Associations between neurofilament light chain levels, disease activity and brain atrophy in progressive multiple sclerosis

Jarmila Szilasiova[a,e], Pavol Mikula[b], Jaroslav Rosenberger[c,d], Miriam Fedicova[e], Peter Urban[f], Lydia Frigova[g], Marianna Vitkova[a,e], Zuzana Gdovinova[a,e], Jozef Hanes[h,i], Eva Stevens[i]

Background. Neurofilament light chain is a promising biomarker of disease activity and treatment response in relapsing-remitting multiple sclerosis (MS). Its role in progressive MS is less clear.
Aim: The aim of the study was to assess the relationship between plasma neurofilament light chain (pNfL) and disease activity as defined by the concept NEDA-3 (No Evident Disease Activity), and brain volumetry, in a cohort of patients with the progressive disease form (PMS).
Methods. Levels of pNfL (SIMOA technology) were examined in 52 PMS patients and analysed in relationship to NEDA-3 status and annual brain volume loss (BVL) during the last 12 months. The statistical model was developed using logistic regression analysis, including demographic, clinical and magnetic resonance imaging (MRI) data as independent variables. Dependent variables were NEDA-3 status and BVL.
Results. The mean age of the study participants (n=52, 50% females) was 45.85 (SD, 9.82) and the median disability score was 5.0 (IQR: 5.0–5.5). ROC analysis showed that pNfL predicts NEDA-3 (the sensitivity and specificity of the model were 77.8% and 87.6%, respectively, $P<0.001$) and abnormal BVL (the sensitivity and specificity were 96.6% and 68.2%, respectively, $P<0.001$).
Conclusions. The results show that pNfL levels are a useful biomarker of disease activity determined by NEDA-3 status, including brain MRI-volumetry, in patients with the progressive form of MS.

Key words: multiple sclerosis, progressive MS, neurofilament light chain, no evident disease activity, brain volume loss

[a]Department of Neurology, Pavol Jozef Safarik University in Kosice, Slovak Republic
[b]Department of Social and Behavioral Medicine, Pavol Jozef Safarik University in Kosice, Slovak Republic
[c]Department of Health Psychology and Methodology of Research, II. Internal Clinic, Pavol Jozef Safarik University in Kosice, Slovak Republic
[d]Olomouc University Social Health Institute, Palacky University Olomouc, Czech Republic
[e]Department of Neurology, L. Pasteur University Hospital, Kosice, Slovak Republic
[f]Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Safarik University in Kosice, Slovak Republic
[g]Pro Magnet Kosice, Slovak Republic
[h]Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovak Republic
[i]AXON Neuroscience R&D Services SE, Bratislava, Slovak Republic
Corresponding author: Jarmila Szilasiova, e-mail: jarmila.szilasiova@upjs.sk

INTRODUCTION

Current research in multiple sclerosis (MS) is focused on the identification of sensitive biomarkers related to disease activity in all MS phenotypes[1-6]. In MS patients treated with DMTs (disease modifying therapy) treatment response is measured using NEDA (No Evident Disease Activity) status[7]. Three-compound NEDA-3 status takes account of the absence of relapse, brain magnetic resonance imaging (MRI) activity and worsening disability during the last year. Some researchers point out that NEDA-3 status mostly reflects inflammatory activity and overlooks ongoing neurodegenerative processes.

In progressive MS (PMS) forms, which include secondary progressive MS (SPMS) and primary progressive MS (PPMS), treatment effect is evaluated using NEDA-3 status despite the fact that inflammatory activity is lower. Following the NEDA-3 concept may not capture the whole disease activity in PMS, in that PMS is characterised by a steady accumulation of disability independent of relapses[8].

One proposal to address these limitations is the measurement of brain volume loss (BVL), a marker of neurodegeneration. Clinical drug trials that involved BVL as an outcome parameter have shown that the effect of treatment on BVL correlated with the effect on disability progression[9-11].

Neurofilament light chain (NfL), cytoskeletal proteins confined to the neuroaxonal compartment, has been identified as a promising biomarker in MS related to neuronal damage, disease activity and treatment response[1-6,9-13]. Brain volume loss, determined as a mean annual BVL rate (AR-BVL) of <0.4%, was identified as the annual threshold BVL rate in MS patients in several studies[12,14-16].

Sormani et al. concluded that NfL could possibly be used as a substitute marker for brain volume loss[11].

