Neuroendocrinology Letters Volume 41 No. 1 2020
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

ORIGINAL ARTICLE

Neurofilament light chain and MRI volume parameters as markers of neurodegeneration in multiple sclerosis

Pavol Filippi[1], Veronika Vestenická[1], Pavel Šiarnik[1], Monika Siváková[1], Daniela Čopíková-Cudráková[1], Víťazoslav Belan[2], Jozef Hanes[3], Michal Novák[3], Branislav Kollár[1], Peter Turčáni[1]

1 1st Department of Neurology, Faculty of Medicine, Comenius University, Bratislava, Slovakia
2 Dr. Magnet - Kramáre, Bratislava, Slovakia
3 Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia

Correspondence to: Pavol Filippi MD.
1st Department of Neurology, Faculty of Medicine, Comenius University, Mickiewiczova 13, 81369, Bratislava, Slovakia
TEL.: +421 948 766 105; E-MAIL: pavolfilippi@gmail.com

Submitted: 2020-01-17 Accepted: 2020-02-23 Published online: 2020-04-03

Key words: **Multiple Sclerosis; Neurofilament Light Chain; Neurodegeneration; Single-molecule Array (SIMOA®); Magnetic Resonance Imaging; MRI Volume Parameters; Icobrain; Biomarker.**

Neuroendocrinol Lett 2020; **41**(1):17–26 **PMID:** 32338853 NEL410120A02

Abstract

BACKGROUND: Neurofilament light chain (NfL) is considered a major marker of neurodegeneration and disease activity. Higher levels of NfL are associated with worse clinical outcomes and increased brain atrophy. In treated patients with Relapsing-Remitting Multiple Sclerosis (RRMS), we aimed to determine the level of NfL, an association between NfL and demographic, clinical and magnetic resonance imaging (MRI) characteristics as well as brain volume parameters. We wanted to confirm that level of NfL is clinically useful as biomarker of neurodegeneration and disease activity.

METHODS: 56 treated RRMS patients were enrolled. Plasmatic levels of NfL (pNfL) were measured by SIMOA® technique. Clinical severity of MS was expressed by Expanded Disability Status Scale (EDSS), and volumetric analysis of MRI data was performed using Icobrain software.

RESULTS: The mean pNfL level was significantly higher in MS patients than in healthy controls (14.73 ± 6.38 versus 6.67 ± 3.9, $p<0.001$). In patients, we did not find association between pNfL and MRI activity, number of new T2 lesions, and number of enhancing lesions. Levels of pNfL correlated significantly with atrophy of whole brain volume (Wbv), atrophy of grey matter volume (Gmv), and negatively with Wbv. We found significantly positive correlation between pNfL levels and EDSS.

CONCLUSION: Study shows association of pNfL with Wbv, presence of brain atrophy and EDSS, and strong correlation of EDSS with multiple MRI volume parameters. We did not confirm association pNfL with disease activity. Our data suggest that pNfL and MRI volume parameters could be considered as biomarkers of neurodegeneration in MS.

Abbreviations:

CNS	- central nervous system
CSF	- cerebrospinal fluid
DMT	- disease-modifying therapies
EDSS	- Expanded Disability Status Scale
Gmv	- grey matter volume
IQR	- interquartile range
MRI	- magnetic resonance imaging
MS	- multiple sclerosis
NfL	- neurofilament light chain
pNfL	- plasmatic levels of neurofilament light chain
RRMS	- relapsing-remitting multiple sclerosis
SD	- standard deviation
Wbv	- whole brain volume

INTRODUCTION

Multiple sclerosis (MS) is a chronic immune-mediated demyelinating disease of the central nervous system (CNS). Focal demyelination, consequently followed by neurodegeneration affecting axons of the neurons, is typical for the disease. Inflammation dominates in the clinical course of the early MS, but early signs of neurodegeneration are also present in this stage of the disease (Charil & Filippi, 2007).

Understanding the mechanisms causing neurodegeneration may be fundamental for the development of therapies that could influence this process, and thereby prevent the progression of disability. It remains unknown whether the degeneration is secondary to inflammation or if it is an independent process in the pathogenesis of MS (Novakova *et al.* 2018).

The monitoring of neuroaxonal damage remains the key challenge in MS. There was a need to search a biomarker of longitudinal monitoring of the disease.

Neurofilament light chain (NfL) is a major component of the axonal cytoskeleton and plays an important role in axonal growth, stability, and intracellular transport (Yabe *et al.* 2001). Neuro-axonal damage leads to release NfL into the interstitial fluid, cerebrospinal fluid (CSF), and consequently into the blood in low levels (Teunissen & Khalil, 2012). Recently, an ultrasensitive antibody-based analytic method, Single-Molecule Array (SIMOA®)(Disanto *et al.* 2017), was developed to quantify low levels of NfL in a serum or plasma blood samples. This technology provides the basis for assessment NfL as an important step towards its use as a biomarker in clinical research and practice.

NfL is considered as one of the major markers of neurodegeneration (Novakova *et al.* 2018). Several studies reported that higher NfL levels were associated with worse clinical outcomes and increased brain atrophy (Disanto *et al.* 2017; Kuhle *et al.* 2017).

Magnetic resonance imaging (MRI) was shown to be a useful tool in diagnosing MS and also monitoring the disease progression and therapeutic response. New T2 lesions, enlargement of existing ones, and presence of T1 gadolinium-enhancing lesion belong to the signs of the disease activity in MS. MRI volume parameters considered as markers of neurodegeneration is not yet a part of clinical routine.

