Elevated CSF neurofilament proteins predict brain atrophy: a 15–year follow–up study

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Axel Petzold[1,2], Martijn D. Steenwijk[3,4], Judith M. Eikelenboom[5], Mike Wattjes[3] and Bernard M.J. Uitdehaag[1]

Abstract

Background Body fluid and structural imaging biomarkers give information on neurodegeneration.The relationship over time is not known in multiple sclerosis.

Objective To investigate the temporal relationship of elevated cerebrospinal fluid (CSF) neurofilament protein (Nf) levels, a biomarker for axonal loss, with magnetic resonance imaging (MRI) atrophy measures.

Methods In patients with multiple sclerosis CSF Nf heavy chain phosphoform levels were quantified at baseline and dichotomised into 'normal' and 'high'. Atrophy was assessed by MRI at baseline and 15–year follow–up using SIENAX and FreeSurfer software.

Results High baseline CSF NfH-SMI35 levels predicted pronounced atrophy at 15–year follow up (OR 36, $p<0.01$), in absence of baseline brain atrophy (OR 28, $p<0.05$), for the averaged MRI normalised brain volume (1.44 L vs 1.33 L, $p<0.05$), normalised grey matter volume (0.77 L vs 0.69 L, $p<0.01$) and putamen (12.7 mL vs 10.7 mL, $p<0.05$). Region specific calculations including the spinal cord showed that a power of $>80\%$ is reached with 14–50 patients.

Conclusion These data suggest that high CSF NfH levels are an early predictor of later brain and spinal cord atrophy using structural imaging biomarkers and can be investigated in reasonably sized patient cohorts.

Keywords
neurodegeneration, multiple sclerosis, cerebrospinal fluid, CSF, biomarker, neurofilaments

Introduction

The assessment of clinical function in patients suffering from multiple sclerosis (MS) relies on validated clinical scales[1]. The major pathological process driving irreversible loss of function is progression of neurodegeneration[2]. The *in vivo* quantification of mechanisms of neurodegeneration relies on indirect data from biomarkers[3]. A strong study design in demyelination combines the well timed use of body fluid biomarkers and structural imaging biomarkers[4]. Neurofilament (Nf) proteins have been validated as a body fluid biomarker for neurodegeneration in multiple sclerosis[3;5]. Disintegration of neurons and axons causes proteolysis and release of neurofilament proteins into the adjacent body fluid compartment[6]. In the acute phase inflammation related oedema and glial activation can mask imaging biomarker quantification of atrophy[7]. With time and resolution of inflammation atrophy becomes more visible and quantification more reliable[8–10]. Therefore a logical sequence of events is that neurodegeneration in MS causes a rise of Nf and irreversible disability which is followed over time by central nervous system (CNS) atrophy[4].

Consistent with this line of argumentation cross–sectional data from patients with MS did show elevated Nf levels[11]. The baseline data did however fail to show a relationship between CSF NfH protein concentration and brain atrophy. Subsequent 3–year follow–up data demonstrated that elevated CSF Nf heavy chain (NfH) protein concentrations were significantly related to irreversible disease progression[12]. This relationship between CSF NfH and disability has since been confirmed by other groups[3;5]. Long–term clinical data of our original cohort confirmed the prognostic value of CSF NfH levels[13]. Importantly,

[1]Department of Neurology and Ophthalmology, Neuroscience Amsterdam, VUmc MS Centre Amsterdam, VU University Medical Center, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands.
[2]UCL, ION, Department of Molecular Neuroscience, Queen Square, London, WC1N 3BG, UK. [3] Department of Radiology and Nuclear Medicine, VU University Medical Center, De Boelelaan 1117, 1081 HV Amsterdam, NL. [4] Department of Physics and Medical Technology, VU University Medical Cente, De Boelelaan 1117, 1081 HV Amsterdam, NL. [5] Westfriesgasthuis, Maelsonstraat 3, 1624 NP Hoorn, NL.