We investigated in a PMS (SPMS and PPMS) cohort plasma NfL (pNfL) levels, parameters of disease activity as defined by NEDA-3 and brain volumetry, including whole brain volume, grey matter volume and BVL. We then analysed the relationship between pNfL and demographic, clinical and radiological parameters, as well as associations with NEDA-3 and BVL in our PMS cohort. To the best of our knowledge these variables have not been studied in such a context.

METHODS

Study Population

Fifty-two consecutive patients with PMS (5 with PMS and 47 with SPMS) were eligible to participate in the study and enrolled in a cohort study at the Department of Neurology of Louis Pasteur University Hospital in Košice (also see Fig. 1 for the study design). The study was approved by the Louis Pasteur University Hospital Ethics Committee (2017/EK/4005) and was performed in accordance with the Good Clinical Practice standard and the Declaration of Helsinki.

The inclusion criteria were the following: (1) diagnosis of primary progressive MS or secondary progressive MS in accordance with the phenotypic MS classification[8], (2) being older than 18 years, and (3) the ability to give written informed consent. The exclusion criterion was a severe comorbidity. Of the patient population involved in the study (n=52), 23 were males and 29 were females. All patients were treated with DMTs according to Slovak MS treatment criteria. The study period was January 2019 – February 2020.

Patients underwent clinical neurological examination, including expanded disability status scale (EDSS) (ref.[17]), plasma NfL (pNfL) sampling and brain MRI. Patient characteristics are presented in Table 1. Disease duration was considered the time from the first symptoms of MS to the date of the pNfL examination.

Study design

The baseline visit was 12 months before the follow-up visit. The baseline visit included compliance with the inclusion criteria (see above), demographic data (age, gender), clinical data (disease duration, EDSS score), MRI with MRI volumetry and current DMT. The follow-up visit included a pNfL sample, history in the last 12 months relapse, EDSS score and EDSS change in the last 12 months, MRI with MRI volumetry and current DMT. EDA (Evident disease activity) status was unfilled NEDA-3 status.

NfL measurements

The NfL sample was taken at the follow-up visit (12 months after the baseline visit). Samples of 3 ml of venous blood were collected into vacutainer tubes including an anticoagulant (sodium citrate) by the treating physicians and processed at room temperature within two hours. The samples were then spun at 4000 rpm for 10 minutes, and the collected plasma was divided into polypropylene tubes and stored at –80 °C. The blood samples were pseudonymized and analysed without clinical data.

The pNfL were analysed by SIMOA™ (Single Molecule Array) NfL assay using the NF-Light Advantage Kit and the SIMOA HD-1 analyser, protocol of Quanterix, Lexington, MA, USA (ref.[18]). The inter-assay coefficients of variation for three native serum samples were 5.3%, 1.9% and 5.3% for 6.2, 21.7 and 256 pg/mL, respectively. The intra-assay coefficients of variation (n=30) for three native serum samples were 6.1%, 6.8% and 7.3% for 6.2, 21.7 and 256 pg/mL, respectively.

MRI measurements

Brain MRI was performed using a standardized three-dimensional (3D) T1-weighted magnetization-prepared rapid gradient-echo sequence and a 3D T2-weighted fluid-attenuated inversion recovery (FLAIR) sequence with MS protocol in all PMS patients. Lesion maps were initially drawn on T2-weighted 3D FLAIR images using a PHILIPS Ingenia 3.0T Omega HP (Philips North America Corporation, dStream, Direct digital technology). Longitudinal coregistration fusion was used for identification of T2 lesions, FLAIR lesions, T1 lesions, T1 gadolinium-enhancing lesions and their occurrence,
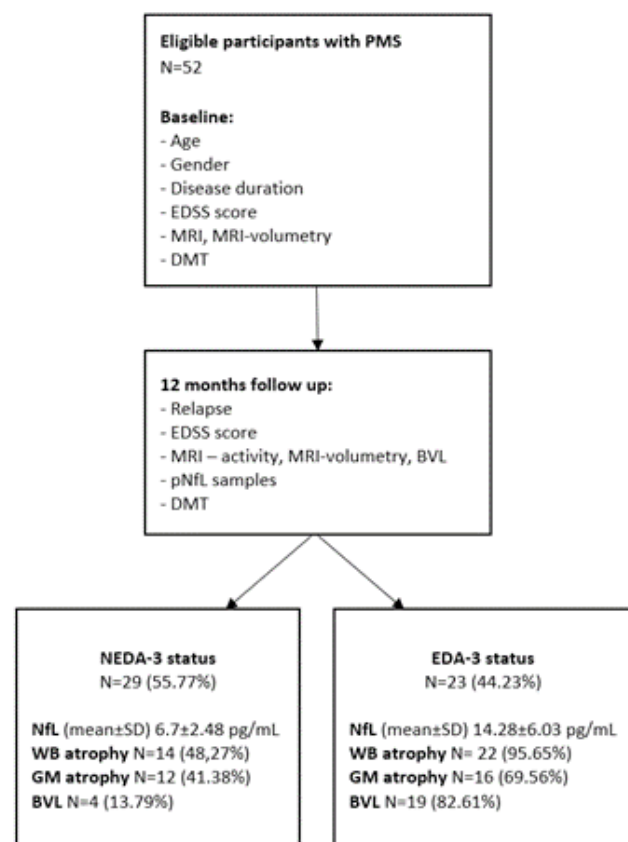
Fig. 1. A schematic flowchart diagram showing the procedure by which data were tested in MS patients.
EDA, Evident disease activity; NEDA, No evident disease activity; BVL, Brain volume loss; MS, Multiple sclerosis.

