Chitinase 3–like 1 and neurofilament light chain in CSF and CNS atrophy in MS

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Abstract

Objective

To investigate cross-sectional associations of CSF levels of neurofilament light chain (NfL) and of the newly emerging marker chitinase 3–like protein 1 (CHI3L1) with brain and spinal cord atrophy, which are established MRI markers of disease activity in MS, to study CHI3L1 and NfL in relapsing (RMS) and progressive MS (PMS), and to assess the expression of CHI3L1 in different cell types.

Methods

In a single-center study, 131 patients with MS (42 RMS and 89 PMS) were assessed for NfL and CHI3L1 concentrations in CSF, MRI-based spinal cord and brain volumetry, MS subtype, age, disease duration, and disability. We included 42 matched healthy controls receiving MRI. CHI3L1 expression of human brain cell types was examined in 2 published single-cell RNA sequencing data sets.

Results

CHI3L1 was associated with spinal cord volume (B = −1.07, 95% CI −2.04 to −0.11, $p = 0.029$) but not with brain volumes. NfL was associated with brain gray matter (B = −7.3, 95% CI −12.0 to −2.7, $p = 0.003$) but not with spinal cord volume. CHI3L1 was suitable to differentiate between progressive or relapsing MS ($p = 0.015$, OR 1.0103, CI for OR 1.002–1.0187), and its gene expression was found in MS-associated microglia and macrophages and in astrocytes of MS brains.

Conclusions

NfL and CHI3L1 in CSF were differentially related to brain and spinal cord atrophy. CSF CHI3L1 was associated with spinal cord volume loss and was less affected than NfL by disease duration and age, whereas CSF NfL was associated with brain gray matter atrophy. CSF NfL and CHI3L1 measurement provides complementary information regarding brain and spinal cord volumes.

Classification of evidence

This study provides Class II evidence that CSF CHI3L1 is associated with spinal cord volume loss and that CSF NfL is associated with gray matter atrophy.

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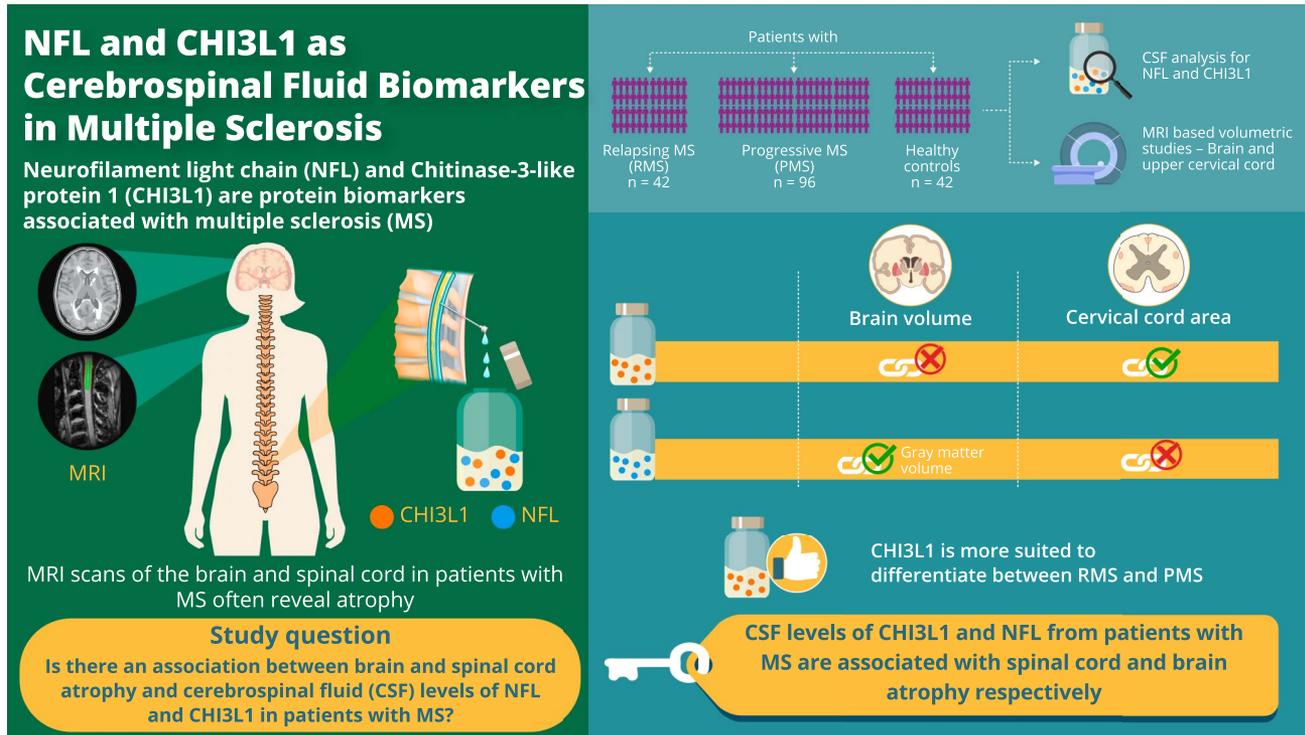
Glossary

