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Original Research Paper

Neurofilaments and 10-year follow-up in multiple sclerosis

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Abstract

Background: The role of biomarkers to predict clinical outcome in multiple sclerosis (MS) is still debated.
Objective: To test whether cerebrospinal fluid (CSF) light-chain neurofilament (NfL) levels in newly diagnosed patients with MS could predict clinical outcome over a 10-year period.
Methods: Patients with newly diagnosed MS underwent standardized clinical assessments at baseline and 5 and 10 years of follow-up. Expanded Disability Status Scale (EDSS) progression between assessments was defined as an increase in one point or more if <6 and 0.5 or more if ≥6. CSF obtained at baseline was analyzed for levels of NfL using enzyme-linked immunosorbent assay technology.
Results: A total of 44 patients were included. In all, 35 patients (80%) had relapsing–remitting multiple sclerosis (RRMS). Patients who progressed in EDSS showed a trend for higher median baseline CSF-NfL levels than patients who did not progress after 5 years (947 ng/L vs 246 ng/L, $p = 0.05$), and although not statistically significant, after 10 years (708 ng/L vs 265 ng/L, $p = 0.28$). Patients who converted from RRMS to secondary-progressive multiple sclerosis (SPMS) at 5 years had a statistical significant higher median CSF level of NfL (2122 ng/L vs 246 ng/L, $p = 0.01$).
Conclusion: CSF levels of NfL at the time of diagnosis seems to be an early predictive biomarker of long-term clinical outcome and conversion from RRMS to SPMS.

Keywords: Multiple sclerosis, biomarkers, neurofilaments, cerebrospinal fluid, disease progression, magnetic resonance imaging

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Introduction

Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS), characterized by inflammatory demyelination with axonal loss and subsequent loss of function.[1] Onset of the disease is frequently in early adulthood and is more predominant among females. Norway is a high-risk area with a prevalence of above 200 per 100,000 inhabitants.[2] Disease severity is only moderately correlated to magnetic resonance imaging (MRI) findings with notably white matter lesions on T2 sequences.[3,4] During the last years, it has become evident that permanent disability is associated with axonal loss and atrophy of the brain and the spinal cord. Clinical and laboratory research through years still have not found clinical useful prognostic markers for the disease course of the individual patient, but clinical parameters have shown value on group level.[5] Neurofilaments are important parts of the cyto-axonal cell structure as they constitute a major component of the axoskeleton. There is increasing evidence that neurofilaments can be regarded as a biomarker for neuro-axonal damage, as axonal destruction results in disintegration of the axon membrane and neurofilament breakdown and subsequent release into the cerebrospinal fluid (CSF), where it can be quantified.[6,7] Neurofilaments are subdivided into light chains (NfL), medium chains, and heavy chains (NfH) according to protein size. NfL has been found to represent early axonal damage and has been shown to be modulated by disease-modifying agents like natalizumab.[8] NfH has been regarded as a possible prognostic marker for chronic disability as

measured by the Kurtzke Expanded Disability Status Scale (EDSS).[9,10] Both NfH and NfL seem to be associated with gadolinium-enhanced MRI lesion activity, and recent findings indicate that an increase in serum NfL may predict the appearance of such lesions.[11] However, the relationship between neurofilament levels in CSF and long-term disease progression has not been extensively examined yet.

Our objective in this prospective study was therefore to evaluate whether CSF-NfL levels in newly diagnosed MS patients could predict clinical outcome over a 10-year period.

Materials and methods

Participants

All participants were derived from a prospective population-based longitudinal cohort study of patients with newly diagnosed MS in Western Norway. The study design has been described elsewhere in detail.[3] In brief, we sought to recruit all patients with a first-time diagnosis of MS during 1998 to 2000 in a well-defined geographical area comprising the county of Hordaland and southern parts of the county of Rogaland, Western Norway. A total of 108 patients were identified in the study area. In all, 3 had moved out of the study area, 1 had died, and 11 refused to participate, leaving 93 patients available for the study. Out of those, 44 patients provided CSF at baseline (Figure 1). A total of 43 were re-assessed after 5 years and 37 after 10 years. All patients met diagnostic criteria of Poser[12] and provided written informed consent in study participation. The study has been approved by the Western Norway Regional Committee for Medical and Health Research Ethics.

Clinical assessment

Clinical assessment at baseline, at 5-year, and 10-year follow-up included a full neurological examination with EDSS scoring, and recording of disease duration, time since last attack, classification of subtype of disease, use of MS therapies, comorbidities, and concomitant medication, as well as sociodemographic variables including education level and marital status. EDSS progression was defined as an increase in one point or more between the assessments if initial EDSS was under 6.0 or 0.5 points or more if initial EDSS was 6.0 or higher.