Corresponding author:
Axel Petzold, Queen Square House, 9th floor, room 914, London, WC1N 3BG, United Kingdom. Phone: +44 7903364197, FAX: +44 207 837 8553
Email: a.petzold@ucl.ac.uk or a.petzold@vumc.nl

the 15 year follow–up data revealed that the strongest relationship of phosphorylated CSF NfH levels was with the visual, pyramidal, cerebellar and sensory systems[13]. It was discussed that degeneration of the very long axons of the posterior columns and spinothalamic tracts may be a relevant source for releasing CSF NfH.

The pathological process responsible for brain atrophy following axonotmesis is bi–directional trans–synaptic axonal degeneration[14]. Therefore, extending on our 3–year follow–up data, the aim of the present study was to test if high CSF NfH levels at baseline would predict more severe CNS atrophy after 15–years.

Material and Methods

This study was approved by the Institutional Review Board and written informed consent was obtained from all patients.

Patients Fifteen patients from a previously reported cohort[13] with clinically definite MS[15] agreed to participate in brain and spinal cord magnetic resonance imaging (MRI). All patients have been started with disease modifying treatment (interferon beta). The Expanded Disability Status Scale score (EDSS)[16] was assessed at baseline and follow–up. The disease course was classified as relapsing remmitting (RR), primary or secondary progressive (PP/SP)[11].

MRI At baseline a 1.5 T system (Siemens AG, Erlangen, Germany) and consisted of an axial T1- and T2-weighted spin echo MRI with 3 mm slice thickness and 1x1 mm in-plane resolution. Brain atrophy and lesion load measurements were analysed as described[11]. More severe atrophy was indicated by a smaller brain parenchymal fraction (BPF $= \frac{whole\,brain\,parenchymal\,volume}{intracranial\,volume}$).

At 15–years follow up MRI was performed on a 3 T whole body system (GE Signa HDxt, Milwaukee, WI, USA). The detailed acquisition parameters have been described previously[17]. Lesion volumes were automatically quantified using 'k nearest neighbour classification with tissue type priors' (kNN-TTP)[18]. Normalized brain volumes (NBV), grey matter volumes (NGMV) and white matter volumes (NWMV) were determined with SIENAX (part of FSL 5.04, fsl.fmrib.ox.ac.uk) after lesion filling[19]. We did use FIRST to determine the normalised deep grey matter volumes (NDGMV). In addition, mean upper cervical cord area (MUCCA) was measured as a measure of spinal cord atrophy[20;21].

CSF Samples of CSF were obtained by routine lumbar puncture. Aliquots of CSF were stored at -80°C until assayed. Levels of NfH phosphoforms were quantified by ELISA[22]. Phosphoforms were indicated with the capturing antibody in the superscript,

with SMI34 for hyperphosphorylated NfH (NfH^{SMI34}) and SMI35 for phosphorylated NfH (NfH^{SMI35}) adhering to a published nomenclature[22]. Consensus guidelines were followed for analysis and classification of CSF oligoclonal bands (OCB)[23]. General CSF findings were summarised as recommended[24].

Data analysis SAS software (v9.4) was used for Statistical analyses and preparation of figures. Non–Gaussian data were reported as median and 25–75 % interquartile range (IQR), normally distributed data as mean ± standard deviation (SD). Two tailed tests were used and a p–value of <0.05 accepted as statistically significant.

Our previously published hypothesis on the prognostic value of CSF NfH levels was tested. Consistent with our previous publications high CSF NfH^{SMI35} levels were defined as $\geq$ 20 pg/mL and high CSF NfH^{SMI34} levels as $\geq$ 11 pg/mL[12;13]. Group comparisons were made according to distribution either using the exact non–parametric Wilcoxon Two–Sample Test or the t–test. In addition, general linear models (GLM) were applied to the segmented FreeSurfer data to detect possible localised atrophy. Power calculations were performed using proc power in SAS with alpha set to 0.05 to calculate the total sample size required to reach a power of 80%.

