Research Paper

Multiple Sclerosis

Multiple Sclerosis
16(3) 287–292

DOI: 10.1177/1352458509359725
msj.sagepub.com
SAGE

Neurofilament light as a prognostic marker in multiple sclerosis

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Abstract

Relapsing-remitting multiple sclerosis has a variable prognosis and lacks a reliable laboratory prognostic marker. Our aim in this study was to investigate the association between neurofilament light levels in cerebrospinal fluid in early multiple sclerosis and disease severity at long-term follow-up. Neurofilament light levels in cerebrospinal fluid collected at diagnostic lumbar puncture were measured in 99 multiple sclerosis cases. Clinical data were obtained from 95 out of those at follow-up visits made 14 years (range 8–20 years) after disease onset. Significant correlations between neurofilament light levels and the multiple sclerosis severity score were found for all cases ($r = 0.30$, $p = 0.005$), for relapsing-remitting multiple sclerosis cases ($r = 0.47$, $p < 0.001$) and for cases with a recent relapse ($r = 0.60$, $p < 0.001$). In the multivariate logistic regression analysis, neurofilament light levels >386 ng/L (median value of cases with detectable levels) increased the risk for severe multiple sclerosis fivefold (odds ratio 5.2, 95% confidence interval 1.8–15). Kaplan–Meier analysis showed that conversion to secondary-progressive multiple sclerosis was more likely in cases with neurofilament light levels >386 ng/L than in those with neurofilament light levels <60 ng/L ($p = 0.01$) or 60–386 ng/L ($p = 0.03$). We conclude that elevated levels of neurofilament light in cerebrospinal fluid collected at diagnostic lumbar puncture were associated with unfavourable prognosis. These data suggest that the neurofilament light level could be used as a prognostic marker in early relapsing-remitting multiple sclerosis.

Keywords

Multiple sclerosis, cerebrospinal fluid markers, neurofilament light, NFL, prognosis

Date received: 29th September 2009; accepted: 6th December 2009

Introduction

Multiple sclerosis (MS) is regarded as a chronic autoimmune disease of the central nervous system (CNS) causing demyelination. Typically, it starts as a relapsing-remitting disease with inflammatory attacks causing CNS dysfunction that resolve within days to months. Later in the course of the disease a secondary progressive phase occurs in most individuals, with a continuous deterioration of CNS function.[1] The prognosis is highly variable, depending on many different factors. A strong indicator of poor prognosis is primary progression (progression from onset) or, among cases with relapsing-remitting MS (RRMS), short time to secondary progressive MS (SPMS).[2] Other markers for poor prognosis are: (1) young age at disease onset (slower progression but younger age at disability landmarks); (2) early motor, cerebellar and sphincter dysfunction; (3) frequent relapses early; (4) early persisting disability; and (5) cigarette smoking.[3–6] However, none of these are very accurate at predicting outcome on an individual basis. This means that we lack a good prognostic marker for people with RRMS, who generally are the ones eligible for disease-modifying treatment (DMT). With the emergence of new drugs with higher efficacy but potentially serious side effects, we need to carefully select the most suitable treatment for each patient. For that purpose, a reliable prognostic marker is essential.

Neurofilament light protein (NFL) is an intracellular protein supplying structural strength to neurons. During axonal damage, NFL is degraded by a calpain-mediated pathway and secreted into the cerebrospinal fluid (CSF).[7] While healthy individuals have no NFL in their CSF, most people with neurological disorders, such as amyotrophic lateral sclerosis, stroke, MS and Alzheimer's disease, have elevated levels.[8] In MS, approximately 80% of the cases have elevated levels of NFL. The levels seem to increase during a relapse of the disease and then gradually decrease with time until the next relapse.[9]

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In 2004, Norgren et al. published a study in which 99 patients with MS in an early phase of the disease had been tested for levels of NFL in the CSF.[10] As a measure of disease severity, progression index (PI) was calculated by dividing the current degree of disability using Kurtzke's expanded disability status scale (EDSS) by the duration of the disease in years. At a median of five years (median year 1998) after disease onset, significant correlations between PI and NFL levels was found (Spearman's correlation coefficient, $r = 0.52$ for those with a relapse within 3 months before lumbar puncture (LP), and $r = 0.29$ for those without). The multiple sclerosis severity score (MSSS)[11] is an algorithm used to assess disease severity based on the EDSS and disease duration. It has advantages over PI such as being more stable longitudinally and being more accurate when comparing disease severity using single assessment data.

Our hypothesis was that long-term prognosis as estimated by MSSS and risk for conversion from RRMS to SPMS can be predicted by NFL levels early in the disease.