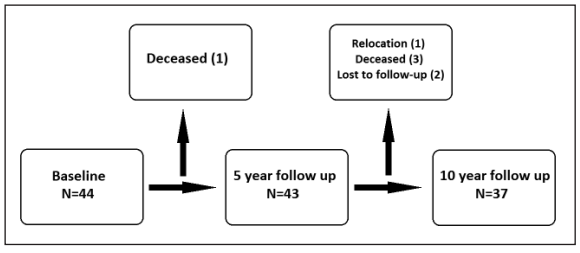

Figure 1. Flow chart of patient inclusion at baseline, 5-year, and 10-year follow-up.

CSF handling and NfL measurements

The CSF was routinely analyzed for cells and oligoclonal bands and then kept frozen at −70°C and had gone through one freeze–thaw cycle before we performed our study. We used the commercially available UmanDiagnostics NF-light® (Umeå, Sweden) enzyme-linked immunosorbent assay (ELISA), which was performed according to the kit instructions, and is also described elsewhere.[13] Repeated freeze–thaw cycles have been shown to not have a considerable effect on the measurement of NfL.[14] Intra-assay coefficients of variation were below 15%, and inter-assay coefficients of variation were below 10%.

Statistical analysis

Statistical analyses were performed using SPSS version 23 (IBM Corp., Armonk, NY, USA). Means are presented for variables showing normality, while medians for variables showing non-normality. Due to small numbers non-parametric tests such as Kruskal–Wallis and Mann–Whitney *U* test were used. Comparisons of categorical variables were done using the chi-square test. Relationship between continuous variables was evaluated using the Spearman correlation coefficient. The association between baseline NfL levels and the longitudinal measures of EDSS was assessed by generalized estimating equations (GEE) analysis. The exchangeable working correlation structure was deemed optimal by the Quasi-likelihood under Independence Model Criterion (QIC) for the full model, thus was used throughout. Log transformation of the NfL values did not improve the model, as assessed by the Corrected Quasi-likelihood under Independence Model Criterion (QICC). Spaghetti plots were created using R version 3.4.1 with package *lattice*. Estimated marginal mean trajectories for specified NfL values were obtained from the GEE solution.

Results

Clinical and demographic characteristics

A total of 44 patients were included at baseline, with a mean age of 41.9 years (standard deviation (SD) 9.6), and 30 (68%) females. Clinical characteristics are shown in Table 1.

Table 1. Demographics and clinical characteristics.

	Baseline *N*=44	5 year *N*=43	10 year *N*=37
Age in years at baseline, mean (SD)	41.9 (9.6)	41.8 (9.7)	40.8 (9.0)
Female, *n* (%)	30 (68)	30 (70)	25 (68)
NfL in ng/L at baseline, median (IQR)	413 (1276)	369 (1149)	310 (1043)
Disease duration in months at baseline, median (IQR)	60 (132)	60 (132)	60 (90)
Months since last attack at baseline, median (IQR)	9.0 (15)[a]	8.5 (15)[a]	10 (16)[b]
EDSS, median (IQR)	3.5 (2.0)	3.5 (2.5)	3.5 (4.0)
Disease course, *n* (%)			
RRMS	35(80)	29 (67)	21 (57)
SPMS	7 (16)	12 (28)	12 (32)
PPMS	2 (4)	2 (5)	4 (11)
Patients on DMT, *n* (%)	7 (16)	18 (42)	20 (54)
Interferons	6	12	9
Glatiramer acetate	1	5	5
Mitoxantrone		1	1
Natalizumab			4
Fingolimod			1

NfL: neurofilament; SD: standard deviation; IQR: interquartile range; RRMS: relapsing–remitting multiple sclerosis; PPMS: primary-progressive multiple sclerosis; SPMS: secondary-progressive multiple sclerosis; DMT: disease-modifying therapy.
[a]Seven patients missing.
[b]Five patients missing.

Baseline NfL CSF levels and conversion to secondary-progressive multiple sclerosis

Out of 44 patients, 35 (80%) had a relapsing–remitting course of disease at baseline, and the proportion of patients with relapsing–remitting multiple sclerosis (RRMS) decreased during the study period. At the 5-year follow-up, 29 patients remained with RRMS, whereas 6 patients had converted to secondary-progressive multiple sclerosis (SPMS). Patients who converted had a statistical significant higher median baseline NfL concentration of 2122 ng/L (interquartile range (IQR) 1921 ng/L) compared to non-converters at 246 ng/L (IQR 885 ng/L, $p=0.01$) (Figure 2). Throughout the study period of 10 years, patients who remained with RRMS ($n=21$) had a lower median baseline concentration of NfL of 265 ng/L (IQR 943) compared to those patients ($n=10$) who converted to SPMS of 926 ng/L (IQR 2357), although this was not significant ($p=0.25$).

