Association of intrathecal pleocytosis and IgG synthesis with axonal damage in early MS

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Abstract

Objective

To investigate the association of serum neurofilament light chain (sNfL) levels with CSF parameters in clinically isolated syndrome (CIS) and early relapsing-remitting MS (RRMS), taking into account radiologic and clinical parameters of disease activity.

Methods

Simultaneously collected serum and CSF samples of 112 untreated patients newly diagnosed with CIS or RRMS were included in this cross-sectional study. CSF parameters were obtained as part of routine diagnostic tests. sNfL levels of patients and of 62 healthy donors were measured by highly sensitive single molecule array (SiMoA) immunoassay.

Results

Patients with RRMS ($n = 91$, median 10.13 pg/mL, interquartile range [IQR] 6.67–17.77 pg/mL) had higher sNfL levels than healthy donors ($n = 62$, median 5.25 pg/mL, IQR 4.05–6.81 pg/mL, $p < 0.001$) and patients with CIS ($n = 21$, median 5.69 pg/mL, IQR 4.73–9.07 pg/mL, $p < 0.001$). Patients positive for oligoclonal bands (OCBs) ($n = 101$, median 9.19 pg/mL, IQR 6.34–16.38 pg/mL) had higher sNfL levels than OCB-negative patients ($n = 11$, median 5.93 pg/mL, IQR 2.93–8.56 pg/mL, $p = 0.001$). sNfL levels correlated with CSF immunoglobulin G (IgG) levels ($r = 0.317$, $p = 0.002$), IgG ratio (QIgG) ($r = 0.344$, $p < 0.001$), and CSF leukocyte count ($r = 0.288$, $p = 0.002$). In linear regression modeling, the CSF leukocyte count combined with the number of contrast-enhancing lesions in MRI predicted sNfL levels best.

Conclusions

In active MS, sNfL levels correlate with intrathecal pleocytosis and IgG synthesis, indicating that axonal damage is associated with both acute and chronic CNS-intrinsic inflammation.

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Glossary

BBB = blood-brain barrier; **CEL** = contrast-enhancing lesion; **CIS** = clinically isolated syndrome; **EDSS** = Expanded Disability Status Scale; **IgG** = immunoglobulin G; **IQR** = interquartile range; **OCB** = oligoclonal band; **ON** = optic neuritis; **NfH** = neurofilament heavy chain; **NfL** = neurofilament light chain; **sNfL** = serum neurofilament light chain; **Qalb** = CSF/serum albumin ratio; **QIgG** = CSF/serum IgG ratio; **RRMS** = relapsing-remitting MS.

Neurofilament light chain (NfL) subunits represent one of the main constituents of the neuronal cytoskeleton, which are released into the CSF and, to a lesser extent, into the peripheral blood, following axonal injury.¹ The development of highly sensitive single molecule array (SiMoA) technology now enables the detection even of small changes in peripheral NfL concentrations.² As it has been demonstrated recently that serum and CSF NfL levels are highly correlated,³ serum neurofilament light chain (sNfL) has emerged as an easily accessible biomarker of neuroaxonal damage. Consequently, the past few years have witnessed a surge in the number of publications on sNfL in a variety of neurologic disorders.^{2,4}

In MS, NfL levels increase during relapses and are positively associated with MRI lesion load and the presence of contrast-enhancing lesions (CELs).^{5–7} However, recent studies provide little and inconsistent information about the impact of CSF parameters that reflect inflammatory processes within the CNS compartment on NfL levels in the periphery. Therefore, we aimed to investigate the association between sNfL and markers of acute and chronic CNS inflammation assessed by routine CSF diagnostics in patients with MS. To rule out confounding effects of immunosuppressive or immunomodulatory therapies, we only included untreated patients at the time point of diagnosis of clinically isolated syndrome (CIS) or relapsing-remitting MS (RRMS).