BGM = brain gray matter; **BWM** = brain white matter; **CHI3L1** = chitinase 3–like protein 1; **CIS** = clinically isolated syndrome; **DD** = disease duration; **EDSS** = Expanded Disability Severity Scale; **HC** = healthy control; **MUCCA** = mean upper cervical cord area; **NfL** = neurofilament light chain; **PMS** = progressive MS; **RMS** = relapsing MS; **RRMS** = relapsing-remitting MS; **scRNA-seq** = single-cell RNA sequencing; **T1w** = T1 weighted.

NFL and CHI3L1 as Cerebrospinal Fluid Biomarkers in Multiple Sclerosis

Neurofilament light chain (NFL) and Chitinase-3-like protein 1 (CHI3L1) are protein biomarkers associated with multiple sclerosis (MS)

MRI scans of the brain and spinal cord in patients with MS often reveal atrophy

Study question
Is there an association between brain and spinal cord atrophy and cerebrospinal fluid (CSF) levels of NFL and CHI3L1 in patients with MS?

CSF levels of CHI3L1 and NFL from patients with MS are associated with spinal cord and brain atrophy respectively

CHI3L1 is more suited to differentiate between RMS and PMS

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In recent years, MS research has progressed, especially in the field of immune therapies of MS, raising the need for reliable markers of individual disease activity and treatment response.[1]

Neurofilament (NF) proteins, as part of the neuronal cytoskeleton,[2] have been proposed as markers of neuronal damage in MS and various other neurologic disorders. Because neuroaxonal degeneration causes their release into the adjacent body fluid compartment, NF levels can be detected in the CSF and in the blood serum. In MS, associations have been reported between NF light chain (NfL) levels and disease activity, progression, and long-term clinical outcome.[3,4] Increased CSF NfL levels also predicted the risk of conversion from a clinically isolated syndrome (CIS) to relapsing-remitting MS (RRMS).[5] Recently, the relevance of serum NfL for discerning CIS from newly diagnosed MS has been demonstrated[6]; however, CSF NfL displayed a higher sensitivity for disease activity than serum NfL.[7,8] Moreover, NfL levels were related with MRI-derived markers, such as brain MS lesion load, atrophy of the brain and spinal cord, and with previous radiologic disease activity.[4,7,9,10]

Besides NfL, other CSF markers of tissue damage have been proposed in MS, e.g., chitinase 3–like protein 1 (CHI3L1), a glycoprotein which is secreted by activated glia cells in the CNS. Increased CHI3L1 levels have been associated with disease progression[11,12] and with conversion from CIS to MS.[13] Recently, the benefit of combining NFL and CHI3L1 measures in CSF to discriminate MS phenotypes and to predict disease progression in RRMS was demonstrated.[14]

In the present study on patients with MS, we studied the associations of CHI3L1 and NfL levels in CSF with atrophy of the brain and spinal cord. Brain and spinal cord atrophy served as established paraclinical markers of disease progression that are known to be associated with axonal degeneration and demyelination in early and chronic MS subtypes.[15,16] We hypothesized that the CSF CHI3L1 and NfL levels were related to both brain and spinal cord atrophy. A second aim was to test whether CSF levels of NfL and CHI3L1 acquired in the mixed cohort of

Table 1 Demography and MRI results of patients and HCs, clinical status, and CSF results for patients and patient subgroups

Variable (unit); No. of participants in the groups	All patients (n = 131)	RMS (n = 42)	PMS (n = 89)	HC (n = 42)	Within-group p value
Female (%)	57	55	57	48	0.582[a]
Age (y)	49.9 (10.9); <0.001[b]	42.2 (12.7); 0.552[b]	53.7 (7.6); <0.001[b]; 0.001[d]	39.4 (11.4)	<0.001[c]
EDSS	5.5 [1.0, 8.5]	3.0 [1.0, 6.5]	6.0 [3.0, 8.5]; <0.001[d]	n.a.	—
Disease duration (y)	12 (10)	7 (9)	15 (9); <0.001[d]	n.a.	—
CHI3L1 (ng/mL); 126/37/89/—	211 [96, 342]	186 [96, 282]	227 [104, 342]; 0.001[d]	n.a.	—
NfL (pg/mL); 93/34/59/—	1,038 [252, 6,617]	857 [252, 5,590]	1,319 [358, 6,617]; 0.008[d]	n.a.	—
MUCCA (mm²); 130/41/89/36	67.9 (11.3); <0.001[b]	75.3 (12.0); 0.248[b]	64.5 (9.3); <0.001[b]; <0.001[d]	78.8 (6.7)	<0.001[c]
BGM (mL); 109/29/80/42	577.1 (62.1); 0.001[b]	599.1 (43.7); 0.502[b]	569.2 (65.9); <0.001[b]; 0.021[d]	609.9 (33.3)	<0.001[c]
BWM (mL); 110/30/80/42	571.3 (47.6)	577.4 (47.2)	569.1 (47.9)	583.8 (30.2)	0.196[c]
BLL (mL); 111/31/80/—	9.6 [0, 91]	2 [0, 89]	14 [0, 91]; <0.001[d]	n.a.	—