Analyzing the different subgroups of MS patients, median NfL CSF levels did not differ between patients with RRMS, primary-progressive multiple sclerosis (PPMS), and SPMS at baseline ($p=0.57$), but the RRMS group were younger (42.5 vs 37.5 years, $p=0.12$) and had lower median EDSS at baseline ($p=0.03$) (Table 2). Male patients had higher concentrations of NfL with a median of 1388 ng/L (IQR 2262) compared to 245 ng/L for female patients (IQR 788, $p<0.01$). We found a statistical non-significant trend that NfL levels declined with increasing disease duration at baseline ($r=-0.28$, $p=0.06$), as well as with increasing age at baseline ($r=-0.27$, $p=0.08$), and declining NfL levels with increasing time interval since last attack ($r=-0.05$, $p=0.38$).

Baseline CSF-NfL levels and EDSS progression after 5 and 10 years

GEE analyses indicated that higher baseline NfL levels predicted a steeper trajectory in EDSS progression during the follow-up of 10 years than did lower NfL levels (Figure 3). Expected increase in EDSS per 5 years was 0.47 greater per 1000 ng/L increase in NfL level at baseline, which was statistically significant, and did not change after adjusting for gender, age at diagnosis, and disease duration (95% confidence interval (CI) 0.25–0.69, $p<0.01$). A higher baseline NfL also predicted higher EDSS at baseline by 0.33 (95% CI 0.13–0.52) per 1000 ng/L increase in NfL, and adjusted for gender, age at diagnosis, and disease duration, the association was even stronger at 0.57 (95% CI 0.34–0.79, $p<0.01$) (Table 3).

EDSS progression at the 5-year follow-up showed a trend that those who progressed had a higher median