MRI volumetry became a clinically relevant component of disease assessment because of its high sensitivity and specificity in detecting volumetric changes of the brain (Wattjes *et al.* 2015; Rocca *et al.* 2017). Brain atrophy, as a macroscopic reflection of the neuroaxonal damage, became one of the most important markers of neurodegeneration and clinical disease progression in MS patients (Koudriavtseva & Mainero, 2016; Zivadinov *et al.* 2016).

Icobrain (previously called MSmetrix by Icometrix Company) is MRI software for brain volume and atrophy assessment that are fast, fully automated, accurate, reproducible, and that apply to clinical research and routine clinical practice (www.icometrix.com).

Based on this, we decided to compare the relationship between pNfL and MRI volume parameters and verify whether they are clinically useful as biomarkers of neurodegeneration and disease activity in treated RRMS patients.

Our primary aim was to assess the level of pNfL in treated RRMS patients, in comparison to age-matched controls, and to assess the role of pNfL as a potential biomarker of neurodegeneration. The secondary aim was to determine the association between pNfL, demographic data, disability status, and MRI volumetric data in treated RRMS patients.

MATERIALS AND METHODS

Patients, controls, and clinical assessment

We consecutively enrolled 56 patients with RRMS, currently treated with various disease-modifying therapies (DMT) and 27 sex and age-matched healthy controls. The patients were selected from the population of the patients followed in the Centre for Multiple Sclerosis at the 1st Department of Neurology, Faculty of Medicine, Comenius University in Bratislava between May 2018 and November 2018. The followed MS patients have control MRI once in a year.

All patients fulfilled the revised McDonald's criteria (Polman *et al.* 2011). Patients underwent clinical neurological examination, including the Expanded Disability Status Scale (EDSS)(Kurtzke, 1983).

Patients before pNfL and MRI measurements were attack-free at least for 6-months. Exclusion criteria included current use of intravenous corticosteroids and presence of other neurodegenerative and cerebrovascular disease. All patients provided written informed consent, and the local ethics committee approved the study. We present a cross-sectional study.

Plasma NfL measurement

In patients without acute clinical relapses, blood samples were obtained on the same day when clinical visit and MRI was performed. The total amount of 3 ml of blood was taken into a tube with EDTA. The blood samples

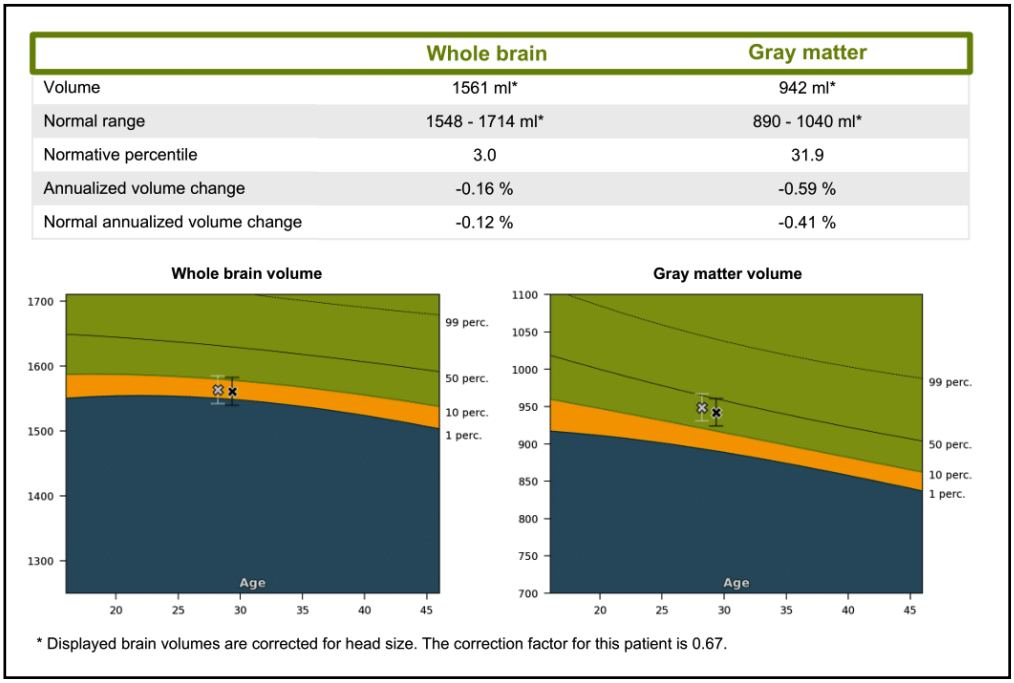

Fig. 1. Volume parameters of whole brain volume (Wbv), grey matter volume (Gmv), and population graph corrected for head size (report of Icobrain software).

were centrifuged at the speed of 4000g rpm for 10 minutes to obtain plasma. Subsequently, they were stored at -70°C and shipped on dry ice in a temperature-controlled container to the Institute of Neuroimmunology of the Slovak Academy of Sciences, where concentrations of neurofilament light chain were measured. The analysis was performed by a laboratory technique called Single-Molecule Array (SIMOA®), with the NF-Light Advantage Kit on the Simoa-HD1 analyzer, according to the protocol issued by the manufacturer (Quanterix, Lexington, MA, USA)(www.quanterix.com)(Kuhle *et al.* 2016; Disanto *et al.* 2017). We measured the concentration of plasma levels of NfL (pNfL). All laboratory personnel remained blinded to the diagnosis and had no access to the clinical data.