Abbreviations: BGM = brain gray matter volume normalized to intracranial cavity volume, corrected for physiologic aging; BLL = brain lesion load (FLAIR contrast); BWM = brain white matter volume normalized to intracranial cavity volume, corrected for physiologic aging; CHI3L1 = chitinase 3–like 1 protein in CSF; EDSS = Expanded Disability Severity Scale; HC = healthy control; max = maximum; min = minimum; MUCCA = mean upper cervical cord area normalized to C1-C3 length; n.a. = not applicable; NfL = neurofilament light in CSF; PMS = progressive MS; RMS = relapsing MS.
EDSS, CHI3L1, NfL, and BLL presented as median [min/max]; MUCCA, BGM, and BWM presented as mean (SD).
[a] p Value of group differences assessed by the χ^2 test.
[b] p Value of comparison patients vs controls (all patients vs HCs: 1-factorial analysis of variance; RMS or PMS vs HC: post hoc pairwise comparisons of "c").
[c] p Value of comparison within groups (HC, RMS, and PMS) using 1-factorial analysis of variance with post hoc pairwise Games-Howell tests.
[d] p Value of comparison PMS vs RMS by Mann-Whitney U tests.

patients with relapsing MS or progressive MS can distinguish between these 2 subtypes. Finally, the expression of CHI3L1 in different cell types from healthy and MS brains was examined to further investigate its relevance with respect to MS pathogenesis.

Methods

Participants

A total of 131 patients (table 1) with clinically definite MS or CIS with subsequent conversion to MS,[17] availability of CSF samples for assessment of CHI3L1 and NfL, and an associated MRI examination were assessed for the analysis. Clinical data, i.e., Expanded Disability Severity Scale (EDSS) and disease duration (DD) at the times of CSF assessment and MRI, were extracted from the patient files.

For the evaluation of CNS atrophy, MRI data of 42 healthy controls (HCs), which had been acquired with the same scanner and identical MRI protocol as the patients, were included from a preexisting in-house MRI database. Patient and control recruitment was performed over a 4-year period from 2011 to 2014.

Standard protocol approvals, registrations, and patient consents

The study protocol was approved by the ethics committee of the Medical Faculty of the Ruhr University Bochum (Approval No.

15-5268) and the research ethics boards of all participating centers. All individuals gave their written informed consent according to the Declaration of Helsinki.

CSF analysis and NfL/CHI3L1 measurements

The concentrations of CHI3L1 (also called YKL-40) and NfL in CSF were analyzed using the assays MicroVueTM YKL-40 EIA (Quidel, San Diego, CA) and NF-light ELISA (IBL International, Hamburg, Germany), respectively. The limit of detection was 5.4 ng/mL for CHI3L1 and 32 pg/mL for NfL. The intra- and interassay coefficients of variation for Micro-VueTM YKL-40 EIA were <5.6%–6.6% and <6.0%–7.0%. The intra- and interassay coefficients of variation for NF-light ELISA were <5% and <9%.

MRI and quantitative image analysis

All participants underwent MRI scans at a single 1.5T scanner, which included 3D T1-weighted (T1w) and T2-weighted fluid-attenuated inversion recovery imaging. The MRI protocol is provided in the electronic supplement (links.lww.com/NXI/A329).

The MRI analysis aimed at quantification of the mean upper cervical cord area (MUCCA), the volumes of brain gray and white matter (BGM and BWM), and the brain MS lesion load. MUCCA was calculated on the basis of the T1w brain image series using a previously described semi-automated method and normalized relative to the distance between the C1 and

C3 vertebrae to compensate for physiologic differences related to body size.[15]

To calculate volumes of BGM and BWM, we used the VBM8 preprocessing and segmentation tools with default parameters.[18] All 3D T1w series were corrected for WM lesions before volumetric analysis using the lesion growths algorithm as implemented in the LST toolbox (version 1.2.2).[19] Intracranial cavity volumes were determined by summation of the volumes of GM, WM, and CSF.

We normalized BGM and BWM with regard to head size differences by normalization according to the intracranial cavity volume and corrected for physiologic aging effects compared with HC.