as well as new or enlarged lesions, and their volume was measured.

Whole brain (WB) volume and grey matter (GM) volume were measured using the Icobrain program (ICOMETRIX). Volumetric parameters were calculated by automatic brain volume quantification using FLAIR and T1-weighted scans using longitudinal, trans-sectional and segmentation techniques[19]. The Icobrain program compares the measured values of brain volumes in patients with those of healthy controls and calculates the deviation from the standard values evaluated in healthy controls (the database is mainly from Europe and North America), with the average expected annual change in volume for controls that match in the age and gender category.

Whole brain volume and grey matter volume parameters were adjusted for skull size using Icobrain, and the normal range and normative volume percentile change in healthy controls was used as a reference.

WB atrophy was determined as abnormal annual WB volume change. This parameter is based on the annual percentage of whole brain volume change as a value above the normal range of reference values in healthy controls (database), in accordance with gender and age (Icobrain program). GM atrophy was determined as abnormal annual GM volume change based on the annual percentage grey matter volume change with value above the normal range of reference values in healthy controls (database), in accordance with gender and age (Icobrain program).

BVL (brain volume loss) was defined as an annual BVL rate (AR-BVL) above 0.4% according to the study of De Stefano et al.[14]. Patients were dichotomized based on an AR-BVL <0.4% or ≥0.4% (BVL).

Brain MRI was performed within the last 3 months before the baseline visit and the follow-up visit (Fig.1). MRI data were analysed by blinded radiologists and Icobrain raters, who had no information about disease activity and NfL levels.

NEDA-3 definition

NEDA-3 status was evaluated using data from the last 12 months. NEDA-3 status was defined as the absence of relapse, EDSS worsening and MRI activity.

EDSS worsening was defined as an increase in the EDSS score of 1.5 points, if the previous EDSS score was (a baseline score) 0; an increase of ≥1.0 point, if EDSS ≤5.0; or an increase of ≥0.5 points, if EDSS ≥5.5, confirmed at 6 months[20,21].

MRI activity was defined as having at least two or more new/or enlarging T2-hyperintense lesions or the presence of a gadolinium (Gd)-enhancing T1 lesion in the brain MRI (at follow-up visit) compared with the previous scan (baseline visit).

Statistical analysis

Descriptive statistics were compiled to provide basic information about the patients. Summary statistics are presented as the mean ± SD, median (range) and percentage, where applicable categorical variables are number and percentage. Thereafter, binary logistic regression analysis was performed. The model included demographic (age, gender), laboratory (pNfL), clinical (EDSS, disease duration) and MRI variables as independent variables. Dichotomized WB atrophy, GM atrophy, NEDA-3 status and BVL status were used as the dependent variables. The conditional backward stepwise method was used to select the model with the best predictors. Consequently, for evaluation of the predictive value of pNfL levels as a predictor of WB atrophy, GM atrophy, NEDA-3 status and BVL status, we plotted the ROC (Receiver operating characteristics) curve and calculated the area under curve (AUC) with a 95% confidence interval (CI). Optimal NfL cut-offs were defined based on the Youden index. From the pNfL cut-off values, we selected the optimal one with the highest discriminant accuracy (sensitivity and specificity).

Statistical analyses were performed at the 0.05 level/ values of significance using the IBM SPSS (Statistical Package for the Social Science) software version 23.0.