MRI examination

MRI was performed using a 3-Tesla device in all MS patients (Philips Ingenia 3.0T, Omega HP, Philips North America Corporation), according to the standardized MRI protocol for MS (Lövblad *et al.* 2010; Filippi *et al.* 2011). All MRI exams were performed on the same scanner. MRI data were evaluated by a specially trained radiologist, who has no information about patients' clinical status and levels of pNfL. Radiologists provide written MRI findings, according to a standardized MS protocol, and an MS report from the Icobrain program. Patients monitored at our Centre for Multiple Sclerosis have an MRI examination, including volumetry once a year. From MRI, we analyzed multiple parameters, including the number of new T2 lesions or enlargement of older T2 lesions and the number of gadolinium-enhancing lesions. MRI activity was defined as the presence of at least two new T2 lesions or one enhancing lesion. Previous MRI examination in our patients did not contain the Icobrain protocol, just conventional MRI MS protocol.

Icobrain MS report provide a whole brain volume, volume of grey matter, data from regional volume changes in white matter, but does not provide data from regional volume changes in grey matter. The volume of FLAIR lesions, hypointensities in T1 weighted image (so-called "black holes"), and volume of enhancing lesions were reported from the first MRI examination. Whole brain and grey matter volume parameters were in the software corrected for head size. The normal range and normative percentiles of the healthy controls served as a reference. Atrophy of whole brain volume and grey matter volume was defined when a value was outside of the reference limits (normal range), provided from age and sex-matched healthy controls. Figure 1 displays the age- and gender-matched average and the normal range for the whole brain (left) and grey matter (right) volume of healthy controls. The current (black) and previous (white) time points are marked with an 'x'. The error bars are computed from the pooled results of several test-retest experiments. They represent the interval in which the differences between test- and retest results can be found with 90% confidence (www.icometrix.com)(Jain *et al.* 2015; Wang *et al.* 2016; Beadnall *et al.* 2019).

Statistical analysis

The statistical analyses were performed with SPSS program version 25 (SPSS Inc., Chicago, IL, USA). Categorical variables were expressed as numbers and

Tab. 1. Baseline characteristics of MS patients and controls

	MS patients (n= 56)	Controls (n= 27)
Male	18 (32 .14%)	9 (33.3%)
Female	38 (67.86%)	18 (66.7%)
Age (years)	41.43 ± 11.15	41.59 ± 14.42
Disease duration (years)	10 (8.50, 1-38)	NA
EDSS	3.25 (2.50, 1-5.5)	NA
pNfl (pg/ml)	14 .73 ± 6.38	6.67 ± 3.92
Disease-modifying therapies (DMT):		
Interferon beta[1]	12 (21.42%)	NA
Glatiramer acetate[1]	14 (25.1%)	NA
Teriflunomide[1]	5 (8.93%)	NA
Dimethyl fumarate[1]	3 (5.26%)	NA
Fingolimod[2]	8 (14 .29%)	NA
Natalizumab[2]	13 (23.21%)	NA
Alemtuzumab[2]	1 (1.79%)	NA

pNfl: plasma neurofilament light chain, EDSS: Expanded Disability Status Scale, Categorical variables expressed as number and proportions (%), Continuous variable as mean with standard deviation, or median, interquartile range, minimal and maximal values,
[1]: First-line treatment, [2]: Second-line treatment, NA: not applicable.

percentages (%). Numerical variables in normally distributed data were presented as mean and standard deviations (SD) or median and interquartile ranges (IQR) if they did not follow a normal distribution using the Kolmogorov-Smirnov normality test. For comparison of the variables in particular subgroups, Student's t-test were used for parametric variables and Mann-Whitney U-test for non-parametric variables.

Non-parametric bivariate correlation analysis (Spearman) was used for correlation analysis. Spearman correlation coefficients (rho) were used to determine relationships between pNfL, baseline characteristics, and MRI data. Multiple linear regression analysis was performed with pNfL as dependent variable and EDSS, Wbv, Gmv, number of enhancing lesions, number of new T2 lesions, volume of FLAIR lesions, volume of enhancing lesions and volume of T1 lesions as independent variables. For control, the age, disease duration, and type of DMT was performed hierarchical regression. The results were similar between the analyses with and without adjustment for age, disease duration and type of DMT. Therefore, we present results not adjusted for these variables. All tests were 2-sided; p-values <0.05 were considered statistically significant.

RESULTS

Baseline characteristics of MS patients and controls

The study population consisted of 38 female (67.86%) and 18 male (32.14%) with the mean age of 41.43 years, ranging from 20 to 64 years. Disease duration was 1 to 38 years, with a median of 10 years. The average EDSS score was 3.25 (range 1-5.5). Patients were treated by seven types of medicaments, of which 34 (60.7%) were on first-line treatment, and 22 (39.3%) were on second-line treatment. The control group consisted of 18 females (66.7%) and 9 males (33.3%) with a mean age of 41.59 years (24 to 67 years). See Table 1.

EDSS in subgroup with Wbv atrophy was significantly higher compared with subgroup without atrophy (3.78 ± 1.32 versus 2.66 ± 1.31, p= 0.03). EDSS in subgroup with Gmv atrophy was significantly higher compared with subgroup without atrophy (4.13 ± 1.1 versus 2.8 ± 1.3, p= 0.001).

pNfL in MS patients and controls

Mean level of pNfL in MS was 14.73 ± 6.38 pg/ml (range 6-34 pg/ml). We did not find any significant difference in pNfL levels between males and females (13.72 ± 6.57 pg/ml versus 15.20 ± 6.31 pg/ml, p= 0.43).
We failed to find any significant difference in a subgroup of the patients with MRI activity compared to the group of patients without MRI activity (17.02 ± 9.14 pg/ml versus 13.81 ± 4.71 pg/ml, p= 0.09). The mean age of the subgroup with MRI activity was significantly lower compared to the subgroup without MRI activity (35.38 ± 7.46 years versus 43.85 ± 11.52 years, p= 0.009).

