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Monitoring of radiologic disease activity by serum neurofilaments in MS

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Abstract

Objective

To determine whether serum neurofilament light chain (sNfL) levels are associated with recent MRI activity in patients with relapsing-remitting MS (RRMS).

Methods

This observational study included 163 patients (405 samples) with early RRMS from the Study of Early interferon-beta1a (IFN-β1a) Treatment (SET) cohort and 179 patients (664 samples) with more advanced RRMS from the Genome-Wide Association Study of Multiple Sclerosis (GeneMSA) cohort. Based on annual brain MRI, we assessed the ability of sNfL cutoffs to reflect the presence of combined unique active lesions, defined as new/enlarging lesion compared with MRI in the preceding year or contrast-enhancing lesion. The probability of active MRI lesions among patients with different sNfL levels was estimated with generalized estimating equations models.

Results

From the sNfL samples ≥90th percentile, 81.6% of the SET (OR = 3.4, 95% CI = 1.8-6.4) and 48.9% of the GeneMSA cohort samples (OR = 2.6, 95% CI = 1.7-3.9) was associated with radiological disease activity on MRI. The sNfL level between the 10th and 30th percentile was reflective of negligible MRI activity: 1.4% (SET) and 6.5% (GeneMSA) of patients developed ≥3 active lesions, 5.8% (SET) and 6.5% (GeneMSA) developed ≥2 active lesions, and 34.8% (SET) and 11.8% (GeneMSA) showed ≥1 active lesion on brain MRI. The sNfL level <10th percentile was associated with even lower MRI activity. Similar results were found in a subgroup of clinically stable patients.

Conclusions

Low sNfL levels (≤30th percentile) help identify patients with MS with very low probability of recent radiologic disease activity during the preceding year. This result suggests that in future, sNfL assessment may substitute the need for annual brain MRI monitoring in considerable number (23.1%–36.4%) of visits in clinically stable patients.

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Glossary

DMT = disease-modifying treatment; **EDSS** = Expanded Disability Status Scale; **GeneMSA** = Genome-Wide Association Study of Multiple Sclerosis; **IFN-β1a** = interferon-beta1a; **NfL** = neurofilament; **RRMS** = relapsing-remitting MS; **SET** = Study of Early interferon-beta1a (IFN-β1a) Treatment; **sNfL** = serum neurofilament light chain; **T1-WI** = T1-weighted image.

Standard clinical follow-up in patients with MS includes regular assessment for new neurologic signs and symptoms of MS. However, only a small proportion of CNS lesions, detected with MRI, are symptomatic.[1,2] Thus, MRI is the most commonly used, sensitive surrogate of subclinical MS activity.[3]

Nevertheless, serial MRI follow-up has its limitations, including the unsolved challenges of accurate measurement of brain atrophy, the need for standardized MRI scanning protocols, cost, and the fact that MRI is frequently not covering the spinal cord in routine settings.[4,5] These limitations, together with a continued need for an accessible measure of neurodegeneration, have stimulated a search for new sensitive biomarkers.

Neurofilaments (NfLs) are the most promising biomarker of neuroaxonal injury in MS.[6–8] Increased concentration of the NfL light chain in the blood and the CSF are closely associated with relapse activity, worsening of disability, occurrence of active MRI lesions and predicts brain and spinal cord atrophy.[9–14] NfL light chain has recently become more accessible, as reliable methods for quantification of NfL light chain levels in serum (sNfL) have emerged.[10,13]

However, its potential role as a new and easily accessible biomarker of subclinical disease activity has been less studied. In this context, the role of sNfL in substitution of monitoring for detection of subclinical disease activity in MRI in certain clinical scenarios is still to be established.

In this study, we investigated the capacity of sNfL assessment as a marker of radiologic disease activity, including the sensitivity and specificity of different sNfL cutoffs in identifying the presence or absence of combined unique active lesions on brain MRI during the preceding year. We performed this study in 2 observational cohorts of patients with relapsing-remitting MS (RRMS): a discovery cohort of patients from the Study of Early interferon-beta1a (IFN-β1a) Treatment (SET), and a validation cohort of patients from the Genome-Wide Association Study of Multiple Sclerosis (GeneMSA).

Methods

Study population

SET cohort

The SET was an investigator-initiated, prospective observational study that involved 8 centers (NCT01592474) in the Czech Republic.[15–17] Enrollment started in October 2005 and was completed in July 2009.

In this cohort were included patients with complete sNfL and MRI data, age 18–55 years, enrollment within 4 months from the demyelinating event, Expanded Disability Status Scale (EDSS) score ≤3.5, at least 2 T2 hyperintense lesions on MRI performed before steroid treatment, and with ≥2 CSF-restricted oligoclonal bands at the screening visit.

SET patients were originally diagnosed with clinically isolated syndrome according to the McDonald 2005 criteria.[18] Patients' diagnosis was reclassified to RRMS based on the 2017 McDonald criteria.[19] All patients were treated with IV steroids following screening and subsequently started the treatment at baseline with 30 mg of IM IFN-β1a once a week (Avonex).