Expression of CHI3L1 in different cell types from healthy and MS brains

CHI3L1 expression of the human brain was examined in 2 published single-cell RNA sequencing (scRNA-seq) data sets (Jäkel et al. and Masuda et al. data sets).[20,21] Both data sets were analyzed using the RaceID algorithm. The Jäkel et al.[20] data set was prepared from white matter areas of postmortem human brain from patients with MS and from unaffected controls using a nuclear sequencing protocol. It provided an overview of different cell types from healthy and MS brains and was acquired from different MS lesions.[20] The findings of the Jäkel et al. data set were validated in an scRNA-seq data set that was acquired from stereotactic biopsies and fluorescence-activated cell sorting sorted for CD45^{+} cells of patients with MS and HCs (Masuda et al. data set).[21] This data set contained cells from chronically active lesions. The results were grouped into 10 clusters (Hu-C1 to Hu-C10), representing the transcriptome signatures of different cell types.

Statistical analysis

Association study between MRI and CHI3L1 and NfL concentrations in CSF

We used IBM SPSS for Windows version 24 (IBM Corp., Armonk, NY) for statistical analyses. Results were considered statistically significant at $p < 0.05$.

All parameters were tested for normality with a Kolmogorov-Smirnov test. Intergroup comparisons of CNS volumes, age, and DD were assessed using 1-way analysis of variance (normally distributed data), with post hoc pairwise tests adjusted for multiple comparisons with Games-Howell correction when more than 2 groups were compared. Intergroup differences of relapsing MS (RMS) and progressive MS (PMS) regarding EDSS, brain lesion load, and CHI3L1 and NfL levels were studied by using Mann-Whitney U tests (ordinal or not normally distributed data). We used χ^2 tests to investigate differences in the male-female distribution within the subgroups.

Because we detected no differences related to sex in the variables (CHI3L1, NfL, age, DD, EDSS, MUCCA, BGM, or BWM by using Mann-Whitney U tests or Student t tests), the results of female and male patients were pooled for further analyses.

We evaluated potential associations between CHI3L1, NfL, and clinical variables (age, DD, and EDSS) and between CHI3L1 and NfL using Spearman rank correlation analyses. Estimates of standard errors and CIs for the correlation coefficients were derived using the bootstrapping method implemented in SPSS (1,000 samples).

Univariate simple linear regression and multiple linear regression analyses with backward selection of variables (exclusion criteria: probability of $F \geq 0.1$) for CHI3L1 or NfL as dependent variables and MUCCA, BGM, and BWM as independent variables served to investigate whether the CSF biomarker levels were related brain and/or spinal cord atrophy.

Figure 1 CHI3L1 (A) and NfL (B) concentrations in CSF of PMS and RMS
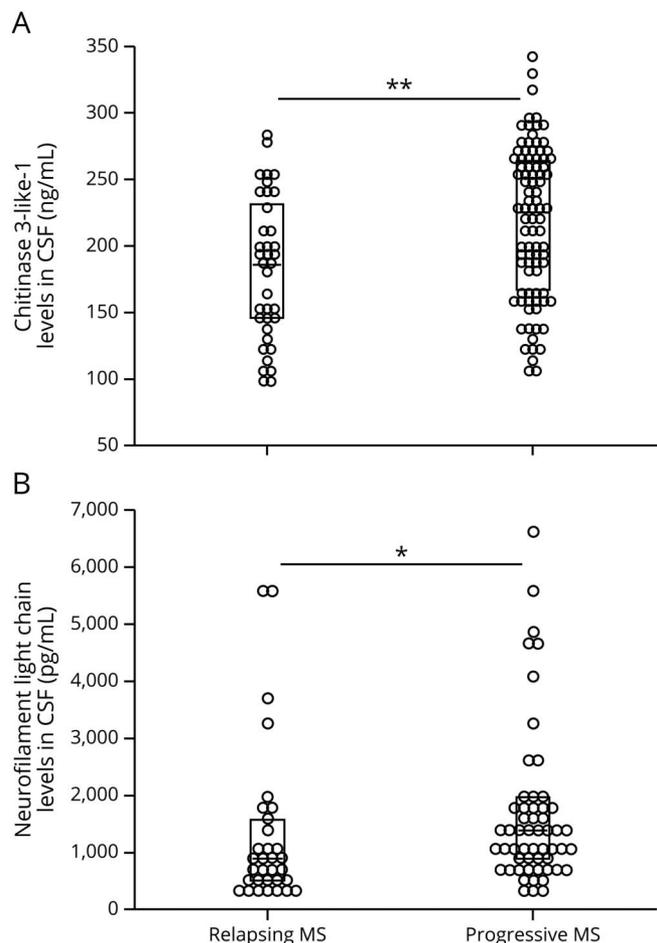
The 2D plots depict the distribution of the results; the boxes mark the median and interquartile ranges. The 2D plots depict the distribution of the results; the boxes mark the median and interquartile ranges. *$p < 0.050$, **$p < 0.001$ in Mann-Whitney U tests. CHI3L1 = chitinase 3–like protein 1; NfL = neurofilament light chain; PMS = progressive MS; RMS = relapsing MS.