Beyond the exclusion of differential diagnoses, CSF analysis is essential in diagnosing MS. Typical CSF findings in MS include a slightly elevated leukocyte count, the presence of mononuclear cells, and of oligoclonal bands (OCBs), elevated immunoglobulin G (IgG) synthesis, and increased synthesis of intrathecally produced immunoglobulins against measles, rubella, and varicella zoster (MRZ) viruses. Because of its prognostic value, the presence of OCBs in CSF has been incorporated into the 2017 revised McDonald criteria for MS diagnosis as a marker of dissemination in time.⁸ The CSF/serum albumin ratio (Qalb) as a marker of blood-brain barrier (BBB) integrity is mainly within normal ranges, which is in line with the very focal and transient BBB disruption in MS, but may also be elevated in some patients.^{9,10}

An increase in the CSF/serum IgG ratio (QIgG) and the presence of OCBs reflect chronic CNS-intrinsic immune reactions,¹¹ whereas the CSF leukocyte count is a dynamic parameter of acute inflammatory activity.^{12,13} We hypothesized that both chronic and acute inflammation influence sNfL levels in patients with MS.

Methods

Patients and healthy controls

A cross-sectional cohort (n = 112) was recruited between 2011 and 2018 at the Department of Neurology at the University Medical Center Mainz (Germany). After informed consent was obtained from all patients, paired serum and CSF samples were prospectively collected and stored. Routine spinal tap was performed as part of the diagnostic workup. Inclusion criteria were (1) a new diagnosis of CIS or RRMS (all diagnoses were reclassified according to the 2017 revised McDonald criteria⁸); (2) availability of paired serum and CSF samples at the time of diagnosis; (3) availability of demographic and clinical data at the time of diagnosis; (4) availability of brain and preferably also spinal cord MRI data acquired as part of the diagnostic workup at the time of diagnosis; and (5) no immunosuppressive or immunomodulatory treatment before sample collection. Samples from patients who had received steroid treatment before sample collection were excluded. A total of 11 patients with the presence of OCBs in CSF were diagnosed with CIS, as they did not fulfill the criteria for dissemination in space.⁸

In addition, serum samples from 62 healthy controls were collected and stored after informed consent was obtained.

Standard protocol approvals, registrations, and patient consents

The study was approved by the local ethics committee (number 837.019.10); written informed consent was obtained from all patients.

CSF analyses

CSF analyses were performed in a standardized fashion as part of routine diagnostic workup. CSF concentrations of albumin (CSF Alb), immunoglobulin (Ig) A (CSF IgA), IgG (CSF IgG), and IgM (CSF IgM) were determined with immunonephelometry. Quotients of albumin (QAlb), quotient of IgA (QIgA), quotient of IgG (QIgG), and quotient of IgM (QIgM) were defined as the ratios of CSF concentrations to the corresponding serum concentrations of these fractions. Detection of OCBs was performed by isoelectric focusing on agarose gel and subsequent immunoblotting using IgG-specific antibody staining. Reference values were considered according to Berlit.¹⁴

NfL single molecule array of serum samples

sNfL of 112 patients with CIS and MS and of 62 healthy donors was measured by SiMoA technology as previously

described.⁷ Briefly, blood samples were spun at 2000g at room temperature for 10 minutes within 2 hours after withdrawal and stored in polypropylene tubes at -80°C . Serum NfL was measured by SiMoA HD-1 (Quanterix) using the NF-Light Advantage Kit (Quanterix) according to the manufacturer's instructions. Samples were measured in duplicates, and the intra-assay coefficient of variation of all samples was 4.5%. sNfL measurements were performed in a blinded fashion without information about clinical data.

Statistics

Continuous variables are described by their median and interquartile range (IQR), and categorical variables by numbers and percentages. Normal distribution was tested using Kolmogorov-Smirnov and Shapiro-Wilk tests for normal distribution. sNfL levels and the CSF leukocyte count were log transformed to achieve a more normal distribution for subsequent analyses. Yet, for simplicity of notation, we will use the original terms when reporting and discussing results. Comparison of sNfL levels across groups was performed using the Mann-Whitney *U* test for comparison between 2 groups and 1-way analysis of variance for comparison between 3 or more groups, respectively. Correlation was determined by the Spearman rank correlation coefficient. Age as a confounding factor needs to be taken into account in older patients, as sNfL seems to considerably increase in particular above the age of 60 years with rather stable values in younger patients.¹⁵ In agreement, in our cohort of considerably young patients (median age 33.5 years, IQR 28–42 years), we found no significant correlation between age ($r = -0.076$, $p = 0.429$) and sNfL values, and therefore, no age correction was performed. The joint effects of CSF parameters and clinical and radiologic parameters of disease activity on sNfL levels were assessed by regression analysis with forward variable selection (inclusion threshold of 0.5). These effects were validated by regression analysis with backward selection (exclusion threshold of 0.01). *p* Values <0.05 were considered statistically significant. Because of the explorative character of the study, no correction for multiple testing was performed. Statistical analyses were performed using SPSS 23.0 software (IBM Corp, Armonk, NY), SAS software 9.4R4 (SAS Institute Inc., Cary, NC), and GraphPad Prism 7.0 for Windows (GraphPad Software; Microsoft, Redmond, WA).