Results

The baseline characteristics of the entire patient cohort are summarised in Table 1. Patients were dichotomised at baseline according to pre–defined CSF NfH cut-off values[12;13]. Patient characteristics for this biomarker driven dichotomisation are presented for baseline and 15–year follow up.

Patient characteristics at baseline

Five patients had normal and ten had elevated CSF NfH^{SMI35} levels at baseline. For CSF NfH^{SMI34} elevated levels were present in eight and normal in seven patients (Table 2). The disease course was RR in seven, SP in six and PP in two of the patients. The time delay from the last relapse to CSF sampling was long (median 18 months) and not related to CSF NfH^{SMI35} or CSF NfH^{SMI34} levels. Age did not differ significantly between the CSF neurofilament subgroups p=0.84. Patients with high CSF NfH^{SMI35} levels had a smaller BPF (more atrophy) compared to those with normal CSF NfH^{SMI35} levels but this narrowly failed to reach statistical significance (p=0.052, Table 2).

Patients with high CSF NfH^{SMI35} levels had a worse EDSS compared to those with normal CSF NfH^{SMI35} levels (p=0.043).

Table 1. Characteristics of the patients with MS. The median (IQR) or n (%) are shown. Red cell count = RCC, white cell count = WCC, oligoclonal bands = OCB. Type 2 OCB = intrathecal IgG synthesis only, type 4 OCB = more IgG in CSF compared to serum, type 5 OCB = monoclonal band in CSF, MRI = magnetic resonance imaging, BPF = brain parenchymal fraction, CSF = cerebrospinal fluid, NfH = neurofilament heavy chain, EDSS = Expanded Disability Status Scale score. In addition the time interval between the last clinical relapse and sampling of CSF is given in months.

Baseline characteristics	
Female:Male	10:5
Age (years)	45 (35–49)
Age at onset (years)	28.7 (25.4–37.6)
Disease duration (years)	10 (7–20)
Medication	Corticosteroids during relapse n=13 (77%)
	Interferon beta n=5 (33%)
	Endoxan n=1 (6%)
EDSS	4.5 (1.5-6.5)
Last relapse (months)	41 (18–144)
CSF total protein	0.088 (0.075–0.093)
CSF RCC	4 (0–219)
CSF WCC	5 (3–15)
CSF OCB	type 2 n=9 (60%)
	type 4 n=5 (33%)
	type 5 n=1 (7%)
CSF NfH^{SMI35} (pg/ml)	31.0 (147)
CSF NfH^{SMI34} (pg/ml)	11.0 (5–19)
MRI BPF	0.81 (0.79–0.86)

Table 2. High baseline CSF NfH^{SMI35} levels are related to clinical but not MRI parameters. The mean $\pm$ standard deviation and median (range) are shown. * = p$<$0.05, ** = p$<$0.01.

	CSF NfH^{SMI35}		CSF NfH^{SMI34}	
	Normal	High	Normal	High
N	5	10	8	7
Age	42$\pm$8	43$\pm$7	42$\pm$8	44$\pm$7
EDSS	**2.0 (1.5–3.0)**	**6.0 (4.5–6.5)***	4.1 (2.3–5.3)	6.0 (1.0–7.0)
MRI BPF, L	0.84$\pm$0.03	0.79$\pm$0.04	0.81$\pm$0.04	0.81$\pm$0.04

There was no correlation between age and the CSF concentration of either NfH^{SMI35} (R=0.17, p=0.55) or NfH^{SMI34} (R=0.09, p=0.74). There was no correlation of the baseline CSF NfH^{SMI35} or CSF NfH^{SMI34} levels with either the EDSS and the BPF.

Table 3. High baseline CSF NfH^{SMI35} levels predict accelerated CNS atrophy at 15–years follow–up. * = p<0.05, ** = p<0.01. NBV = normalised brain volumes, NGMV = normalised grey matter volumes, NWMV = normalised white matter volumes, MUCCA = mean upper cervical cord area, NDGMV = normalised deep grey matter volumes.