Discrimination between the RMS (CIS/early RRMS and RRMS >2 years) and PMS (primary progressive and secondary progressive) by CHI3L1 and NfL levels was tested through multiple binary logistic regression analyses with MS type as dependent variable (RMS coded: 0, PMS: coded 1) and CHI3L1 and NfL as independent variables using backward conditional exclusion (p_{in} = 0.05, p_{out} = 0.10).

Expression of CHI3L1 in different cell types from healthy and MS brains

Published scRNA-seq data sets were examined for the expression of CHI3L1. Both data sets were analyzed using the RaceID algorithm using standard settings.[22] CHI3L1 expression was presented using mean and standard error of the mean. Data analysis and visualization of the genetic data was performed using the R programming suite and the tidyverse package.[23]

Data availability

Anonymized data used for this study are available from the corresponding author on reasonable request.

Results

Patient characteristics

Of the 131 patients with MS, 42 (32%) had relapsing MS (CIS and early RRMS with DD ≤2 years [n = 17] or RRMS with DD >2 years [n = 25]), and 89 (68%) patients had progressive MS (secondary progressive [n = 69] or primary progressive subtype [n = 20]). Mean/median values of age, DD, and EDSS were higher in the PMS group than in the RMS group. Clinical and demographic details of the entire patient group, RMS, PMS, and HC are shown in table 1.

CSF ELISA analyses of CHI3L1 and NfL concentrations

The median of CHI3L1 concentration was higher in PMS than in the RMS subgroup, and maximal values of CHI3L1 concentrations were observed progressive MS (table 1, figure 1, left panel). Similarly, the median of NfL concentration was higher in the PMS group than in RMS (figure 1, right panel). Compared with CHI3L1, the NfL concentrations of the entire patient group spanned a considerably wider relative range (factor 0.24–6.4 of the whole group median in NfL compared with 0.5–1.6 of the median of the entire group in CHI3L1).

Although correlations between CHI3L1 and age, DD, and EDSS were moderate (Spearman ρ [95% CI], p: 0.241 [0.064–0.419], p = 0.006; 0.198 [0.011–0.368], p = 0.198; 0.217 [0.010–0.407], p = 0.015), NfL showed stronger associations with these clinical parameters (ρ [95%CI], p: 0.556 [0.352–0.715], p < 0.001; 0.452 [0.248–0.624], p < 0.001; 0.279 [0.060–0.469], p = 0.009), especially with age (reflected by higher correlation coefficients and lower p values).

The CSF concentrations of CHI3L1 and NfL were correlated (Spearman ρ [95% CI], p: 0.373 [0.177–0.552], p < 0.001), A

scatter plot of the association between CHI3 and NfL is provided in the supplement (figure e-1, links.lww.com/NXI/A329).

MRI-derived atrophy of the cervical cord and the brain in patients with MS

We observed MUCCA atrophy in the entire MS group as compared to HC (table 1 and figure e-2A, links.lww.com/NXI/A329), which was driven by MUCCA reduction in the PMS subgroup Furthermore, the mean of MUCCA in the PMS group was smaller than in the RMS subtype. In the latter group, no significant group differences compared with HC were observed. Atrophy of BGM was observed in the entire patient group compared with HC and in PMS compared with RMS (figure e-2B). Brain lesion load was higher in chronic MS compared with RMS. We observed no BWM atrophy in the patient groups.

Associations between CSF biomarkers and cervical cord area or brain volumes addressed using backward linear regression analyses

To study associations between the CSF biomarkers and the MRI-derived CNS volumetry measures, we used univariate and multiple linear regression analyses separately for CHI3L1 or NfL as dependent variables including MUCCA, brain GM, and WM as independent variables. The multiple regressions used backward stepwise exclusion (table 2). As a prerequisite for the applicability of the multiple linear regressions, we checked conditions, such as independence and normal distribution of the residuals, no collinearity of the independent variables, and occurrence of potential outliers. As a consequence, we excluded 5 samples with NfL >3,500 ng/mL from the analysis.

CHI3L1: In the univariate regression analyses, MUCCA showed the strongest associations with CHI3L1 (reflected by the t statistic and lowest p value). In the final multiple regression model, MUCCA was the only predictive MRI volumetry variable for CHI3L1. Still, the corrected R^2 of the final model reflected merely a small effect size.[24] According to the negative regression coefficient, a decrease of MUCCA of 10 mm^2 predicted an average increase in CHI3L1 of 11 ng/mL in this regression model.