Mean pNfL level in the control group was 6.67 ± 3.9 pg/ml (range 2.2-18.8 pg/ml). We did not find any significant difference in pNfL levels between males and females (6.36 ± 6.0 pg/ml, versus 7.9 ± 3.4 pg/ml). In control group, pNfL levels significantly correlated with

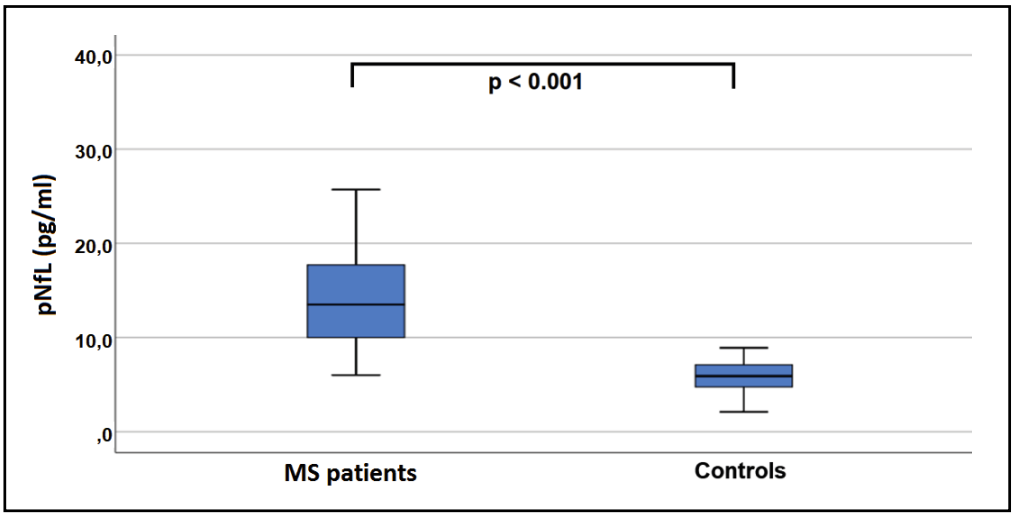

Fig. 2. Levels of pNfL in MS patients and controls.

age (r= 0.63, p <0.001). pNfL levels in our group of MS patients were significantly higher when compared to the control group (14.73 ± 6.37 pg/ml versus 6.67 ± 3.9 pg/ml, p <0.001), see Figure 2. The levels of pNfL in subgroup with Wbv atrophy was significantly higher than a levels in subgroup without atrophy (16.84 ± 6.8 versus 13.02 ± 5.5, p= 0.025). Similarly, the levels of pNfL in subgroup with Gmv atrophy was significantly higher than a levels of pNfL in subgroup without atrophy (17.65 ± 6.0 versus 13.6 ± 6.0, p= 0.037).

MRI data

Our data from MRI in treated RRMS patients present Table 2.

Correlation between pNfL, baseline characteristics, and MRI data

The level of pNfL statistically significantly positively correlated with EDSS (r= 0.377, p= 0.004), disease duration (r= 0.298, p= 0.026), atrophy of Wbv (r= 0.317, p= 0.017), atrophy of Gmv (r= 0.302, p= 0.020), and statis-

Tab. 2. MRI data of MS patients

	n	Value
Whole brain volume (Wbv)(ml)	56	1487 ± 91.5
Normative percentile of Wbv	56	5.65 (31.3, 0.8-91)
Grey matter volume (Gmv)(ml)	56	896 ± 51.3
Normative percentile of Gmv	56	20.80 (50.18, 0.8-96.8)
Number of enhancing lesions	56	0.00 (0.00, 0-4)
Number of new T2 lesions	56	0.00 (1.0, 0-18)
Volume of FLAIR lesions (ml)	56	9.38 (12.83, 0.6-28.7)
Volume of enhancing lesions (ml)	40	0.00 (0.02, 0-2.06)
Volume of T1 lesions (ml)	52	6.11 (8.34, 0.09-24)
MRI activity - present		16 (28.6%)
MRI activity - non-present		40 (71.4%)
Atrophy of Wbv - present		25 (44.6%)
Atrophy of Wbv - non-present		31 (55.4%)
Atrophy of Gmv - present		15 (26 .8%)
Atrophy of Gmv - non-present		41 (73.2%)

Categorical variables expressed as number and proportions (%), Continuous variable as mean with standard deviation, or median, interquartile range, Minimal and maximal values.

Tab. 3. Correlation between pNfL, baseline characteristics, and MRI data

	n	rho	Sig. (2-tailed)
Age	56	0.233	0.084
EDSS	56	0.377 **	0.004
Disease duration	56	0.298 *	0.026
Whole brain volume (Wbv)(ml)	56	-0.270 *	0.044
Normative percentile of Wbv	56	-0.240	0.075
Grey matter volume (Gmv)(ml)	56	-0.222	0.100
Normative percentile of Gmv	56	-0.147	0.280
Number of enhancing lesions	56	-0.082	0.549
Number of new T2 lesions	56	0.157	0.246
Volume of FLAIR lesions (ml)	56	0.191	0.158
Volume of enhancing lesions (ml)	40	0.054	0.739
Volume of T1 lesions (ml)	52	0.194	0.168
Atrophy of Wbv	56	0.317 *	0.017
Atrophy of Gmv	56	0.302 *	0.020

** p <0.01,* p <0.05, rho: Spearman's correlation coefficient.

tically significantly negatively correlated with Wbv (r= -0.270, p= 0.044). We failed to find any statistically significant correlation between pNfL and MRI activity, the number of new T2 lesions and new enhancing lesions. Correlation with age was positive, but not significant (p= 0.084), see Table 3.