	CSF NfH^{SMI35}		CSF NfH^{SMI34}	
	Normal	High	Normal	High
N	5	10	8	7
Age	56±8	57±7	56±7	58±7
EDSS	**3** (2.5–4.3)	**6.3 (5.6–7.5)****	4.5 (2.9–6.0)	7.5 (3 – 7.5)
Δ EDSS	1.0 (1.0–1.0)	0.25 (0–1.5)	0.75 (0-1.75)	1.0 (0–2.5)
MRI NBV, L	**1.44±0.08**	**1.33±0.07***	1.37±0.10	1.37±0.08
MRI NGMV, L	**0.77±0.04**	**0.69±0.05****	0.72±0.06	0.71±0.05
MRI NWMV, L	0.68±0.05	0.64±0.04	0.65±0.04	0.65±0.44
MRI MUCCA, mm^2	70.34±7.96	62.82±8.03	65.88±7.83	65.02±10.19
NDGMV, mL	59.02±8.70	52.77±3.85	54.89±5.62	54.81±7.58
Accumbens, mL	1.13±0.38	0.85±0.29	0.91±0.27	0.98±0.42
Amygdala, mL	3.71±0.58	3.46±3.71	3.52±0.35	3.58±0.57
Caudate, mL	8.56±1.43	7.49±1.13	7.78±1.34	7.93±1.35
Hippocampus, mL	9.79±1.45	8.40±1.25	8.84±1.48	8.89±1.49
Pallidum, mL	4.70±0.91	3.84±0.44	4.17±0.80	4.08±0.70
Putamen, mL	**12.68±2.22**	**10.69±1.37***	11.32±1.63	11.39±2.28
Thalamus, mL	18.44±2.61	18.03±2.12	18.35±1.98	17.95±2.59
Cortical thickness, mm	2.43±0.05	2.39±0.11	2.38±0.08	2.43±0.11
Frontal, mm	2.51±0.03	2.41±0.09	2.42±0.10	2.46±0.09
Precentral, mm	2.49±0.14	2.36±0.13	2.37±0.16	2.40±0.12
Postcentral, mm	2.01±0.10	2.00±0.11	1.97±0.07	2.03±0.14
Parietal, mm	2.31±0.05	2.31±0.13	2.28±0.07	2.35±0.15
Temporal, mm	2.73±0.15	2.70±0.14	2.68±0.10	2.75±0.17
Occipital, mm	2.00±0.15	2.06±0.12	1.98±0.10	2.11±0.12
Cingulate, mm	2.66±0.04	2.55±0.14	2.59±0.17	2.56±0.10
Insula, mm	3.05±0.05	2.95±0.10	2.98±0.09	2.98±0.11

Patient characteristics at 15 year follow–up

Patients with high CSF NfH^{SMI35} levels at baseline were significantly more disabled at 15 year follow–up compared to those with normal CSF NfH^{SMI35} levels (p=0.01, Table 3). Progression on the EDSS was, however minimal and changes did not reach statistical significance (Table 3). The disease course changed from RR to SP in two of the patients. One patient thought to have a PP disease course at baseline developed clinical and MRI documented relapses and was reclassified as having a SP disease course. This patient was also treated with corticosteroids during relapses.

Brain atrophy was significantly more pronounced in the patients with high CSF NfH^{SMI35} levels compared to those with normal CSF NfH^{SMI35} levels for the NGMV (p=0.007) and the NBV (p=0.014). Significance was not reached when comparing the NWMV (p=0.11) and MUCCA (p=0.12) between patients with high or normal CSF NfH^{SMI35} levels.

No statistical significant relationships were found for any of the demographic, clinical or MRI parameter for the comparison of patients with high or normal CSF NfH^{SMI34} levels (Table 3). Likewise, a subgroup analysis considering the clinical disease course did not reveal any differences, but statistical power was poor for this subgroup analysis (RR n=5, SP n=9). A subgroup analysis was not possible for PP (n=1).