RESULTS

The demographic, clinical, laboratory and radiological (MRI) characteristics of the patients with PMS are provided in Table 1. The mean age was 45.87 ± 9.82 years, and 29 (55.77%) patients were female. The median disease duration was 16.0 (IQR: 9.25–21.75) years, and the median pNfL was 11.1 pg/mL (IQR: 5.2–11.13). Patients were treated with DMTs in the following proportions: 22 patients (42.31%) were on first line DMTs and 30 (57.69%) on second line DMTs (Table 1).

From a total of 52 (100%) patients, 29 (55.77%) had NEDA-3 status in the past evaluated year, while 23 (44.23%) patients showed EDA-3 status. The pNfL were significantly higher in the EDA-3 group than in the NEDA-3 group (14.28 ± 6.03 pg/mL vs 6.7 ± 2.48 pg/ml; *P*<0.001).

Thirty-six (69.23%) patients met the criteria for WB atrophy and 28 (53.85%) patients met the criteria for GM atrophy. Twenty-three (44.23%) patients met the criteria for BVL (AR-BVL ≥ 0.4%); 4 of them had NEDA-3 status, and 19 had EDA-3 status (Fig. 1.).

From the subgroup of patients with NEDA-3 status (n=29), 4 (13.79%) patients had BVL, 12 (41.38%) had GM atrophy and 14 (48.27%) had WB atrophy.

From the subgroup of patients with EDA-3 status (n=23), 19 (82.61%) patients had BVL, 16 (69.56%) had GM atrophy, and 22 (95.65%) had WB atrophy (Fig. 1).

Multivariable logistic regression analysis, a model consisting of age, gender, disease duration, EDSS (at follow-up visit) and pNfL as independent variables and NEDA-3 status as the dependent variable, showed that the pNfL value (B=-0.563, Exp(B) 0.57; *P*<0.001) is a significant predictor of NEDA-3 status. ROC curve analysis showed the pNfL to be a predictor of NEDA-3 status (AUC=0.848; 95% CI:0.739–0.957; *P*<0.001), and the sensitivity and specificity of the predictive model were 77.8%

Table 1. Demographic, clinical, laboratory and MRI characteristics of the study sample.

Demographic characteristics	
Sample size, n	52
Female, n (%)	29 (55.77)
Age (years), mean (SD)	45.87 (9.82)
Clinical characteristics	
Disease duration (years), median, IQR	16 (9.25–21.75)
EDSS, median, IQR	5.0 (5.0–5.5)
Proportion of patients with last year relapse, n (%)	20 (38.46)
Proportion of patients with EDSS worsening, n (%)	18 (34.62)
Laboratory characteristics	
pNfL levels, pg/mL, median, IQR	11.1 (5.2–11.13)
MRI measures	
WB volume, median, IQR	1461 (1403.25–1461)
WB volume normative percentile, median, IQR	0.98 (0.8–6.7)
WB annual volume change, median, IQR	–0.52 (–0.79––0.23)
GM volume, median, IQR	858.5 (826–888)
GM volume normative percentile, median, IQR	2.6 (0.9–15.5)
GM annual volume change, median, IQR	–0.4 (–0.71––0.21)
Proportion of patients with MRI activity, n (%)	14 (26.92)
Proportion of patients with BVL, n (%)	23 (44.23)
Proportion of patients with WB atrophy, n (%)	36 (69.23)
Proportion of patients with GM atrophy, n (%)	28 (53.85)
NEDA-3 status	
Proportion of patients with NEDA-3, n (%)	29 (55.77)
Proportion of patients with EDA-3, n (%)	23 (44.23)
Therapy	
Proportion of patients with first line DMT, n (%)	22 (42.31)
Proportion of patients with interferon-beta n (%)	4 (7.69)
Proportion of patients with teriflunomide, n (%)	6 (11.54)
Proportion of patients with dimethylfumarate, n (%)	12 (23.08)
Proportion of patients on second line DMT, n (%)	30 (57.69)
Proportion of patients with fingolimod n (%)	7 (13.46)
Proportion of patients with ocrelizumab, n (%)	7 (13.46)
Proportion of patients with cladribine, n (%)	10 (19.23)
Proportion of patients with alemtuzumab, n (%)	6 (11.54)