Correlation between EDSS, baseline characteristics, and MRI data

EDSS statistically significantly correlated with pNfL, age, disease duration, FLAIR lesion volume, the volume of T1 lesions, and with the presence of Wbv and Gmv atrophy. EDSS statistically significantly negatively correlated with Wbv, normative percentile of Wbv, and Gmv (Table 4).

Correlation between Wbv, baseline characteristics, and MRI data

Wbv statistically significantly negatively correlated with age, disease duration, and pNfL level. Wbv statistically significantly correlated with the number of enhancing lesions, and the number of new T2 lesions. Wbv sta-

Tab. 4. Correlation between EDSS, baseline characteristics, and MRI data

	n	rho	Sig. (2-tailed)
Age	56	0.580 **	0.001
pNfl	56	0.377 **	0.004
Disease duration	56	0.485 **	< 0.001
Whole brain volume (Wbv)(ml)	56	-0.501 **	< 0.001
Normative percentile of Wbv	56	-0.302 *	0.024
Grey matter volume (Gmv)(ml)	56	-0.453 **	< 0.001
Normative percentile of Gmv	56	-0.236	0.080
Number of enhancing lesions	56	-0.152	0.265
Number of new T2 lesions	56	-0.188	0.164
Volume of FLAIR lesions (ml)	56	0.302 *	0.024
Volume of enhancing lesions (ml)	40	-0.117	0.473
Volume of Tl lesions (ml)	52	0.275 *	0.049
Atrophy of Wbv	56	0.395 **	0.003
Atrophy of Gmv	56	0.405 **	0.002

** p <0.01,* p <0.05, rho: Spearman's correlation coefficient.

Tab. 5. Correlation between Wbv, baseline characteristics, and other MRI data

	n	rho	Sig. (2-tailed)
Age	56	-0.491 **	0.001
pNfl	56	-0.270 *	0.044
EDSS	56	-0.501 * *	< 0.001
MRI activity	56	0.306 *	0.022
Disease duratio	56	-0.371 * *	0.005
Normative percentile of Wbv	56	0.863 **	< 0.001
Grey matter volume (Gmv)(ml)	56	0.843 **	< 0.001
Normative percentile of Gmv	56	0.670 **	< 0.001
Number of enhancing lesions	56	0.270 *	0.045
Number of new T2 lesions	56	0.317 *	0.017
Volume of FLAIR lesions (ml)	56	-0.682 * *	< 0.001
Volume of enhancing lesions (ml)	40	0.259	0.106
Volume of T1 lesions (ml)	52	-0.664 * *	< 0.001

** p <0.01,* p <0.05, rho: Spearman's correlation coefficient.

tistically significantly negatively correlated with FLAIR lesions volume and volume of T1 lesions (Table 5).

Gmv was statistically significantly associated with EDSS (r= 0.563 p <0.001). Gmv statistically significantly negatively correlated with age (r= -0.654, p <0.001), with FLAIR lesions volume (r= -0.507, p <0.001), and volume of T1 lesions (r= -0.470, p <0.001).

According to the multiple linear regression analysis we found, that the EDSS and volume of enhancing lesions were the only independent variables significantly associated with the levels of pNfL (Table 6).

DISCUSSION

MS is characterized by both the inflammatory and neurodegenerative processes (Giorgio & De Stefano, 2018). For a long time, it has been regarded as a chronic inflammatory disease of the cerebral and spinal white matter that leads to demyelination and eventually to neurodegeneration. In the past decade, several aspects of MS pathogenesis have been challenged, and degenerative changes, especially of the cerebral grey matter, which are independent of demyelination, have become a topic of interest (Mandolesi *et al.* 2015).

In our study, we focused on the use of pNfL in MS patients, in whom it could serve as a promising blood-based marker of neuro-axonal damage in MS, and predictor of brain volume loss (Charil & Filippi, 2007; Disanto *et al.* 2017; Novakova *et al.* 2018; Piehl *et al.* 2018; de Flon *et al.* 2019; Kuhle *et al.* 2019; Sormani *et al.* 2019).

To date, little has been reported about the association of volumetric data using Icobrain concerning pNfL, EDSS, and other parameters in treated RRMS patients.

Tab. 6. Multiple regression analysis, dependent variable pNfL

	Unstandardized Coefficients		Std. Coefficients			95.0% Confidence Interval for B	
	B	Std. Error	Beta	t	*p*	Lower Bound	Upper Bound
EDSS	1.816	0.857	0.418	2 .118	0.043 *	0.060	3.573
Whole brain volume (Wbv)(ml)	-0.014	0.023	-0.193	-0.621	0.539	-0.061	0.032
Grey matter volume (Gmv)(ml)	0.027	0.043	0.213	0.636	0.530	-0.060	0.114
Number of enhancing lesions	-2.318	1.514	-0.307	-1.531	0.137	-5.421	0.784
Number of new T2 lesions	0.456	0.419	0.242	1.088	0.286	-0.403	1.315
Volume of FLAIR lesions (ml)	0.324	0.596	0.414	0.544	0.591	-0.897	1.546
Volume of enhancing lesions (ml)	6.643	2.912	0.359	2.281	0.030 *	0.678	12.608
Volume of T1 lesions (ml)	-0.465	0.700	-0.486	-0.665	0.512	-1.898	0.968

We searched for the relationship of pNfL levels with the baseline characteristics of the treated MS patients, as well as with MRI volumetric data. We were interested, whether pNfL levels are related to the MRI volumetric data obtained by Icobrain software. Similarly, we searched for the association of EDSS with volumetric data from Icobrain.