There was an inverse correlation of the absolute concentration of baseline CSF NfH^{SMI35} levels with the follow–up NGMV (Figure 1, Spearman R=-0.62, p=0.014). No significant correlation was found for absolute concentration of CSF NfH^{SMI34} . There were no significant correlations of either neurofilament phosphoform with age or EDSS at follow–up.

The relationship between dichotomised CSF NfH^{SMI35} levels and progression of brain atrophy on MRI is summarised in Figure 2. At baseline 12/15 (80%) of MS patients had a normal BPF. There were signs of atrophy only in 3/15 (20%) all of whom had high CSF NfH^{SMI35} levels (Figure 2, red dots in yellow shaded area). At 15–year follow–up six patients with high CSF NfH^{SMI35} levels developed more pronounced atrophy of the NGMV (Figure 2, red dots in blue shaded area). Four patients with normal CSF NfH^{SMI35} levels remained within the normal range for age (Figure 2, green triangles in white area). Taken together high baseline CSF NfH^{SMI35} levels have an odds-ratio of 36 (95%CI 1.8-731.6, p=0.0067) for more pronounced NGMV atrophy after 15–years. If the three cases with manifest brain atrophy at baseline are excluded the odds ratio is 28 (95%CI 1.4-580.6, p=0.0149).

There was significant more atrophy of the putamen in patients with high baseline CSF NfH^{SMI35} levels compared to those with normal CSF NfH^{SMI35} levels ($p<0.05$, supplementary Table 1). For all other regions of interest statistical significance was not reached probably due to small group size. Consistently, however smaller average thickness data, suggestive of localised atrophy were observed in patients with high CSF NfH^{SMI35} levels for the accumbens, amygdala, caudate, hippocampus, pallidum and thalamus (supplementary Table 1). Likewise absolute cortical thickness values were smaller, suggesting localised atrophy, in patients with high CSF NfH^{SMI35} levels for the frontal, precentral, postcentral, temporal areas, the cingulate and insula.

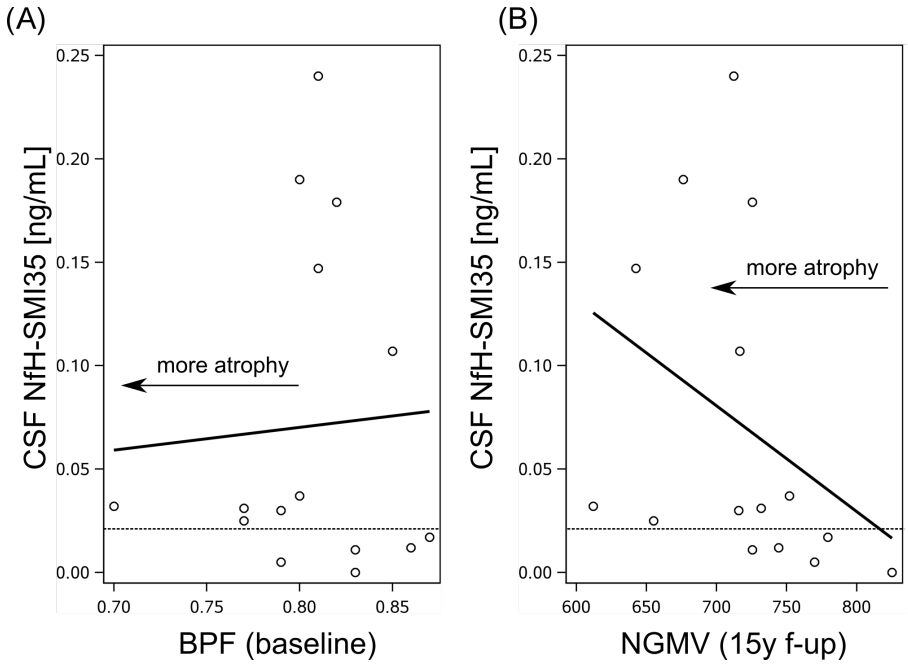

Figure 1. Brain atrophy and CSF NfH^{SMI35} levels. **(A)** Higher CSF NfH^{SMI35} levels were seen in those patients with a higher BPF indicating absence of significant brain atrophy at baseline (Table 2). **(B)** 15 years later those patients with high CSF NfH^{SMI35} levels developed more pronounced atrophy of the NGMV ($p<0.01$, Table 3) which also correlated inversely with the absolute CSF NfH^{SMI35} levels ($R=-0.62$, $p=0.014$). The dashed horizontal reference line indicates the predefined CSF NfH^{SMI35} cut–off value of 20 pg/mL [12].