Methods

The MS cases had been identified in an epidemiological study estimating the incidence (1988–1997) of MS in Västerbotten County in Sweden.[12] CSF collected between 1989 and 2002 was available for 99 out of 133 cases from the incidence population. The levels of NFL in these 99 cases was retrospectively measured as reported in a previous study[10] with an in-house developed enzyme-linked immunosorbent assay described elsewhere.[8] The sensitivity of the assay was 60 ng/L.

In this follow-up report, we were able to include 95 of the 99 cases from the previous study (Table 1). Information on EDSS enabling calculation of the MSSS was retrospectively collected from medical records and the Swedish Multiple Sclerosis Register ($n = 72$ and $n = 3$, respectively). In cases where recent (≤ 2 years) information on EDSS was not available from these sources, an extra follow-up visit with neurological examination ($n = 15$) or telephone interview ($n = 5$) was performed by the first author. For 89 cases the exact EDSS was established. In the remaining six cases (five interviewed by telephone, one with data from medical records), it was not possible to establish the exact EDSS; however, a value of less than 3 or less than 4 was assigned based on the available information. Together with the disease duration, it was possible to establish the maximum possible MSSS. These six cases were included in the logistic regression analyses where MSSS was dichotomized (all six cases were below median), but not in the correlation analyses where the exact MSSS was needed. Data on smoking habits were available from a local MS database and in some cases updated at extra follow-up visits and telephone interviews. Smoking habits were known for 94 cases. Data on past and current immunomodulatory treatment were available from medical records. Information from follow-up interviews and examinations were used together with medical records to determine whether cases had converted from RRMS to SPMS. The term 'recent relapse' was defined as a relapse within 3 months before time of LP, and 37 of the followed-up cases had experienced such an event. The female-to-male ratio was 1.8 (61 : 34).

Table 1. Characteristics of 95 multiple sclerosis cases at diagnostic lumbar puncture and after a median of 14 years disease duration

	Lumbar puncture		Follow-up	
Number of:				
Clinical subtype				
Relapsing-remitting	65		39	
Secondary progressive	10		36	
Progressive from onset	20		20	
Clinical characteristics:				
Median disease duration (range)	3	(0–10)	14	(8–20)
Median age (range)	34	(14–63)	49	(27–73)
Median MSSS (range)			3.25	(0.13–10)
Median EDSS (range)			3.5	(0–10)
Performed year:				
Median (range)	1996	(1989–2002)	2007	(2002–2008)

EDSS, Kurtzke's expanded disability status scale; MSSS, multiple sclerosis severity score.
Data on disability were not recorded at lumbar puncture.

The associations between MSSS and NFL were analysed using Spearman's correlation coefficient. The risk for high MSSS (above median among 95 cases) was estimated in bivariate and multivariate logistic regression analyses in terms of odds ratios (ORs) and 95% confidence intervals (CIs). The variables that were statistically significant in the bivariate analysis were included in the multivariate analysis. Three NFL categories were used: high (>386 ng/L, above median among those with detectable levels), intermediate (60–386 ng/L) and undetectable (<60 ng/L). For the logistic regression analysis, the intermediate and undetectable categories were merged. Kaplan-Meier curves were used to illustrate the conversion from RRMS to SPMS using the same NFL categories as above. The Log Rank test (Mantel–Cox) was used to test whether the differences between the curves were due to random variation or not. The Mann–Whitney test was performed to test the difference between median values. Only cases with MSSS estimates were included in the comparison between median values at 14- and 5-year follow-up.

Results

At long-term follow-up, there were significant correlations between NFL levels at diagnosis and MSSS among all MS cases ($r = 0.30$, $n = 89$, $p = 0.005$), among cases with RRMS ($r = 0.47$, $n = 59$, $p < 0.001$) and among cases with a recent relapse ($r = 0.60$, $n = 34$, $p < 0.001$) (Figure 1). There was also a significant correlation found among cases with progressive disease from onset ($r = 0.47$, $n = 20$, $p = 0.04$), but when analysing only the cases without a recent relapse, no significant correlation was found ($r = 0.25$, $n = 55$, $p = 0.07$).