The first significant observation in our study was significantly higher levels of pNfL in RRMS patients compared with controls, even if they were treated and clinically without attack. pNfL levels were significantly higher also in RRMS patients without MRI activity compared to controls. This fact confirms the assumption of ongoing neuroaxonal damage independent of inflammatory activity in MS.

We failed to find any significant association of pNfL levels with age and gender, which is consistent with recent studies (Barro *et al.* 2018; Siller *et al.* 2019).

Another significant observation is the positive correlation of pNfL levels with EDSS, which was also reported in several published works (Kuhle *et al.* 2017; Barro *et al.* 2018). In multiple linear regression analysis, EDSS belong to the independent variables significantly associated with pNfL levels. According to our results, we suggest that pNfL levels could be influenced by the extent of the neurodegenerative process and could reflect the clinical severity of the disease.

NfL in MS has been studied in association with neuro-axonal damage because their levels are elevated during all stages of MS. They could play a role as a prognostic factor and could be related to the treatment response (Håkansson *et al.* 2017; Novakova *et al.* 2017; Piehl *et al.* 2018).

Many studies have reported that pNfL levels correlate with the number of T2 lesions and the number of enhancing lesions (Disanto *et al.* 2017; Novakova *et al.* 2017; Barro *et al.* 2018; Håkansson *et al.* 2018; Kuhle *et al.* 2019). It could be suggested that pNfL is attributed to be the marker of disease activity in MS. We did not find any correlation between pNfL and MRI activity, the number of new T2 lesions, and the number of enhancing lesions. We suggested, this may be caused by the fact, we enrolled DMT treated patients without clinical attacks. In our study, 28.6% of patients have MRI activity, with higher levels of pNfL, compared to the patients without MRI activity, but this difference was not statistically significant.

We found a significantly positive association of levels pNfL with atrophy of Wbv, atrophy of Gmv, and inverse association with Wbv, possibly resulting from the ongoing process of neurodegeneration during the disease, and found no significant association of levels pNfL with age.

The brain volume loss in MS patients is not only due to the aging, but also neurodegeneration caused by MS. The relationship between brain atrophy and pNfL in MS patients has already been reported in several publications (Kuhle *et al.* 2017; Barro *et al.* 2018; Håkansson *et al.* 2018; Siller *et al.* 2019). NfL is consider as a predictor of future brain atrophy, what is in concordance with another significant observation in our study, that the levels of pNfL in patients with Wbv and Gmv atrophy have significantly higher levels of pNfL compared with patients without atrophy.

We did not find any correlation between EDSS and MRI activity. These results support the growing evidence that the extent of clinical disability does not correlate with the number of MRI white matter lesions.

The interesting result of our study is the significant correlation of Wbv with MRI activity, which could be caused by an inflammatory reaction, leading to a temporary increase of brain volume, causing vasogenic edema and immune cells accumulation (Zivadinov *et al.* 2016). In our study, it is most likely because the age in group of patients with MRI activity was significantly lower compared to the group of patients without MRI activity because the Wbv significantly negatively correlated with age.

According to multiple authors, the extent of total axonal injury and brain atrophy correlate with the disease duration and disability. It also strongly correlates with clinical disease progression and could serve as a strong predictor of EDSS worsening (Paolillo *et al.* 2000; Zivadinov *et al.* 2013; Rocca *et al.* 2017).

Considerable finding in our study is a strong correlation between EDSS with almost all brain volumetric data obtained from Icobrain. This finding is consistent with the recently published work, where authors compared normalization of brain atrophy by program Icobrain in MS patients (Beadnall *et al.* 2019).

T1 hypointense lesions belonged to the first MRI measure of neurodegeneration. The relationship between the black holes and disability was assessed in several studies. Higher EDSS were significantly associated with higher numbers and volume of the black holes (Giorgio *et al.* 2014; Filippi, 2015).

It has been reported that the extent of total axonal damage in process of neurodegeneration displayed by MRI shows a strong correlation with clinical progression and is a strong predictor of irreversible disability, expressed by the value of EDSS (Yabe *et al.* 2001; Bakshi *et al.* 2008; Filippi *et al.* 2013; Rocca *et al.* 2017).

Clinical disability worsening in MS patients is not presented only changes in white matter, default observed in conventional MRI. Many studies confirm that cerebral grey matter damage and subsequent grey matter atrophy can be relevant for the development of disability in MS patients (Calabrese *et al.* 2013; Coghe *et al.* 2018). We also found a significant correlation between Gmv and EDSS, which points out that the patients with Gmv atrophy had a significantly worse EDSS.

In MS patients, the brain volume loss per year is significantly higher compared to the healthy gender and age-matched controls (0.5-1.3%, versus 0.1-0.4%). Currently is accepted that the brain volume loss reflects grey

matter atrophy rather than white matter atrophy. Grey matter atrophy shows that the some regions are more susceptible to atrophy than others. The results of studies suggest that early pathomechanisms in MS are relatively homogeneous, while diversity at later stages leads to heterogeneity of disease course. Described as the first regions which have become atrophic in MS patients is the posterior cingulate cortex, precuneus, thalamus, and brainstem. This regions were consistently identified in relapsing and progressive MS, with the addition of insula, accumbens, and caudate. In patients with RRMS, the spread of atrophy was associated with disease duration and worsening of EDSS (Eshaghi *et al.* 2018). This facts proposes a new look at the natural history of neurodegeneration in MS, arguing that it can be viewed as an ordered sequential process, translated into grey matter atrophy progression over time (Stankoff & Louapre, 2018).

In our study used Icobrain software for MRI volumetry, not provide us report of regional grey matter atrophy. That does not allow us to explore and compare these new findings in regional grey matter atrophy in our patients.

