the manuscript.

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DISCLOSURE

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