Continuous data are calculated with mean±SD (standard deviation); pNfL are median (IQR); categorical variables are number (%).
Legend: AR-BVL, Annual Rate Brain Volume Loss; BVL, Brain Volume Loss; DMT, Disease Modyfying Therapy; EDSS, Expanded Disability Status Scale; IQR, Interquartile Range; MRI, Magnetic Resonance Imaging; NEDA, No Evident Disease Activity; pNfL, Plasma Neurofilament Light Chain; WB, Whole Brain; GM, Grey Matter
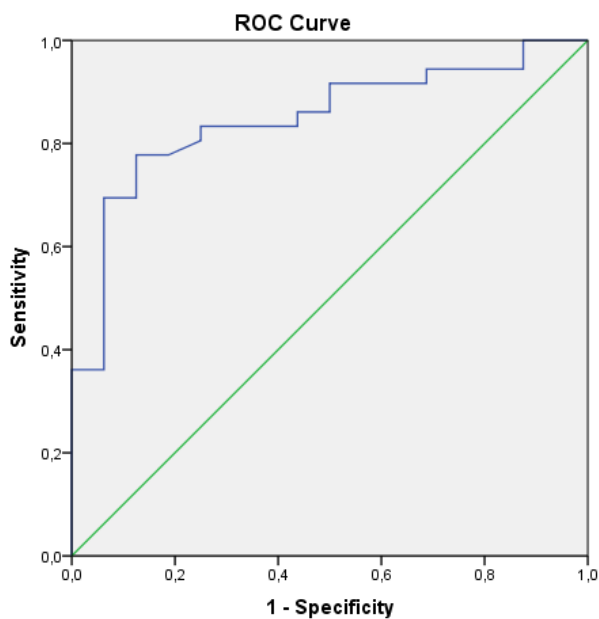
and 87.57%, respectively (Fig. 2). Lower pNfL values indicate stronger evidence for the presence of NEDA-3 status, with a cut-off level of 9.1 pg/mL for pNfL.

Multivariable logistic regression analysis, a model consisting of age, gender, disease duration, EDSS and pNfL as independent variables and WB atrophy as the dependent variable, showed that the pNfL value (B=0.528, Exp(B) 1.695; *P*<0.05) is a significant predictor of WB atrophy. ROC curve analysis showed the pNfL value to be a predictor of WB atrophy (AUC=0.905; 95% CI: 0.803–1.0; *P*<0.001), and the sensitivity and specificity of the predictive model were 93.5% and 75%, respectively (Fig. 3). Higher pNfL values indicate stronger evidence for the presence of WB atrophy, with a cut-off level of 8.5 pg/mL for pNfL.

Multivariable logistic regression analysis, a model consisting of age, gender, disease duration, EDSS and pNfL as independent variables and GM atrophy as the dependent variable, showed that the pNfL value (B=0.129, Exp(B) 1.137; *P*=0.097) is a predictor of GM atrophy but did not show a level of statistical significance. ROC curve analysis showed the pNfL value to be a predictor of GM atrophy (AUC=0.738; 95% CI: 0.587–0.809; *P*<0.01), and the sensitivity and specificity of the predictive model were 71.9% and 73.7%, respectively (Fig. 4). Higher pNfL values indicate stronger evidence for the presence of GM atrophy, with a cut-off level of 9.55 pg/mL for pNfL.