NfL: For NfL, the univariate regressions showed the strongest associations between BGM and NfL. BGM was found to be the only predictive MRI volumetry variable in the final multiple regression model. Within this model, a decrease of 10 mL in BGM predicted an average increase of 73 pg/mL NfL. The final model explained 12% of the variance of NfL, corresponding to a small to medium effect size[24]; figure 2, A and B further illustrates the associations between CHI3L1 and MUCCA and between NfL and BGM.

Discrimination between the RMS and chronic MS types by CHI3L1 and NfL CSF levels

To address the impact of CHI3L1 and NfL levels in CSF to discriminate between the RMS and progressive MS types, we

Table 2 Univariate and multiple linear regression analyses between CHI3L1 and NfL concentrations in CSF and CNS volumes (including MUCCA, BGM, and BWM) using backward stepwise exclusion

	Linear regression	MRI parameter	Regression coefficient, B	95% CI of B Lower	95% CI of B Upper	Standardized coefficient, β	t	p Value
CHI3L1 (ng/mL)	Univariate simple regressions	MUCCA	−1.07	−1.96	−0.19	−0.210	−2.39	0.018
		BGM	0.017	−0.18	0.21	0.016	0.17	0.869
		BWM	−0.08	−0.34	0.18	−0.060	−0.63	0.533
	Multiple: final model	MUCCA	−1.07	−2.04	−0.11	−0.210	−2.21	0.029
		Constant	284	215	352		8.21	<0.001
NfL (pg/mL)	Univariate simple regressions	MUCCA	−0.88	−20.4	18.7	−0.01	−0.09	0.929
		BGM	−7,31	−11.9	2.66	−0.36	−3.14	0.003
		BWM	3.11	−3.55	9.77	0.11	0.93	0.345
	Multiple: final model	BGM	−7.3	−12.0	−2.7	−0.361	−3.14	0.003
		Constant	5,077	2,627	7,527		4.14	<0.001

Abbreviations: BGM = brain gray matter volume (ml); BWM = brain white matter volume (ml); CHI3L1 = chitinase 3–like protein 1; NfL = neurofilament light chain; MUCCA = mean upper cervical cord area (mm^2).
CHI3L1: final model (includes 109 patients with all parameters available) corrected R^2 = 0.04.
NfL: final model (includes 69 patients with all parameters available) corrected R^2 = 0.12.

used multiple binary logistic regression analyses (backward conditional exclusion) with type (RMS coded: 0, PMS: coded 1) as dependent variable and CHI3L1 and NfL as independent variables (table 3). CHI3L1 turned out to be most suitable for differentiating between PMS or RMS types. Thus, in the final model ($\chi^2(1)$ = 6.445, p = 0.011, N = 88), the odds of having PMS are 1.11 times larger for a patient with a 10 ng/mL higher CHI3L1 level, Nagelkerke R^2 = 0.097 of the final model corresponded to a medium effect size, Cohen f = 0.33.[24]

Expression of CH3L1 in different cell types from healthy and MS brains

In the Jäkel et al. data set, reflecting CH3L1 in MS and healthy BWM, CHI3L1 was mainly expressed in 2 astrocyte subsets

Figure 2 Association between (A) CHI3L1 and MUCCA and (B) NfL and BGM
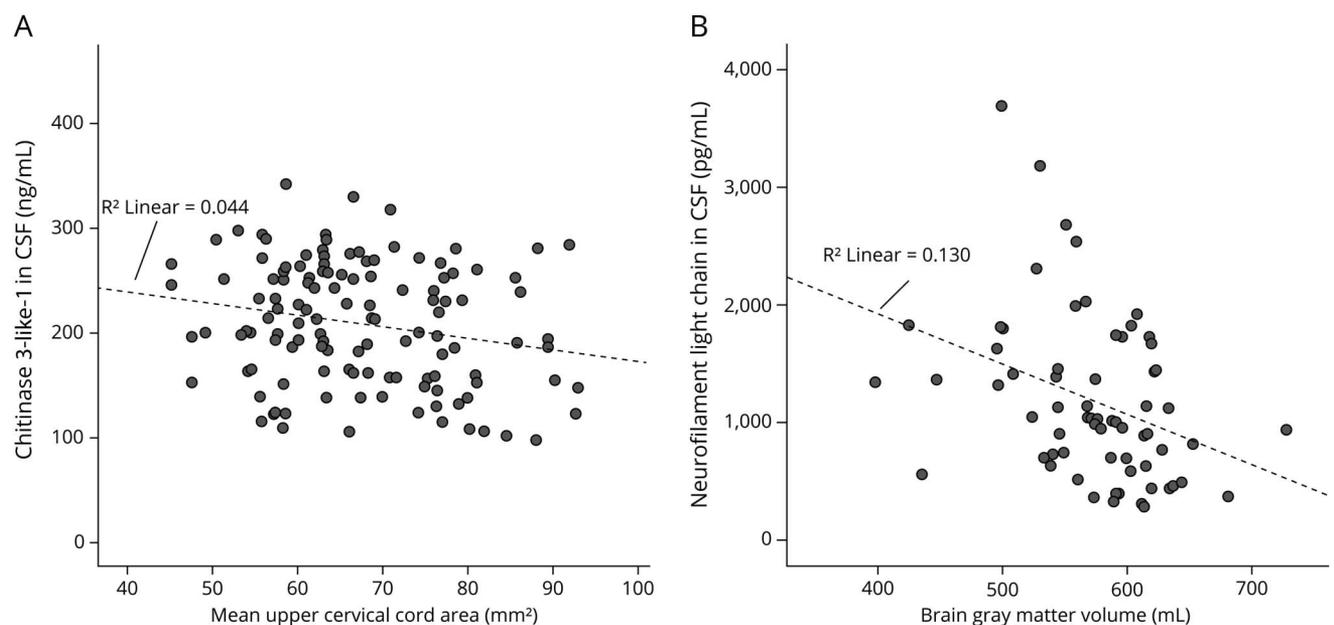
The line represents simple linear regressions between the variable pairs with R^2: coefficient of determination. BGM = brain gray matter; CHI3L1 = chitinase 3–like protein 1; NfL = neurofilament light chain; MUCCA = mean upper cervical cord area.