Power calculations for detecting localised atrophy

Power calculations were performed to estimate how many patients were needed to test whether or not there is a statistical relationship between dichotomised baseline CSF NfH levels and localised atrophy on MRI. Table 4 summarises the actual power of the present study and numbers needed to reach a desired power of 80%.

The power calculations are grouped into those areas which will be potentially be possible testing given a original cohort size of n=51 and those were testing will likely be futile.

A sample size of $n < 51$ is sufficient to test the predictive value of dichotomised baseline CSF NfH^{SMI35} levels for atrophy of the MRI NMV, NGMV, MUCCA, hippocampus, pallidum, putamen, frontal lobe, precentral cortical thickness, cingulate and insula.

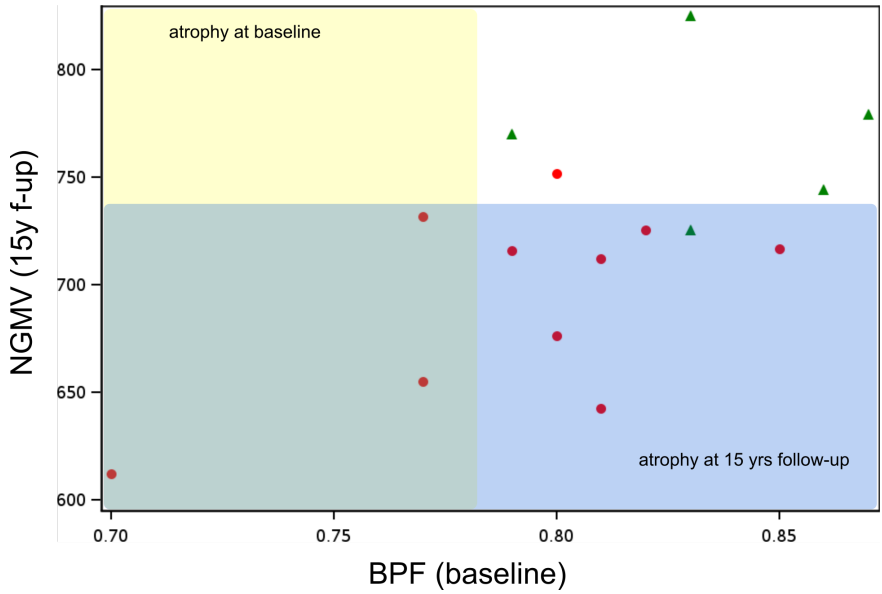

Figure 2. Progression of atrophy between baseline (yellow shaded area) and 15–year follow–up (blue shaded area) in relation to dichotomised CSF NfH^{SMI35} levels (high = red dots, normal = green triangles).

Discussion

The long–term follow–up data of this cohort are unique because they provide evidence that development of brain atrophy is preceded by rise of a clinically relevant biomarker for axonal damage. To better develop the argument one has to recall the principal hypothesis underlying this research. An increase of CSF neurofilament levels was thought to reflect on axonal disintegration[3;5;25]. Logically, therefore one would expect that those patients who suffer axonal degeneration at baseline also suffer from disability as axonal loss is hold responsible[26]. The inflammatory plaques found in MS can however not only cause axonal degeneration, but also demyelination and conduction block from which patients may recover. To be attributable to axonal degeneration disability will therefore need to be sustained, which it was in this study. To close the argument one needs to demonstrate that elevated CSF neurofilament levels also relate over time to development of more severe brain and or spinal cord atrophy. This relationship we were unable to demonstrate at baseline and three year follow–up[11;12]. Only 15–years later the association between rise of CSF neurofilament levels and subsequent brain atrophy emerged.