Multivariable logistic regression analysis, a model consisting of age, gender, disease duration, EDSS and pNfL as independent variables and BVL as the dependent
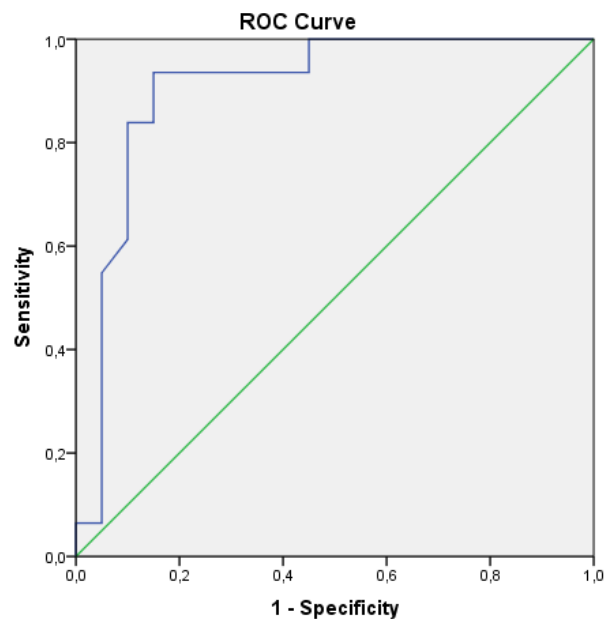
Fig. 2. ROC analysis of the pNfL of patients with and without NEDA-3 status. The sensitivity and specificity of the predictive model were 77.8% and 87.5%, respectively, with a cut-off level of 9.1 pg/mL for pNfL, and an area under curve (AUC) of 0.848 (95% CI: 0.739–0.957; $P<0.001$). Lower pNfL values indicate stronger evidence for the presence of NEDA-3 status.
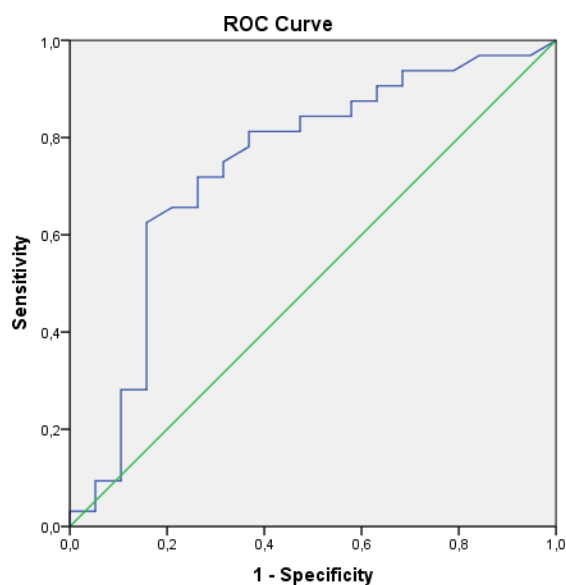
AUC, Area under curve; NEDA, No evident disease activity; pNfL, plasma neurofilament light chain; ROC, Receiver operating characteristics.

Fig. 3. ROC analysis of the pNfL of patients with and without WB atrophy. The sensitivity and specificity of the predictive model were 93.5% and 75%, respectively, with a cut-off level of 8.5 pg/mL for NfL, and an area under curve (AUC) of 0.905 (95% CI: 0.803–1.0; $P<0.001$). Higher pNfL values indicate stronger evidence for the presence of WB atrophy.
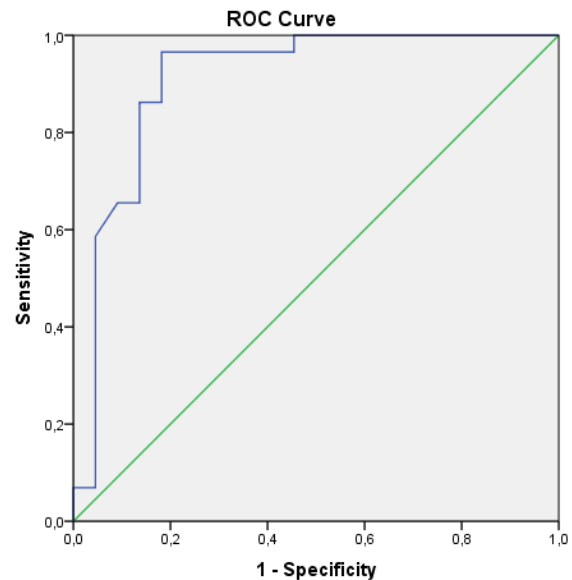
AUC, Area under the curve; NEDA, No evident disease activity; pNfL, plasma neurofilament light chain; ROC, Receiver operating characteristics; WB, Whole brain.

Fig. 4. ROC analysis of the pNfL of patients with and without GM atrophy. The sensitivity and specificity of the predictive model were 71.9% and 73.7%, respectively, with a cut-off level of 9.55 pg/mL for NfL, and an area under curve (AUC) of 0.738 (95% CI: 0.587–0.809; $P<0.01$). Higher pNfL values indicate stronger evidence for the presence of GM atrophy.

AUC, Area under the curve; GM, Gray matter; NEDA, No evident disease activity; pNfL, plasma neurofilament light chain; ROC, Receiver operating characteristics.

Fig. 5. ROC analysis of the pNfL of patients with and without BVL. The sensitivity and specificity of the predictive model were 96.6% and 68.2%, respectively, with a cut-off level of 8.25 pg/ml for NfL, and an area under curve (AUC) of 0.909 (95% CI: 0.815–1.0; $P<0.001$). Higher pNfL values indicate stronger evidence for the presence of BVL.