Data availability

The raw data used in preparation of the figures and tables will be shared in anonymized format on request of a qualified investigator to the corresponding author for purposes of replicating procedures and results.

Results

Patient characteristics and CSF analysis

Patient characteristics including demographic, clinical, and MRI parameters are reported in table 1. Briefly, 21 patients with CIS and 91 patients with RRMS (according to the 2017

revised McDonald criteria⁸) were included in this study. Of the patients, 70.5% experienced an acute relapse within 90 days^{16,17} before sample collection; the median time between onset of relapse and spinal tap was 14 days. CELs in MRI at the time of diagnosis were detectable in 44.6% of the patients. 34.8% of the patients showed clinical relapse and CELs in MRI, whereas 19.6% showed neither (figure 1A). 26.6% of the patients with relapse presented with optic neuritis (ON), 39.2% with cerebral, and 34.2% with spinal clinical manifestation. Type of relapse manifestation was defined by clinical presentation and, whenever available, MRI findings.

sNfL levels in patients with RRMS ($n = 91$, median 10.13 pg/mL, IQR 6.67–17.77 pg/mL) were significantly higher than those of healthy donors matched for sex and age ($n = 62$, median 5.25 pg/mL, IQR 4.05–6.81 pg/mL, $p < 0.001$) and those of patients with CIS ($n = 21$, median 5.69 pg/mL, IQR 4.73–9.07 pg/mL, $p < 0.001$) (figure 1B). Within the patient group, we observed no sex-specific differences of sNfL levels (men: $n = 36$, median 10.95 pg/mL, IQR 6.93–16.82 pg/mL; women: $n = 76$, median 8.54 pg/mL, IQR 5.63–14.89 pg/mL; $p = 0.350$); also, sNfL levels showed no correlation with age in our cohort of young patients ($r = -0.079$, $p = 0.407$).

CSF measurements for leukocyte count, total protein concentration, Qalb, QIgG, QIgA, QIgM, and OCBs are also presented in table 1. OCBs were present in 90.2% of the patients of the whole group and in nearly all of the patients of the MS group (98.9%).

sNfL level increase is associated with cerebral and spinal relapse manifestation

sNfL levels tended to be higher during clinical relapse ($n = 79$, median 9.91 pg/mL, IQR 6.05–17.77 pg/mL) in comparison to samples taken from clinically stable patients ($n = 33$, median 8.43 pg/mL, IQR 5.41–11.51 pg/mL), but the difference failed to reach significance ($p = 0.095$). We also observed no significant correlation for sNfL levels between the time since symptom onset and spinal tap ($r = 0.064$, $p = 0.579$). However, when stratifying patients for relapse localization, we found that patients with spinal or cerebral relapse showed higher sNfL levels ($n = 58$, median 10.95 pg/mL, IQR 6.62–24.61 pg/mL) than those without relapse ($p = 0.028$) and those with ON ($n = 21$, median 8.43 pg/mL, IQR 5.35–11.86 pg/mL, $p = 0.022$) (figure 1C).

We did not observe an association between clinical prognostic factors (sensory compared with motor symptoms and complete compared with incomplete remission) and sNfL levels in patients with cerebral relapses. sNfL levels also demonstrated no significant correlation with the Expanded Disability Status Scale (EDSS), independent of whether the cohort was stratified for disease activity at the time of sample collection. Furthermore, there was no significant correlation between any of the inflammation-related CSF parameters and the EDSS, except for Qalb ($r = 0.211$, $p = 0.026$) (table e-1, links. [lww.com/NXI/A191](https://www.lww.com/NXI/A191)).