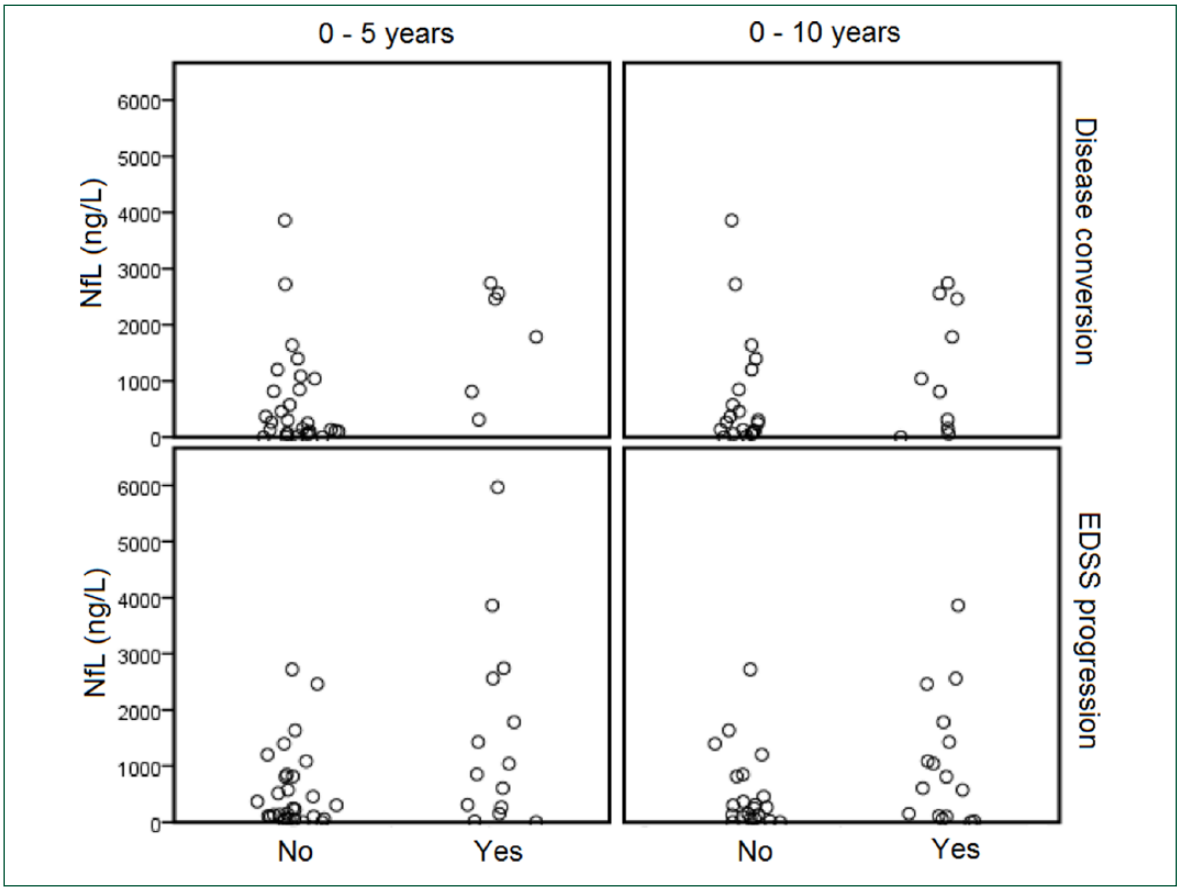

Figure 2. Observed NfL values at baseline for disease converters from RRMS to SPMS and EDSS progression at 5 and 10 years, defined as an increase in one point or more between the assessments if initial EDSS was under 6.0, or 0.5 points or more if initial EDSS was 6.0 or higher. Out of 35 patients, 6 converted to SPMS by 5 years, and these patients had a statistical significant higher median baseline NfL than the non-converters ($p=0.01$). By 10 years, 10 out of 31 patients who had converted also had higher median baseline NfL than non-converters, but this was not significant ($p=0.25$). At 5 years, 14 out of 43 patients had progressed, and they had higher median baseline NfL than the non-progressors ($p=0.05$). At 10 years, 16 out of 37 patients had progressed. They also had higher median baseline NfL than the non-progressors, but this difference was not significant ($p=0.28$).

NfL level at baseline (947 ng/L, IQR 2369, $n=14$) compared to those without progression (246 ng/L, IQR 751, $n=29$, $p=0.05$). Although not statistical significant, it was also a difference at the 10-year follow-up, with higher median NfL level among patients who progressed (708 ng/L, IQR 1592, $n=16$) compared to those without progression (265 ng/L, IQR 750, $n=21$, $p=0.28$).

There were no statistically significant differences in NfL levels ($p=0.22$), EDSS scores ($p=0.26$), or disease duration at baseline ($p=0.66$) between patients who were lost at the 10-year assessment and patients who remained in the study throughout the study period.

Discussion

We found that baseline CSF-NfL levels in newly diagnosed MS patients predicted the clinical outcome as measured by EDSS and conversion from RRMS to SPMS at 10-year follow-up. Limited data on NfL and long-term follow-up in MS are available, but our results are in line with another cohort followed for a median time period of 14 years.[15] NfL is thought to reflect ongoing axonal degeneration, which dominates early in the disease phase, and our results support that increased early disease activity as identified by increased levels of CSF-NfL has a prognostic effect several years later. This emphasizes the need of early diagnosis and early treatment initiation. There were some indications of nonlinear effects of NfL, in which the association to EDSS progression was more marked for higher levels of NfL; however, the sample size limited further analyses of this issue.

We found a statistical significant correlation between NfL levels at baseline and EDSS progression and conversion from RRMS to SPMS at the 5-year follow-up,

Table 2. NfL and EDSS according to gender, disease course, and age association at baseline.

	NfL (ng/L), median (IQR)	*P* value	EDSS, median (IQR)	*P* value
Gender				
Male ($n=14$)	1388 (2262)	<0.01	3.5 (3.0)	0.85
Female ($n=30$)	245 (788)		3.5 (1.6)	
Disease course				
RRMS ($n=35$)	310 (1102)	0.57	3.5 (2.0)	0.03
SPMS ($n=7$)	515 (1224)		4.0 (1.5)	
PPMS ($n=2$)	1018		4.8 (-)	
Age				
≤35 ($n=12$)	1122 (2333)	0.16	2.5 (2.0)	<0.01
36–49 ($n=20$)	413 (1172)		3.0 (2.4)	
≥50 ($n=12$)	236 (526)		3.75 (1.5)	

NfL: neurofilament; IQR: interquartile range; RRMS: relapsing–remitting multiple sclerosis; PPMS: primary-progressive multiple sclerosis; SPMS: secondary-progressive multiple sclerosis.