AUC, Area under the curve; BVL, Brain volume loss, NEDA-No evident disease activity; pNfL, plasma neurofilament light chain; ROC, Receiver operating characteristics.

variable, showed that the pNfL value (B=0.564, Exp(B) 1.758; *P*<0.001) is a significant predictor of BVL. ROC curve analysis showed the pNfL level to be a predictor of BVL (AUC=0.909; 95% CI=0.815–1.0; *P*<0.001), and the sensitivity and specificity of the predictive model were 96.6% and 68.2%, respectively (Fig. 5). Higher pNfL values indicate stronger evidence for the presence of BVL, with a cut-off level of 8.25 pg/mL for pNfL.

DISCUSSION

The results of our study showed that plasma neurofilament light chain levels are predictors of disease activity in progressive MS forms, as measured by 3-domain NEDA-3 status, consisting of the absence of relapse, disability (EDSS) worsening and brain MRI activity. The second main result is that the pNfL level predicts brain volume parameters, specifically whole brain atrophy, gray matter atrophy and brain volume loss (≥ 0.4%). From a total of 52 patients with PMS on DMT treatment, more than half (55.8%) had NEDA-3 status, and 86.2% of them were without BVL. More than half (55.8%) of all the patients with PMS in the sample were without BVL, and 44.2% met the criteria for BVL.

The results of ROC curve analyses showed that pNfL predicts BVL with higher sensitivity (96.6%) when compared to WB atrophy (93.5%), GM atrophy (71.9%) and NEDA-3 (77.8%). This may also be reflected by the AUC values: BVL-AUC (0.909) was higher than WB atrophy-AUC (0.905), GM atrophy-AUC (0.738) and NEDA-3-AUC (0.848). Our results show good potential for pNfL levels to discriminate between patients with and without NEDA-3 status and the good performance of pNfL levels in discriminating between patients with and without abnormal BVL.

A comparison of pNfL levels in the NEDA-3 and EDA-3 subgroups showed significant higher levels in the EDA-3 group (6.7 ± 2.48 vs 14.28 ± 6.03). These results are consistent with the findings of several studies[5,22-25] and they seem to indicate that pNfL reflects disease activity measured by 3-domain NEDA-3 status. In addition, it may support ability of immunosuppressive DMTs to supress NfL.

In the NEDA-3 group 13.8% of patients had BVL, while in EDA-3 group BVL was present in 82.6%. This shows that BVL probably well captures the disease activity in PMS; similar results have been presented in a few studies with progressive MS (ref.[24-27]). In fact, this allows us to conclude that an analysis with pNfL as the dependent variable may better quantify the association between pNfL and recent BVL and indicate whether pNfL and BVL may be somehow complementary. Based on this observation, BVL, along with pNfL, appears to be a useful tool in evaluating progressive MS.

Progressive multiple sclerosis (PMS) is characterised by a steady accumulation of disability largely independent of relapses[8]. Progression in both SPMS and PPMS may occur either in association with inflammatory activity or in the absence of such inflammatory activity[8]; therefore, there is a need to determine sensitive biomarkers pointing to disease activity particularly in "non-active" PMS.

NEDA-3 status is a widely used target of DMT in MS patients in clinical studies as well as in routine practice. Three-domain NEDA status is considered annually in a patient before a decision is made regarding the continuing or switching of DMT therapy. One of the known disadvantages of NEDA-3 is that it mainly reflects the inflammatory component, while neurodegenerative processes can progress undetected; additionally, the EDSS scale (used for disabilty evaluation) may not accurately detect the progression of disability. It is known that disability progression in patients, despite their meeting the criteria of NEDA-3, is possibly a consequence of brain and spinal cord atrophy[27]. Thus, we chose BVL status for analysis of the relationship between pNfL and disease activity.

In contrast to other studies in patients with early MS and RRMS, we studied a cohort of patients with PMS with ongoing disease who were on DMT treatment, and our results show that the plasma NfL level at any point during the disease course is a sensitive marker of disease activity and is associated with brain volume loss.

The main contribution of this study is that the strength of the association between the brain volume loss (MRI volumetry) seems to be higher than the association between pNfL and the "gold standard" measurement of current disease activity by NEDA-3. This is supported by results of the area under the ROC curve. We hypothetize that one of the advantages of pNfL is that unlike NEDA-3, pNfL also appears to cover false NEDA-3, as it appears to include spinal cord pathology and subclinical disease activity. On the other hand, it should be clarified in the future, whether pNfL reflects brain diseases other than MS (comorbidities) and the influence of age. However, because this is only an observational study, it is clearly necessary to verify the validity of our findings in the future in a wider range of SPMS and PPMS cohorts.