Our study had some limitations. We are missing longitudinal observations regarding pNfL levels and MRI parameters. We don't design the study to investigate the treatment effects of DMT. We suppose that the presence of newly diagnosed patients without treatment could help to elucidate studied associations, especially pNfL, as a marker of disease activity.

Ongoing, not yet published, is our study about pNfL in newly diagnosed RRMS patients. In this group is high rate of patients with enhancing lesions. We observed in group of newly diagnosed RRMS patients with enhancing lesions statistically significantly higher level of pNfL compared to the patients without enhancing lesions. Number of enhancing lesions statistically significantly correlated with level of pNfL. These results are in line of many previous studies of NfL as marker of disease activity. Future longitudinal data is needed.

CONCLUSION

We found that pNfL levels in a group of patients with RRMS treated with DMT are significantly higher compared to the healthy controls. Their levels do not significantly correlate with disease activity. On the other hand, pNfL levels significantly correlated with EDSS, atrophy of Wbv, atrophy of Gmv, and with Wbv. In our study, we found that EDSS, and volume of enhancing MRI lesions were the only independent variables significantly associated with pNfL levels in multiple linear regression analysis. Multiple volume parameters obtained from Icobrain, including Wbv, Gmv, atrophy of Wbv, and Gmv, the volume of FLAIR lesions and volume of T1 lesions significantly correlated with EDSS. We failed to find any significant association between EDSS, number, and volume of new T2 and enhancing lesions.

Our findings suggest that RRMS patients treated with DMT suffer brain volume loss associated with disability worsening. Therefore, the process of neurodegeneration seems to play an important role in MS. pNfL levels could be considered as a biomarker of neurodegeneration similarly to MRI volumetric parameters. Compared to MRI examination, monitoring of NfL levels is time-saving and less costly.

CONFLICT OF INTEREST

The authors reported no potential conflict of interest.

GRANT SUPPORT

The APVV-15-0228 grant supported this research.

REFERENCES

1 Bakshi R, Thompson AJ, Rocca MA, Pelletier D, Dousset V, Barkhof F, et al (2008). MRI in multiple sclerosis: current status and future prospects. Lancet Neurol. **7**: 615–25.
2 Barro C, Benkert P, Disanto G, Tsagkas C, Amann M, Naegelin Y, et al (2018). Serum neurofilament as a predictor of disease worsening and brain and spinal cord atrophy in multiple sclerosis. Brain. **141**: 2382–2391.
3 Beadnall HN, Wang C, Van Hecke W, Ribbens A, Billiet T, Barnett MH (2019). Comparing longitudinal brain atrophy measurement techniques in a real-world multiple sclerosis clinical practice cohort: towards clinical integration? Ther Adv Neurol Disord. **2019**: 12.
4 Calabrese M, Romualdi C, Poretto V, Favaretto A, Morra A, Rinaldi F, et al (2013). The changing clinical course of multiple sclerosis: a matter of gray matter. Ann Neurol. **74**: 76–83.
5 Charil A, Filippi M (2007). Inflammatory demyelination and neurodegeneration in early multiple sclerosis. J Neurol Sci. **259**: 7–15.
6 Coghe G, Fenu G, Lorefice L, Zucca E, Porta M, Pilloni G, et al (2018). Association between brain atrophy and cognitive motor interference in multiple sclerosis. Mult Scler Relat Disord. **25**: 208–211.
7 de Flon P, Laurell K, Sundström P, Blennow K, Söderström L, Zetterberg H, et al (2019). Comparison of plasma and cerebrospinal fluid neurofilament light in a multiple sclerosis trial. Acta Neurol Scand. **139**: 462–468.
8 Disanto G, Barro C, Benkert P, Naegelin Y, Schädelin S, Giardiello A, et al (2017). Swiss Multiple Sclerosis Cohort Study Group. Serum Neurofilament light: A biomarker of neuronal damage in multiple sclerosis. Ann Neurol. **81**: 857–870.
9 Eshaghi A, Marinescu RV, Young AL, Firth NC, Prados F, Jorge Cardoso M, et al (2018). Progression of regional grey matter atrophy in multiple sclerosis. Brain. **141**: 1665–1677.
10 Filippi M, Rocca MA, De Stefano N, Enzinger C, Fisher E, Horsfield MA, et al (2011). Magnetic resonance techniques in multiple sclerosis: the present and the future. Arch Neurol. **68**: 1514–20.
11 Filippi M, Preziosa P, Copetti M, Riccitelli G, Horsfield MA, Martinelli V, et al (2013). Gray matter damage predicts the accumulation of disability 13 years later in MS. Neurology. **81**: 1759–67.
12 Filippi M (2015). MRI measures of neurodegeneration in multiple sclerosis: implications for disability, disease monitoring, and treatment. J Neurol. **262**: 1–6.
13 Giorgio A, Stromillo ML, Bartolozzi ML, Rossi F, Battaglini M, De Leucio A, et al (2014). Relevance of hypointense brain MRI lesions for long-term worsening of clinical disability in relapsing multiple sclerosis. Mult Scler. **20**: 214–9.
14 Giorgio A, De Stefano N (2018). Effective Utilization of MRI in the Diagnosis and Management of Multiple Sclerosis. Neurol Clin. **36**: 27–34.