Table 4. Sample sizes required to test the predictive value on dichotomised CSF NfH^{SMI35} levels on subsequent localised CNS atrophy. The original size of this cohort was n=51. Power calculations were based on alpha=0.05 for a two–sample t Test.

MRI Readout	Actual power	Desired power	Numbers required
MRI NBV, L	61%	80 %	21
MRI NGMV, L	84%	80 %	14
MRI NWMV, L	27%	80 %	52
MRI MUCCA, mm^2	33%	80 %	43
NDGMV , mm	24%	80 %	55
Accubens , mm	24%	80 %	59
Amygdala , mm	5%	80 %	>100
Caudate , mm	24%	80 %	59
Hippocampus , mm	36%	80 %	39
Pallidum, mm	37%	80 %	35
Putamen, mm	34%	80 %	39
Thalamus, mm	6%	80 %	>100
Cortical thickness, mm	14%	80 %	>100
Frontal, mm	82%	80 %	14
Precentral, mm	33%	80 %	41
Postcentral, mm	5%	80 %	>100
Temporal, mm	6%	80 %	>100
Cingulate, mm	56%	80 %	25
Insula, mm	65%	80 %	20

There is a debate on the time course of axonal degeneration in the central nervous system (CNS). The relative timing of these cellular events differs considerably between the CNS and peripheral nervous system[27;28]. In general terms the CNS response is much slower[29–31]. In MS there is emerging evidence that this is related to presence of trans–synaptic axonal degeneration[14;32]. The association of development of disability related to long tract spinal cord axons[13] suggests that the dominant source of CSF NfH^{SMI35} levels at baseline is likely to be of spinal cord origin. Therefore axonal damage far remote from the brain grey matter would be a convenient explanation for high CSF NfH^{SMI35} levels at baseline, association with functional system disability on the EDSS and a long time lag through trans–synaptic axonal degeneration until brain grey matter atrophy becomes manifest on MRI.

Conceptionally comparable, but and on a much shorter time scale, this association was already shown for the visual system. An elevation of plasma NfH^{SMI35} had been associated with poor visual acuity following optic neuritis and preceded optic disc pallor on funduscopy[33]. This clinical observation has since been confirmed quantitatively using optical coherence tomography[34]. But then distance from the optic nerve to the

brain is much closer compared to the distal spinal cord, substantially shortening time requirements for trans–synaptic axonal degeneration.

Importantly, the power calculations preformed suggest that it will be possible testing the hypothesis that dichotomised baseline CSF NfH^{SMI35} levels may be predictive of more localised CNS atrophy (Table 4). The association with the putamen, where motor fibres interconnect has been shown in this study. Extending on present finding, it would be particularly interesting testing a likely association of high baseline CSF NfH^{SMI35} levels with atrophy of the precentral gyrus (Brodman area 4) as the pathway of trans–synaptic axonal degeneration would predict. Another interesting new finding was the unexpected strong association of CSF NfH^{SMI35} levels with frontal lobe atrophy. This has not previously been reported in MS, but was earlier brought out be a systematic review on the CSF neurofilament data in dementia[35]. Impairment of frontal lobe function and cognition is also an important feature of MS pathology.

This study did not find CSF Nf levels to be influenced by factors previously reported in the literature, such as time from last relapse or disease course. This former was most likely due to the long delay between relapse on CSF sampling, far exceeding the reported crucial three months. The latter might be a negative finding due to the low statistical power for subgroup analyses of clinical groups. Of note those patients with high CSF NfH^{SMI35} levels had a, non-significant, lower MRI BPF at baseline compared to those with normal CSF NfH^{SMI35} levels. Also significance was missed in this study, this is likely related to power.

In the present study findings were only significant for CSF NfH^{SMI35} but not for CSF NfH^{SMI34} . The argument might be valid that this could only be a statistical phenomenon. A known autoimmune phenomenon might also be considered[25]. Presence of auto-antibodies against NfH can cover epitopes relevant to detection by immunological techniques. Because hyperphosphorylation of tissue NfH has been related to pathology in many neurodegenerative diseases (reviewed in[25]) further research in this field is warranted.