Table 1 Patient characteristics: demographic, clinical, MRI, and CSF data

Parameter	Number (%)	Median (IQR)	
Age (y)		33.5 (28–42)	
Sex			
Male	36 (32.1%)		
Female	76 (67.9%)		
Diagnosis			
CIS	21 (18.8%)		
RRMS	91 (81.2%)		
Relapse			
Yes	79 (70.5%)		
No	33 (29.5%)		
CEL			
Yes	50 (44.6%)		
No	62 (55.4%)		
Relapse and CEL			
Both	39 (34.8%)		
Neither	19 (16.9%)		
T2 lesion/CEL number			
All patients		11 (6–20)/1 (0–2)	
CIS		1 (1–5)/0 (0–1)	
RRMS		13 (8–26)/1 (0–2)	
Days since relapse		14 (5–35)	
Clinical manifestation			
Spinal	27 (34.2%)		
Cerebral	31 (39.2%)		
ON	21 (26.6%)		
EDSS score		2.0 (1.0–2.5)	
CSF parameter	Number (%)	Median (IQR)	Reference range
CSF leukocyte count (/μL)		6 (4–10)	≤4
CSF total protein (mg/dL)		34.1 (27.8–41.7)	15–40
Qalb		5.0 (3.8–6.1)	≤6.5 (<40 years) ≤8 (>40 years)
QIgG		4.0 (2.8–5.4)	<2.3
QIgA		1.5 (1.1–5.4)	<1.3
QIgM		0.4 (0.2–0.9)	<0.3

Table 1 Patient characteristics: demographic, clinical, MRI, and CSF data (*continued*)

CSF parameter	Number (%)	Median (IQR)	Reference range
OCB			
Positive	101 (90.2%)		
Negative	11 (9.8%)		

Abbreviations: CIS = clinically isolated syndrome; CEL = contrast-enhancing lesion; EDSS = Expanded Disability Status Scale; IQR = interquartile range; OCB = oligoclonal band; ON = optic neuritis; QlgG = CSF/serum IgG ratio; RRMS = relapsing-remitting MS. Age, disease duration, and EDSS score are presented as median and IQR; sex, diagnosis, presence of relapse and CELs, and relapse manifestation in absolute numbers and percent.

sNfL level increase is associated with T2 lesion load and the presence of CELs in MRI

We found a highly significant increase in sNfL levels in the presence of CELs in MRI (patients with CELs: $n = 50$, median 10.98 pg/mL, IQR 7.67–26.11 pg/mL; patients without CELs: $n = 62$, median 7.78 pg/mL, IQR 5.54–11.90 pg/mL, $p = 0.001$) (figure 2A). Furthermore, sNfL levels correlated with the number of T2 lesions ($r = 0.496$, $p < 0.001$) (figure 2B) and the number of CELs ($r = 0.489$, $p < 0.001$) (figure 2C).

To investigate the prognostic value of sNfL, we performed subgroup analyses according to established MRI prognostic factors¹⁸ in the group of patients with cerebral relapse manifestation ($n = 31$). Supratentorial MRI lesions were found in 28 of these patients (17 with CEL), and infratentorial lesions were found in 19 patients (6 with CEL). There was no association between the presence of infratentorial MRI lesions and sNfL levels. However, after classifying patients according to the number of cerebral T2 lesions, we found significantly higher sNfL levels in patients with 10 or more T2 lesions ($n = 18$; median 16.33 pg/mL, IQR 7.82–35.68 pg/mL) than in patients with less than 10 lesions ($n = 13$ median 7.50 pg/mL, IQR 5.14–14.52 pg/mL; $p = 0.028$). In addition, we observed that patients with more than 1 CEL ($n = 9$, median 24.58 pg/mL, IQR 9.82–38.77 pg/mL) had higher sNfL levels than patients with 0 or 1 CEL ($n = 22$, median 8.18 pg/mL, IQR 5.61–15.87 pg/mL, $p = 0.26$).

sNfL levels correlate with CSF markers of acute inflammation

The CSF leukocyte count was higher during relapse than in clinically stable patients and correlated with sNfL levels ($r = 0.288$, $p = 0.002$) (figure 3A). In addition, we observed a correlation of the CSF leukocyte count with the number of T2 lesions ($r = 0.209$, $p = 0.027$) and with the number of CELs ($r = 0.215$, $p = 0.023$) in MRI.