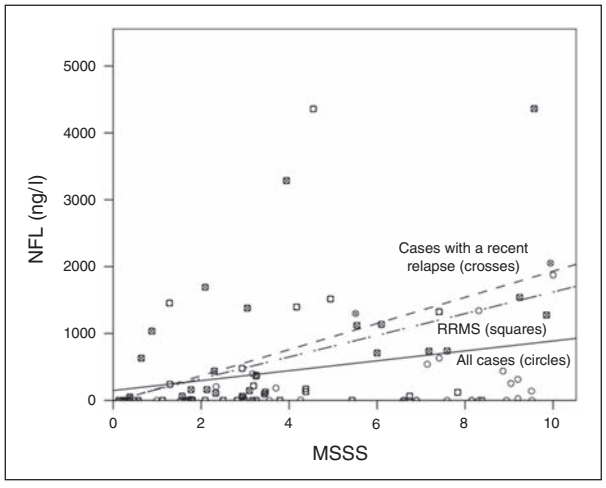

Figure 1. Neurofilament light (NFL) levels in cerebrospinal fluid collected at diagnostic lumbar puncture (LP) in all multiple sclerosis (MS) cases, denoted with a circle (○), in relation to multiple sclerosis severity score (MSSS) after median 14 years disease duration (Spearman's rho 0.30, $n = 89$, $p = 0.005$). Cases with relapsing-remitting MS (RRMS), median disease duration 13 years, are denoted with a square (□) (Spearman's rho 0.47, $n = 59$, $p < 0.001$). Cases with a recent relapse (defined as a relapse within 3 months before LP), median disease duration 13 years, are denoted with a cross (×) (Spearman's rho 0.60, $n = 34$, $p < 0.001$).

In the logistic regression analysis, the risk for high MSSS was increased threefold (OR = 3.2, 95% CI 1.3–8.1) for cases with high NFL levels. Among the other studied prognostic variables, only progressive disease at time of LP increased the risk for high MSSS (OR = 9.8, 95% CI 3.3–29). These risk factors for poor prognosis remained significant with even higher ORs in the multivariate analysis (Table 2). Conversion from RRMS to SPMS was more likely in cases with high NFL levels ($n = 21$) when compared with those with undetectable ($n = 22$, $p = 0.01$) or intermediate NFL levels ($n = 22$, $p = 0.03$) (Figure 2).

Median MSSS was not different at 14 versus 5-year follow-up (MSSS = 3.55, $n = 89$ versus MSSS = 4.27, $n = 91$, $p = 0.98$). Median PI was significantly lower at 14 than at 5-year follow-up (PI = 0.27, $n = 89$ versus PI = 0.44, $n = 91$, $p < 0.001$).

Discussion

In the present study we showed that high levels of NFL in the CSF collected at diagnostic LP are associated with worse outcome in MS. This association has been shown previously when clinical follow-up was made 5 years after onset.[10] Here we showed, by using three different statistical methods, that the association remains after a median of 14 years disease duration. High NFL levels increased the risk for worse outcome in the studied population where other predictors of bad prognosis failed to reach statistical significance, probably due to the limited sample size (Table 2). However, early progressive disease was confirmed to be a strong predictor for bad prognosis. The correlation between NFL levels and outcome was present among all cases, and became stronger when excluding cases with signs of progression. The strongest correlation between NFL levels and outcome was found among cases with a recent relapse, and conversely, when analysing cases without a recent relapse, no significant correlation was found. At the same time, progressive disease at diagnostic LP predicted bad outcome independently of NFL levels. Therefore, these two predictors seem to complement each other, as suggested by the increase of ORs in the multivariate analysis compared with the bivariate analysis.

The fact that long-term prognosis correlated better with CSF NFL levels in cases with a recent relapse as compared with those without argues that damage that occurs during an exacerbation is of major importance for the future course of the disease. The negative effect

Table 2. The risk for high multiple sclerosis severity score after a median of 14 years disease duration in 95 multiple sclerosis cases estimated in bivariate and multivariate logistic regression analyses

		MSSS (*n*)		Bivariate analysis		Multivariate analysis	
Variables	Categories	≤3.25	>3.25	OR	95% CI	OR	95% CI
NFL levels	≤386 ng/L	39	27	1.0		1.0	
	>386 ng/L	9	20	3.2	1.3–8.1	5.2	1.8–15
Clinical subtype at time of LP	RRMS	43	22	1.0		1.0	
	PP/PR/SPMS	5	25	9.8	3.3–29	14	4.2–44
Age at onset	≤34 years	30	20	1.0			
	>34 years	18	27	2.3	0.99–5.1		
Smoking	Never	22	15	1.0			
	Ever	25	32	1.9	0.8–4.3		
DMT	Never	29	26	1.0			
	Ever	19	21	1.2	0.5–2.8		
Sex	Female	30	31	1.0			
	Male	18	16	0.9	0.4–2.0		

MSSS, multiple sclerosis severity score; OR, odds ratio; CI, confidence interval; LP, lumbar puncture; NFL, neurofilament light; RRMS, relapsing-remitting multiple sclerosis; PPMS, primary progressive multiple sclerosis; PRMS, progressive relapsing multiple sclerosis; SPMS, secondary progressive multiple sclerosis; DMT, disease-modifying treatment. MSSS and age at onset are stratified at median (3.25 and 34 years, respectively). NFL is stratified at median (386 ng/L) among those with detectable (≥60 ng/L) levels. Smoking habits were known for 94 cases.