Table 3 Binary logistic regression of differentiation between RMS and progressive MS subtypes including CHI3L1 and NfL in CSF as independent variables (backward conditional exclusion; PMS coded: 1, RMS coded: 0)

Binary logistic regression		Regression coefficient B	SE	Wald	p Value	OR	95% CI for OR	
							Lower	Upper
Final model	CHI3L1 (ng/mL CSF)	1.03E-2	4.2E-3	5.946	**0.015**	1.0103	1.0020	1.0187
	Constant	−1.40	0.819	2.937	0.087	0.2457		

Abbreviations: CHI3L1 = chitinase 3–like protein 1; NfL = neurofilament light chain; PMS = progressive MS; RMS = relapsing MS; Wald = square of Wald test statistic for regression coefficients B.
Final model (includes 88 patients with both parameters available): coefficient of determination R^2 = 0.097 (Nagelkerke).
Bold text indicates $p < 0.05$.

and in microglia and macrophages (figure e-2A, links.lww.com/NXI/A329). Of interest, in MS samples, CHI3L1 expression was mainly found in MS-associated microglia and macrophages, but not in other immune cells, such as monocytes and lymphocytes (figure e-2B).

These findings were validated using the Masuda et al. scRNA-Seq data set. Herein, MS-associated microglia and macrophages (clusters Hu-C2, Hu-C3, and Hu-C8) expressed elevated levels of CHI3L1,[21] while CH3L1 was almost undetectable in the homeostatic clusters (figure e-2C, links.lww.com/NXI/A329). Moreover, like in the Jäkel et al. data set, hematogenic immune cells (monocytes and lymphocytes) showed no detectable and barely detectable levels of CHI3L1. Overall, specific CHI3L1 gene expression was found in MS-associated microglia and macrophages, but not in homeostatic microglia or other immune cells. In contrast, astrocytes expressed CHI3L1 in healthy control samples and moreover in active and chronically active MS lesions.

Discussion

Contrary to our hypothesis, CHI3L1 and NfL were not associated equally with both cervical cord area and brain volumes in our study. Although CHI3L1 was associated with cervical cord volume loss (MUCCA reduction) but not with brain volumes, NfL was related to BGM decrease and not to MUCCA.

To date, relations between CSF biomarkers and spinal cord atrophy in MS have rarely been investigated. CSF NF heavy chain levels were associated with late spinal cord atrophy progression, but not with baseline spinal cord measures in a longitudinal study including a small cohort of patients with MS.[25] Similarly, NfL in blood serum was associated with long-term progression of spinal cord volume loss but not with baseline measures in a large longitudinal study including a mixed MS cohort[7] This association between NfL and spinal cord volume might seem contradictory to our findings, but differences in the study design (longitudinal spinal cord volume loss was assessed, while the patient group did not include patients with later-stage secondary PMS) and assessment of serum NfL instead of CSF NfL may be reasons for differing results.[8]

Our findings of associations between NfL levels and baseline reduction of BGM are corroborated by recent findings based on serum NfL.[26] Other studies reported relations between NfL in CSF or serum with longitudinal brain volume changes.[7,8]

These differing patterns of associations, especially referring to the association of CHI3L1 with MUCCA, may be based on different pathophysiologic sources of CHI3L1 and NfL CSF concentrations in the patient subgroups.

In line with previous studies, average levels of CHI3L1 and NfL were higher in PMS compared with RMS.[11,27–29] Still, CHI3L1 was better suited to differentiate between both subtypes than NfL. This might be due to broader ranges of values in NfL and cases of high NfL levels both in the RMS and in the PMS group.