CSF albumin levels ($r = -0.050$, $p = 0.597$), Qalb ($r = -0.055$, $p = 0.562$), and CSF total protein ($r = 0.038$, $p = 0.693$) were

Figure 1 Study design and comparison of sNfL levels according to clinical and MRI parameters

unrelated to sNfL levels independent of the presence of relapse (table e-1, links.lww.com/NXI/A191). However, only 13 patients demonstrated Qalb values above the norm (as per the previously defined cutoff value according to Berlit¹⁴), which may account for the lack of association between Qalb and sNfL levels.

sNfL levels correlate with CSF markers of chronic inflammation

CSF IgG ($r = 0.317$, $p = 0.001$) (figure 3B) and QIgG ($r = 0.344$, $p < 0.001$) (figure 3C) moderately correlated with sNfL, and the presence of OCBs was significantly related to increased sNfL levels (OCB positive: $n = 101$, median 9.19 pg/mL, IQR 6.34–16.38 pg/mL; OCB negative: $n = 11$, median 5.93 pg/mL, IQR 2.93–8.56 pg/mL, $p = 0.001$) (figure 3D). Intrathecal fraction of the immunoglobulin subclasses IgA ($r = -0.030$, $p = 0.753$) and IgM ($r = 0.128$, $p = 0.182$) and their CSF/serum ratios QIgA ($r = 0.106$, $p = 0.267$) and QIgM ($r = 0.170$, $p = 0.072$) were unrelated to sNfL levels (table e-1, links.lww.com/NXI/A191).

sNfL levels are predicted by the CSF leukocyte count and CELs in MRI

Multiple regression analysis using forward selection of CSF parameters and clinical and radiologic parameters of disease activity showed that a total of 2 parameters were retained in the final model for sNfL levels. These included the number of CELs in MRI and the CSF leukocyte count,

which, when combined, predicted sNfL levels best. Backward selection for validation showed identical results. The estimated effects of these parameters, their standard error, and statistical significance are detailed in table e-2, links.lww.com/NXI/A191. To conclude the main results, this model states that each increase in the leukocyte count by 1 cell elevates sNfL levels by 0.434 pg/mL (standardized regression coefficient β 0.332) and that each additional CEL in MRI leads to an increase in sNfL levels by 3.390 pg/mL (β 0.532).

Discussion

In MS, different pathologic processes are linked to overall neuroaxonal damage with a subsequent rise in NfL levels in CSF and serum. These include oxidative stress, mitochondrial damage,¹⁹ direct T cell-mediated neuronal damage,²⁰ and toxic accumulation of synaptic proteins.²¹ It is so far unclear whether the sNfL level increase rather reflects acute focal inflammatory activity or chronic widespread diffuse neurodegenerative processes. Our findings strongly support the view that both contribute to the rise in the sNfL level, as we identified associations with CSF parameters of acute and chronic CNS-intrinsic inflammation. However, markers of acute inflammation outperformed those of chronic inflammation in predicting sNfL levels in our total cohort of mainly active patients,

Figure 2 Correlation of sNfL levels with MRI parameters

Comparison of sNfL levels between patients with (n = 50) and without (n = 62) CEL in MRI (A). Correlation of sNfL levels with the number of T2 lesions (B) and the number of CELs in MRI (C). Y-axes represent log-transformed sNfL levels in A–C. Horizontal line denotes median; boxes extend from the 25th to 75th percentile, whiskers from minimum to maximum; ** $p < 0.01$. CEL = contrast-enhancing lesion; sNfL = serum neurofilament light chain.

who had significantly higher sNfL levels than healthy controls matched for sex and age.

Intrathecal IgG synthesis is an established marker of chronic CSF inflammation. It can be detected by an elevation of

QIgG, the presence of OCBs, or by positive MRZ reaction. Once acquired, it persists mostly unchanged over time and never disappears.¹¹ In our cohort, sNfL levels were significantly higher in OCB-positive patients and correlated with CSF IgG levels and QIgG. The finding that OCB-positive

Figure 3 Associations of standard CSF parameters and sNfL levels

Correlation of sNfL levels with the CSF leukocyte count (A), CSF IgG (B), and QIgG (C). Comparison of sNfL levels between OCB-positive and OCB-negative patients (D). Y-axes represent log-transformed sNfL levels in A–D; in A also, x-axis represents log-transformed CSF leukocyte count. Horizontal line denotes median; boxes extend from the 25th to 75th percentile, whiskers from minimum to maximum; ** $p < 0.01$. CEL = contrast-enhancing lesion; CIS = clinically isolated syndrome; IgG = immunoglobulin G; OCB = oligoclonal band; sNfL = serum neurofilament light chain.