15 Håkansson I, Tisell A, Cassel P, Blennow K, Zetterberg H, Lundberg P, et al (2017). Neurofilament light chain in cerebrospinal fluid and prediction of disease activity in clinically isolated syndrome and relapsing-remitting multiple sclerosis. Eur J Neurol. **24**: 703–712.
16 Håkansson I, Tisell A, Cassel P, Blennow K, Zetterberg H, Lundberg P, et al (2018). Neurofilament levels, disease activity and brain volume during follow-up in multiple sclerosis. J Neuroinflammation. **15**: 209.
17 Jain S, Sima DM, Ribbens A, Cambron M, Maertens A, Van Hecke W, et al (2015). Automatic segmentation and volumetry of multiple sclerosis brain lesions from MR images. Neuroimage Clin. **8**: 367–75.
18 Koudriavtseva T, Mainero C (2016). Neuroinflammation, neurodegeneration and regeneration in multiple sclerosis: intercorrelated manifestations of the immune response. Neural Regen Res. **11**: 1727–1730.
19 Kuhle J, Barro C, Andreasson U, Derfuss T, Lindberg R, Sandelius Å, et al (2016). Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. Clin Chem Lab Med. **54**: 1655–61.
20 Kuhle J, Nourbakhsh B, Grant D, Morant S, Barro C, Yaldizli Ö, et al (2017). Serum neurofilament is associated with progression of brain atrophy and disability in early MS. Neurology. **88**: 826–831.
21 Kuhle J, Kropshofer H, Haering DA, Kundu U, Meinert R, Barro C, et al (2019). Blood neurofilament light chain as a biomarker of MS disease activity and treatment response. Neurology. **92**: e1007–e1015.
22 Kurtzke JF (1983). Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. **33**: 1444–52.
23 Lövblad KO, Anzalone N, Dörfler A, Essig M, Hurwitz B, Kappos L, et al (2010). MR imaging in multiple sclerosis: review and recommendations for current practice. AJNR Am J Neuroradiol. **31**: 983–9.
24 Mandolesi G, Gentile A, Musella A, Fresegna D, De Vito F, Bullitta S, et al (2015). Synaptopathy connects inflammation and neurodegeneration in multiple sclerosis. Nat Rev Neurol. **11**: 711–24.
25 Novakova L, Zetterberg H, Sundström P, Axelsson M, Khademi M, Gunnarsson M, et al (2017). Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. Neurology. **89**: 2230–2237.
26 Novakova L, Axelsson M, Malmeström C, Imberg H, Elias O, Zetterberg H, et al (2018). Searching for neurodegeneration in multiple sclerosis at clinical onset: Diagnostic value of biomarkers. PLoS One. **13**: e0194828.
27 Paolillo A, Pozzilli C, Gasperini C, Giugni E, Mainero C, Giuliani S, et al (2000). Brain atrophy in relapsing-remitting multiple sclerosis: relationship with 'black holes', disease duration and clinical disability. J Neurol Sci. **174**: 85–91.
28 Piehl F, Kockum I, Khademi M, Blennow K, Lycke J, Zetterberg H, et al (2018). Plasma neurofilament light chain levels in patients with MS switching from injectable therapies to fingolimod. Mult Scler. **24**: 1046–1054.
29 Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, et al (2011). Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol. **69**: 292–302.
30 Rocca MA, Battaglini M, Benedict RH, De Stefano N, Geurts JJ, Henry RG, et al (2017). Brain MRI atrophy quantification in MS: From methods to clinical application. Neurology. **88**: 403–413.
31 Siller N, Kuhle J, Muthuraman M, Barro C, Uphaus T, Groppa S, et al (2019). Serum neurofilament light chain is a biomarker of acute and chronic neuronal damage in early multiple sclerosis. Mult Scler. **25**: 678–686.
32 Sormani MP, Haering DA, Kropshofer H, Leppert D, Kundu U, Barro C, et al (2019). Blood neurofilament light as a potential endpoint in Phase 2 studies in MS. Ann Clin Transl Neurol. **6**: 1081–1089.
33 Stankoff B, Louapre C (2018). Can we use regional grey matter atrophy sequence to stage neurodegeneration in multiple sclerosis? Brain. **141**: 1580–1583.
34 Teunissen CE, Khalil M (2012). Neurofilaments as biomarkers in multiple sclerosis. Mult Scler. **18**: 552–6.
35 Wang C, Beadnall HN, Hatton SN, Bader G, Tomic D, Silva DG, et al (2016). Automated brain volumetrics in multiple sclerosis: a step closer to clinical application. J Neurol Neurosurg Psychiatry. **87**: 754–7.
36 Wattjes MP, Rovira À, Miller D, Yousry TA, Sormani MP, de Stefano MP, et al (2015). Evidence-based guidelines: MAGNIMS consensus guidelines on the use of MRI in multiple sclerosis- establishing disease prognosis and monitoring patients. Nat Rev Neurol. **11**: 597–606.
37 Yabe JT, Chylinski T, Wang FS, Pimenta A, Kattar SD, Linsley MD, et al (2001). Neurofilaments consist of distinct populations that can be distinguished by C-terminal phosphorylation, bundling, and axonal transport rate in growing axonal neurites. J Neurosci. **21**: 2195–205.
38 Zivadinov R, Bergsland N, Dolezal O, Hussein S, Seidl Z, Dwyer MG, et al (2013). Evolution of cortical and thalamus atrophy and disability progression in early relapsing-remitting MS during 5 years. AJNR American journal of neuroradiology. **34**: 1931–9.
39 Zivadinov R, Jakimovski D, Gandhi S, Ahmed R, Dwyer MG, Horakova D, et al (2016). Clinical relevance of brain atrophy assessment in multiple sclerosis. Implications for its use in a clinical routine. Expert Rev Neurother. **16**: 777–93.
40 Zivadinov R, Uher T, Hagemeier J, Vaneckova M, Ramasamy DP, Tyblova M, et al (2016). A serial 10-year follow-up study of brain atrophy and disability progression in RRMS patients. Mult Scler. **22**: 1709–1718.