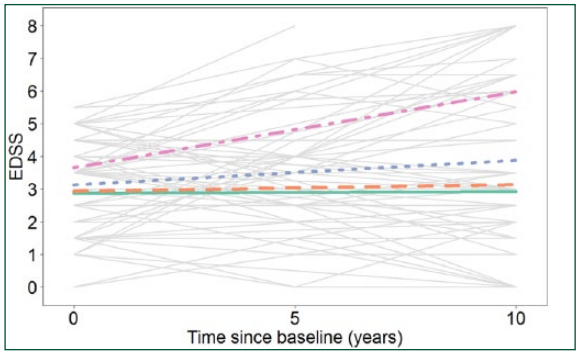

Figure 3. GEE model–based predictions for EDSS development (*Y*-axis) through 10-year follow-up (*X*-axis) given measured NfL concentration in CSF at baseline. The colored lines represent the median EDSS of the four quartiles of NfL concentrations, that is, 56 ng/L (green/solid), 225 ng/L (orange/dashed), 817 ng/L (blue/dotted), and 2461 ng/L (purple/dotted-dashed). The individual trajectories of EDSS over time for the 44 patients included at baseline are shown in gray.

but a weaker correlation at the 10-year follow-up. This may be due to the increasing number of patients on disease-modifying therapy (DMT) throughout the study period, as only 16% received therapy at baseline but 54% at 10-year follow-up. Previous studies have shown that several DMTs that reduce the relapse rate and new MRI disease activity are associated with less axonal destruction and lower NfL levels.[16,17]

We found a median NfL level of 310 ng/L (IQR 1102) among the 35 patients with RRMS at baseline. Among the 18 patients who had lower NfL values than this, only 1 (6%) converted to SPMS by 5 years, whereas 5 out of 17 (29%) of those with above median NfL converted ($p=0.09$). Likewise, at 10 years, we found that 4 out of 18 patients (22%) with lower than median NfL concentration had converted from RRMS to SPMS, while 6 out of 17 (35%) of patients in the high-NfL group converted ($p=0.47$). In our opinion, early initiation of effective DMT for this group would be particularly advisable to avoid disease progression. As more data will be available in the future, narrowing down on reliable cut-off values for CSF-NfL measurements could be vital in utilizing NfL as an aid for clinicians in choosing correct level of therapy for MS patients.

Our patients were diagnosed according to the Poser criteria and had relatively long disease duration at diagnosis, with a median duration of 60 months. The current use of the revised McDonald criteria[18] would probably reduce this pre-diagnostic phase and may also influence the NfL levels as we and others have shown that NfL levels decrease as disease duration increases.[19]

Male patients had a statistically significantly higher median CSF concentration of NfL compared to females. They were also younger and had shorter disease duration at baseline with a median time of 48 months compared to females with a median of 60 months. Still, median EDSS was similar across genders at 3.5. As mentioned, NfL is a product of axonal degradation which is more prominent in the early inflammatory phase of the disease course, and thus, both age and disease duration may explain the observed gender difference.

We have only analyzed baseline levels of CSF-NfL as lumbar puncture is an invasive procedure requiring an

Table 3. GEE model parameter estimates.

	Model 1		Model 2[a]	
	β (95% CI)	*p* value	β (95% CI)	*p* value
Time (per 5 years)	−0.00 (−0.29 to 0.29)	0.99	0.01 (−0.28 to 0.30)	0.94
NfL (per 1000 ng/L)	0.33 (0.13 to 0.52)	<0.01	0.57 (0.34 to 0.79)	<0.01
NfL*time interaction	0.47 (0.25 to 0.69)	<0.01	0.47 (0.25 to 0.68)	<0.01

GEE: generalized estimating equations; CI: confidence interval; NfL: neurofilament.
[a]Model 2 includes adjustment for sex, age at diagnosis, and disease duration.

experienced examiner, resources including time and facilities to perform the examination, and the patients' goodwill to undergo the procedure. The need for repeated lumbar puncture may therefore restrict the use of CSF-NfL as a disease marker in a long-term perspective. However, recent studies have shown the ability to measure NfL in serum, which opens up to possibilities of more readily assessment of longitudinal NfL measurements.[20–24] But, as lumbar puncture usually is a part of the diagnostic procedure, a baseline measurement of NfL in CSF may add some prognostic information and help to sort out the patients who should start high-efficacy DMT as early as possible.

MRI is currently the most common used diagnostic and monitoring tool of disease activity in MS but has limited correlation to disability progression as a clinical outcome.[25,26] At present, there are no non-imaging biomarkers routinely in use to monitor MS. Our study gives further support for an association between CSF-NfL levels and future disability from MS and thereby the use of CSF-NfL as a routinely used prognostic marker.

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