Table 2 Literature overview

Ref. (no.)	Disease	No. of patients	Type of neurofilament	Results
OCB				
Present results	MS (CIS and early MS)	112	Serum NfL	Higher sNfL levels in OCB-positive patients than in OCB-negative patients
Farina et al.²²	MS (disease course not defined)	90	CSF NfL	Higher CSF NfL levels in OCB-positive patients than in OCB-negative patients
Kuhle et al.²³	MS (CIS, RRMS, SPMS, and PPMS)	150	CSF NfH	No association with OCB status
Immunoglobulin levels				
Present results	MS (CIS and early MS)	112	Serum NfL	Correlation with CSF IgG and QIgG
Eikelenboom et al.²⁴	MS (RRMS, SPMS, and PPMS)	51	CSF NfH	No correlation with QIgG
Norgren et al.²⁷	MS (RRMS, SPMS, and PPMS)	99	CSF NfL	No correlation with QIgG
Kuhle et al.²³	MS (CIS, RRMS, SPMS, and PPMS)	150	CSF NfH	No correlation with CSF IgG, IgA, and IgM levels, nor QIgG
Leukocyte count				
Present results	MS (CIS and early MS)	112	Serum NfL	Correlation with leukocyte count
Norgren et al.²⁷	MS (RRMS, SPMS, and PPMS)	99	CSF NfL	Correlation with leukocyte count during relapse
Kuhle et al.⁴¹	MS (CIS, RRMS, SPMS, and PPMS)	148	CSF NfL	Correlation with leukocyte count in CIS and RRMS, but not in SPMS and PPMS
Olesen et al.²⁶	Acute ON	40	CSF NfL	Patients with NfL higher than the median had higher leukocyte counts than those below
Pawlitzi et al.²⁹	PPMS	50	CSF NfL	No correlation with leukocyte count
Qalb				
Present results	MS (CIS and early MS)	112	Serum NfL	No correlation with Qalb
Lycke et al.³⁵	RRMS	60	CSF NfL	No correlation with Qalb
Malmeström et al.³⁹	MS (RRMS and SPMS)	66	CSF NfL	No correlation with Qalb
Petzold et al.³⁶	MS (RRMS, SPMS, and PPMS)	29	CSF NfH	No correlation with Qalb
Novakova et al.¹⁷	MS	158	CSF and serum NfL	No correlation with Qalb
Steinacker et al.³⁸	Motor neuron disease (ALS and PLS)	455	CSF NfL	No correlation with Qalb
Kalm et al.³⁷	Patients with varying degree of BBB impairment	43	Serum NfL	No correlation with Qalb
Kuhle et al.²³	MS (CIS, RRMS, SPMS, and PPMS)	150	CSF NfH	Correlation with Qalb only in patients with RRMS during relapse
Kuhle et al.⁴¹	MS (CIS, RRMS, SPMS, and PPMS)	148	CSF NfL	Correlation with Qalb in CIS, RRMS, and SPMS, but not PPMS
Anesten et al.³⁴	HIV-infected patients	631	CSF NfL	Correlation with Qalb
Skillbaeck et al.⁴²	Dementias	1,590	CSF NfL	Correlation with Qalb in late-onset Alzheimer disease and vascular dementia

Abbreviations: ALS = amyotrophic lateral sclerosis; BBB = blood-brain barrier; CIS = clinically isolated syndrome; IgG = immunoglobulin G; NfH = neurofilament heavy chain; NfL = neurofilament light chain; OCB = oligoclonal band; ON = optic neuritis; PLS = primary lateral sclerosis; PPMS = primary progressive MS; Qalb = CSF/serum albumin ratio; QIgG = CSF/serum IgG ratio; RRMS = relapsing-remitting MS; sNfL = serum neurofilament light; SPMS = secondary progressive MS.