The relevance of CSF CHI3L1 to neurologic disability in PMS was recently reported.[30] In PMS compared with RMS, higher levels of spinal cord atrophy are common findings.[31]

Spinal cord atrophy in MS, correlating with clinical disability, has been shown to be at least in part independent of local inflammation. Thus remote effects of Wallerian degeneration secondary to neuroaxonal tissue damage in the brain have been suggested as drivers of spinal cord atrophy in MS.[15] In PMS, neurodegenerative rather than inflammatory processes are the main drivers of disease progression. Neurodegeneration generates higher relative volume losses in the spinal cord than in the brain due to a lack of compensatory capabilities in this relatively small structure. We hypothesize that diffuse neurodegeneration along with ongoing astrocytic and microglial activation results in higher levels of CSF CHI3L1 in progressive MS, which correlates with cervical spinal cord volume reduction. Thus, CHI3L1 may have a closer association to spinal cord atrophy than NfL.

Brain GM atrophy, on the other hand, is driven by inflammatory activity reflected by new or gadolinium-enhancing brain lesions in relapsing-remitting disease

courses and by diffuse neurodegeneration and chronical inflammation in progressive MS.[16,25,32] NfL has widely been described as a marker for axonal damage secondary to acute inflammatory processes in MS,[2,4,28] which might explain the strong association between CSF NfL and BGM atrophy.

We also could verify a differing pattern of CHI3L1 and NfL related to age and DD. In contrast to CHI3L1, NfL showed stronger associations with age and DD. In line with our CSF-based results, increasing levels of serum NfL with age in HC and in patients with MS have been previously reported,[7] suggesting a limitation for the use of NfL as disease marker in older patients with longer DD.[33,34] The weaker impact of age and DD on CHI3L1 may underline its suitability for use as biomarker in PMS.

Although elevated CHI3L1 and NfL levels are not MS specific,[35,36] both markers have been associated with inflammatory processes in MS, whereby CHI3L1 has a regulatory function on inflammation, whereas NfL is a product of axonal degeneration.[28] Furthermore, the expression of CHI3L1 and NfL can be considerably increased by various inflammatory and immunologic conditions.[37]

The occurrence of CHI3L1 in inflammatory environments as described[38,39] was further substantiated by the analysis of cell-specific CHI3L1 expression patterns: CHI3L1 expression was mainly found in active and chronically active MS lesions in activated macrophages/microglia of MS and in activated astrocytes.

Our study is not without limitations. A drawback is its retrospective design and a lack of CSF probes of HC, which excluded the discrimination between physiologic and disease-related age dependencies. Another limitation is the cross-sectional design. In particular, to clarify whether CHI3L1 baseline levels might be sensitive markers for future spinal cord atrophy progression, longitudinal studies including CHI3L1 and spinal cord atrophy are warranted.

Conclusions

NfL and CHI3L1 in CSF were differentially related to brain and spinal cord atrophy. We demonstrated associations between CHI3L1 and spinal cord volume. In contrast, NfL was not correlated with MUCCA, but we confirmed recently reported correlations of NfL with BGM reduction. CHI3L1 was suited to differentiate between relapsing and progressive MS and was less affected by DD and age than NfL. Consistent with a recent study showing that the pattern of combined measurement of CSF-based NfL and CHI3L1 was useful to discriminate MS phenotypes,[14] the combined measurement of CSF NfL and CHI3L1 provides complementary information with regard to brain and spinal cord volumes.

Appendix Authors

Name	Location	Contribution
Ruth Schneider, MD	Ruhr University Bochum, Germany	Designed and conceptualized the study; acquisition of data; analyzed the data; and drafted the manuscript for intellectual content
Barbara Bellenberg, PhD	Ruhr University Bochum, Germany	Designed and conceptualized the study; acquisition of data; analyzed the data; and revised the manuscript for intellectual content
Barbara Gisevius, PhD	Ruhr University Bochum, Germany	Major role in the acquisition of data
Sarah Hirschberg, PhD	Ruhr University Bochum, Germany	Major role in the acquisition of data
Roman Sankowski, MD	University of Freiburg, Germany	Major role in the acquisition of data
Marco Prinz, MD	University of Freiburg, Germany	Interpreted the data and revised the manuscript for intellectual content
Ralf Gold, MD	Ruhr University Bochum, Germany	Interpreted the data and revised the manuscript for intellectual content
Carsten Lukas, MD	Ruhr University Bochum, Germany	Designed and conceptualized the study; analyzed the data; and revised the manuscript for intellectual content
Aiden Haghikia, MD	Ruhr University Bochum, Germany	Designed and conceptualized the study; analyzed the data; and revised the manuscript for intellectual content

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