There are however important limitations to the present study. First there might be a potential bias towards more severely disabled patients. At both time points those patients with high CSF NfH^{SMI35} levels also were those which had a poorer EDSS. From a biomarker point of view this bias might be expected because of the association between high CSF neurofilament levels and disability[3;5;25]. From a clinical point of view one would like to see these results confirmed in a cohort of less severe disabled patients and also more rapidly progressing patients prior to extrapolating from present data to patients

with MS in general. Likewise, the development of MRI techniques from 1.5 T to 3 T in this study did not permit to calculate progression on the same variable. This will remain a problem as the average life time of a magnet is about 10 years and therefore short of the long–term clinical follow–up time. Another potential bias is the drop out of patients from the original cohort. This drop out was at least in part related to the request for a second lumbar puncture at three years follow–up[12]. This bias might also be related to the trend of current diagnostic criteria relying on non invasive examinations, with MRI representing the current consensus cornerstone of MS diagnostics. We would be hesitant to draw any conclusion from potential effect of treatment on brain atrophy from this small cohort with heterogenous treatments streched over a long time. The drop-out also reduced the power of the study such that our attempts to investigate for more localised atrophy, for example in the primary motor cortex failed to reach statistical significance. This important limitation is further highlighted by the power calculations. The larger standard deviation for the MUCCA already exemplifies to what extend the accuracy of a method impacts on the required sample size. Compared to the tighter, smaller standard deviation, data for brain volumes requiring only about 21 patients, 43 are needed to be in a powerful enough situation to test for the hypothesised association between high baseline CSF NfH^{SMI35} levels and subsequent spinal cord atrophy. These limitations discussed, the present study was sufficiently powered (power 84%, alpha 0.05) to test for a relationship between dichotomised baseline CSF NfH^{SMI35} levels and NGMV atrophy.

Taken together the 15 year follow up data strengthen the argument that elevation of CSF neurofilament protein levels in patients with multiple sclerosis are related to axonal loss responsible for sustained disability and precede CNS atrophy by abuot 3 to 15 years (Figure 3).

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Declaration of conflicting interests

The authors declare no conflict of interests.

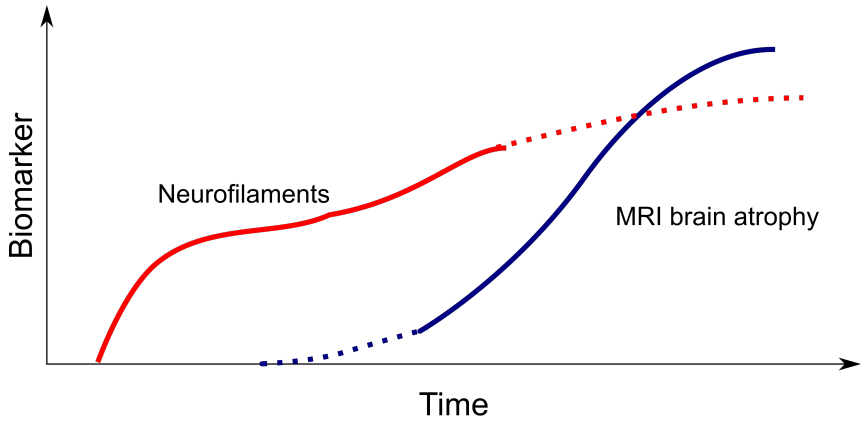

Figure 3. Model suggesting that development of disability related to axonal degeneration directly causes an increase of CSF neurofilament levels. Elevated neurofilament levels precede global brain atrophy on MRI.

Supplemental material

Supplementary data available from the Journal's website.

Table S1 Localised brain atrophy of FreeSurfer data at 15-years follow up for dichotomised CSF NfH phosphoform data at baseline The median (IQR) are shown, *= $p<0.05$.

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