Comparison of earlier and recent findings concerning associations of neurofilament with CSF markers of chronic (A) and acute (B) CNS-intrinsic inflammation.

patients demonstrate higher sNfL levels than OCB-negative patients matches an earlier study, which described the same trend for CSF NfL levels.²² In contrast, others found no association of neurofilament with intrathecal immunoglobulin synthesis,^{23,24} but it should be noted that these studies only examined CSF neurofilament heavy chain (NfH), which may have different characteristics to NfL.

sNfL levels increase during relapses (compared with stable disease phases) and correlate with the number of CELs in MRI, which suggests exacerbated acute focal inflammation as an underlying cause. In CSF, acute inflammation is reflected by an increase in the leukocyte count and Qalb. Indeed, we found a significant correlation between sNfL levels and the CSF leukocyte count. Comparable results have been seen in previous studies for NfL in CSF.^{25–27} In ocrelizumab-treated patients, corresponding decreases in the CSF leukocyte count and sNfL concentrations have been observed after treatment initiation, leading to the suggestion that levels of CSF inflammatory cells may predict axonal damage.²⁸ In progressive MS, on the other hand, no correlation of CSF NfL with CSF cell count could be found,^{25,29} which indicates a less pronounced acute inflammatory activity in progressive MS.

Albumin is exclusively of extrathecal origin, making its concentration in CSF independent of neuronal homeostasis and any underlying neurodegenerative processes.³⁰ Therefore, Qalb is a reliable indicator of BBB integrity.^{31,32} In our study, Qalb did not correlate with sNfL concentrations. However, as expected in a cohort of patients with MS, the majority of patients (88%) had Qalb values within normal ranges. We suggest that NfL levels in blood will only be affected by severe disruption of BBB integrity, as described in certain types of dementia³³ and HIV,³⁴ which might explain the inconsistency of previous reports.^{35–38} We provide an overview of former findings concerning the influence of CSF parameters that are relevant in MS diagnostics on various neurofilament isotypes in CSF and serum within table 2.

Although our study showed an association of both acute and chronic neuroinflammatory processes with sNfL levels, markers of acute inflammation, the CSF leukocyte count and the number of CELs in MRI, were better at predicting sNfL levels. This means that the effect of acute inflammatory activity on sNfL may outweigh the effect of underlying chronic inflammation. As sNfL is being discussed intensely as a prognostic biomarker in various neurologic disorders, it will be important to bear in mind the impact of active inflammatory processes on its concentration.

In addition, our data imply that the location of acute inflammation influences sNfL levels, as we found significantly lower sNfL concentrations in patients presenting with ON than in patients with symptoms suggestive of cerebral or spinal inflammation. One possible explanation could be that

quantitatively larger CNS compartments are able to release higher absolute quantities of NfL protein into the CSF and consecutively to the peripheral blood. An earlier study found that CSF NfH levels were nearly twice as high in patients with spinal cord-located relapses than in patients with brain-located relapses,²³ whereas others found no association of sNfL levels and type of clinical manifestation in MS³⁹ or myelin oligodendrocyte glycoprotein antibody-associated disorders.⁴⁰ When interpreting the current results, the presence and number of CELs in MRI need to be taken into consideration as possible confounders because patients with cerebral or spinal relapse manifestation presented with CEL more often than those with ON or clinically stable disease. The strict inclusion of only untreated patients, to rule out therapy effects on sNfL levels, is one of the main strengths of our study; however, this also resulted in a relatively small sample size not allowing further subgroup analyses. Further studies are needed to gain deeper insight into the pathophysiologic background behind our observations.

To conclude, our results add to the understanding of neurodegenerative processes in MS by conceptually supporting the hypothesis that axonal damage is caused by acute inflammatory activity and diffuse chronic neuroinflammation, both reflected by an increase in sNfL levels. In addition, episodes of disease activity, and possibly also type of clinical relapse manifestation as well as localization of lesions on MRI, need to be taken into consideration when interpreting sNfL values in scientific studies and clinical practice.

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Disclosure

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Falk Steffen	University Medical Center of the Johannes Gutenberg University, Mainz, Germany	Author	Acquisition of data
Timo Uphaus, MD	University Medical Center of the Johannes Gutenberg University, Mainz, Germany	Author	Revision of the manuscript for intellectual content
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Frauke Zipp, MD	University Medical Center of the Johannes Gutenberg University, Mainz, Germany	Author	Design and conceptualization of the study and revision of the manuscript for intellectual content
Stefan Bittner, MD	University Medical Center of the Johannes Gutenberg University, Mainz, Germany	Author	Design and conceptualization of the study and drafting significant proportion of the manuscript
Felix Luessi, MD	University Medical Center of the Johannes Gutenberg University, Mainz, Germany	Author	Design and conceptualization of the study; acquisition and analysis of data; and drafting significant proportion of the manuscript

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