Neurofilaments have been extensively studied in relapsing–remitting multiple sclerosis[1-6]. Their role in progressive MS, where there is a particularly urgent need for sensitive valid biomarkers, is less clear. Several studies have shown that NfL levels in PMS are associated with current inflammatory activity[22,24-26], current disability (EDSS, MSFC) (ref.[6,22,25,26]), future disability worsening and future brain and spinal cord atrophy. A few studies have consistently shown NfL to be a marker of treatment response with immunosuppressive disease-modifying therapies[22,25,27]. Our results support these findings, and moreover we conducted our study to determine the benefit of pNfL levels and BVL for our routine practice at a patient's regular annual evaluation.

While Kapoor et al.[25] showed in the ASCEND study of natalizumab in SPMS that higher baseline blood NfL was associated with greater future 96-week brain atrophy, our study shows that NfL reflects brain atrophy of the past 12 months. This information can be useful in evaluation of treatment response.

Based on the results, we assume that pNfL has the potential to discriminate those patients with NEDA-3 and BVL, and therefore pNfL seems to be supportive biomark-

er in making a treatment decision. We can recommend clinicians to include pNfL evaluation at a patient's regular annual monitoring measures of patients with progressive forms of multiple sclerosis as an auxiliary tool complementing the results of clinical and radiological follow-up. Based on strong association between pNfL levels, NEDA-3 status and abnormal brain volume loss, unchanged NfL level compared to the previous one reflects no recent disease activity including neurodegenerative process. It can be useful for the treatment algorithm and identification of optimal treatment respondes.

There are several limitations of our study: our cohort is small and the follow-up was relatively short; a prospective study with a larger cohort of PMS patients could better explain NfL validity in individual PMS patient's disease course. We did not take into account all of the patients' comorbidities, vascular risk factors and aging, known factors which may affect NfL levels. Regarding brain volume measurement, there are several known obstacles in data interpretation or MRI volumetric techniques.

CONCLUSION

Plasma neurofilament levels, along with brain volume loss measurement, appear to be a useful tool for assessing current disease activity in patients with progressive MS.

ABBREVIATIONS

AR-BVL, Annual Rate Brain Volume Loss; BVL, Brain Volume Loss; CI, Confidence Interval; DMT, Disease Modifying Therapy; EDA, Evident Disease Activity; EDSS, Expanded Disability Status Scale; FLAIR, Fluid-attenuated Inversion Recovery; GM, Gray Matter; MRI, Magnetic Resonance Imaging; MS, Multiple Sclerosis; MSFC, Multiple Sclerosis Functional Composite; NEDA, No Evident Disease Activity; pNfL, Plasma Neurofilament Light Chain; ROC, Receiver Operating Characteristics; PMS, Progressive Multiple Sclerosis; SIMOA, Single Molecule Array; WB, Whole brain.

Author contributions: All authors made substantial intellectual contributions to the study. JS, MV, MF: contributed to data collection; JS, MF: performed the initial literature review and wrote the first draft of the manuscript; JR, ZG, PM: supervised the work and performed statistical analyses; PU, JH, ES: performed and revised the laboratory tests; LF: analysed MRI data; JS, ZG, MV, JH, ES: contributed to revisions. All authors critically reviewed the manuscript and approved the final version of it.

Conflict of interest statement: The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: JS has received personal compensation for consulting, serving on a scientific board, speaking or other activities, with received compensation for serving on a Scientific advisory board from Biogen, TEVA, Sanofi, Novartis, Roche and Merck, and for consultancy from Merck and Biogen. PM has no potential conflict of interest. JR has received personal compensation for consulting and speaking or other activities, with received compensation from Mundipharma. MF has received personal compensation for consulting, speaking or other activities from Biogen, Merck, Roche, Novartis, Sanofi and TEVA. PU has no potential conflict of interest. LF has no potential conflict of interest. MV has received personal compensation for consulting, serving on a scientific board, speaking or other activities, with received compensation for serving on scientific advisory board from Biogen, Merck, Novartis, Roche, Sanofi, and Teva. ZG has received personal compensation for consulting, serving on a scientific board, speaking or other activities, with received compensation for serving on scientific advisory board from Bayer, Biogen, Boehringer-Ingelheim, Merck, MSD, Novartis, Pfizer, Takeda and Teva. JH has no potential conflict of interest. ES has no potential conflict of interest.

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