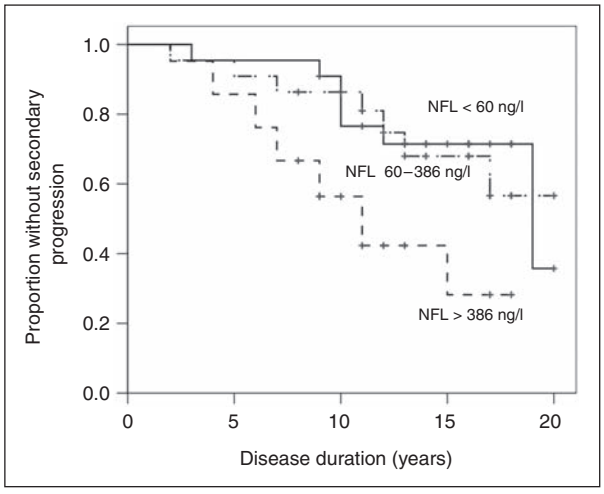

Figure 2. Kaplan-Meier plots of time to secondary progression among multiple sclerosis cases with relapsing-remitting disease at the time of diagnostic lumbar puncture ($n = 65$). Secondary progressive disease was more likely in cases with cerebrospinal fluid neurofilament light protein (NFL) levels >386 ng/L (above median among those with detectable levels, $n = 21$) as compared with those with intermediate or undetectable levels (60–386 ng/L, $n = 22$, $p = 0.03$, and <60 ng/L, $n = 22$, $p = 0.01$, respectively).

on prognosis from elevated NFL levels at diagnostic LP was most evident in those with clearly elevated NFL levels. This is illustrated by the Kaplan-Meier plots in Figure 2, which did not show any difference regarding the risk for conversion to SPMS between RRMS cases with undetectable NFL levels and those with intermediate NFL levels. Future studies are needed on RRMS to investigate how low elevations of NFL relate to prognosis.

Recently, a study showed that in cases with a clinically isolated syndrome, NFL levels were higher in those who converted within 3 years to clinically definite MS than among those who did not. Furthermore, the NFL levels were positively correlated to the amount of pathological MRI findings and EDSS scores. That study supports our findings of an association between NFL levels and future disease severity in RRMS.[13]

In contrast to previous reports, DMTs were not beneficial for the long-term prognosis in this study (Table 2).[14] However, to demonstrate such a treatment effect, we would have needed a larger number of RRMS cases enabling us to match untreated and treated cases for clinical prognostic predictors. If the present analysis is restricted to RRMS cases, there is a trend for a beneficial effect from DMTs (data not shown).

Neuronal damage, such as axonal transections occurring secondary to CNS inflammations in MS, leads to elevated levels of NFL in CSF.[9] The degree of NFL elevation probably reflects the degree of axonal transection, and axonal loss is a major cause of permanent neurological disability in MS. Thus, there is a clear rationale for NFL as a prognostic marker in MS. As argued above, a prognostic marker early in the course of MS, especially in RRMS with its

great variability in prognosis, would be of great value when making treatment decisions.[15] Current DMTs both reduce the number of relapses and the number of active lesions seen on magnetic resonance imaging.[16–19] Many new drugs are being tested, including monoclonal antibodies targeting immune cells. Preliminary data suggest that these new drugs far surpass the effect of the first-line treatments available today.[20,21] These emerging therapies, however, are expensive and have side effects that are not negligible. This makes selection of suitable patients important.

As expected, median MSSS stayed essentially the same between the 5 and 14-year follow-ups, while median PI decreased. This suggests that MSSS is a more reliable estimate of disease severity than PI when comparing groups of cases longitudinally.

The level of NFL in the CSF may become a marker of MS treatment efficacy.[22] It seems reasonable to assume that the levels of NFL would be high in populations with highly active disease entering clinical trials. Since both the number of relapses and the cumulated disability are reduced by DMTs, the levels of NFL would be presumed to decrease. However, to our knowledge, there are no studies that have used NFL levels in CSF as a marker of treatment efficacy, and the value of NFL in this context remains to be tested.

In conclusion, this study provides further support for an association between NFL levels in the CSF and later development of disease severity in MS. We have, in a follow-up study with a median of 14 years disease duration, shown that a high NFL level in early MS is suggestive of bad outcome. These findings need confirmation in a larger population, but data so far suggest that NFL could be used as a prognostic marker, especially in RRMS. An important use of such a marker would be to aid treatment decisions in early MS.

Conflict of interest statement

The authors have no conflicting interests.

Ethics approval

This study was approved by the local ethics committee in Umeå, Sweden.

Acknowledgement

This study was funded by grants from Biogen Idec and Sanofi Aventis.

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