GeneMSA cohort

Patients were recruited in Basel as part of a prospective multicenter study initiated in 2003 (GeneMSA).[9,20,21]

In this cohort were included 179 patients with complete sNfL and MRI data, who were recruited at the Neurologic Clinic and Policlinic, University Hospital Basel (Switzerland) between June 2004 and October 2005.

The GeneMSA cohort consisted of patients with RRMS. A small proportion (4.5%) of the GeneMSA patients were originally diagnosed with clinically isolated syndrome according to the McDonald 2001 criteria.[22] Diagnosis of these patients was reclassified to RRMS based on the 2017 McDonald criteria.[19]

Ethical statement

The SET protocol was approved by the Medical Ethics Committees of the General University Hospital in Prague and by the local ethical committees in the participating centers, and all patients gave their written informed consent. All patients from the GeneMSA cohort provided written informed consent, and the study was approved by the local ethics committee.

MRI acquisition and analysis

SET cohort

The SET protocol stipulates brain MRI scans at baseline and at 12, 24, and 36 months, using a standardized protocol performed on the single 1.5-Tesla scanner (Gyroscan, Philips). Axial brain acquisitions included fluid-attenuated inversion recovery, T1-weighted images (T1-WIs), and pre- and

post-contrast T1 spin-echo images. Semiautomated subtraction image methodology was used to identify combined unique active lesions (active lesions later in the text), defined as current new contrast-enhancing lesions on T1-weighted scans and new or enlarging lesions on T2-weighted scans occurred during the preceding year. A new or enlarging lesion on T2-weighted images was defined as a rounded or oval lesion (≥3 mm of size) arising from an area previously considered as normal-appearing brain tissue and/or showing an identifiable increase in size from a previously stable-appearing lesion.[17] Image analyses were performed at Buffalo Neuroimaging Analysis Center, United States.[15,17]

GeneMSA cohort

Brain MRI scans were performed on all patients at baseline and then yearly in a 1.5-Tesla scanner (Magnetom Avanto, Siemens) equipped with a 12-element head matrix coil. MRI scans were performed on a single scanner at baseline and then annually over 78 months (median). Axial brain acquisitions included a double-echo proton density/T2-weighted sequence, T1-WI (magnetization prepared rapid acquisition with gradient echo), and T1 pre- and post-contrast spin-echo images. Active brain MRI lesions were identified and marked by consensus reading on simultaneously viewed T2-weighted and proton density–weighted images. Qualitative analysis for the presence of gadolinium enhancement was performed on post-contrast T1-WI. Image analyses were performed in the Medical Image Analysis Centre in Basel, Switzerland.[9] The details of the MRI acquisition and analysis are provided in supplementary appendix e-1, links.lww.com/NXI/A233.

Serum neurofilament light chain measurements

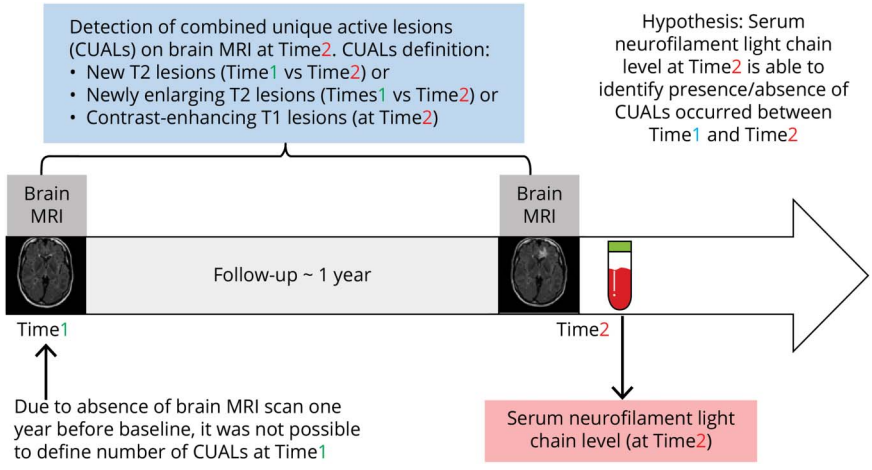
In the SET cohort, serum samples were collected at 12, 24, and 36 months from baseline on the same day as the MRI examination. In the GeneMSA cohort, serum samples were collected annually over 78 months (median) on the same day as the MRI examination. In both cohorts, the samples were stored at −80°C following standard procedures.[23] sNfL levels were measured using a homebrew Single Molecule Array assay at the University Hospital Basel as described previously.[13] Interassay coefficients of variation for 3 native serum samples were below 10% (i.e., 7.8%, 8.8%, and 5.5% for 7.0 pg/mL). The mean intra-assay coefficient of variation of duplicate determinations for concentration was 6.4%. One sample (of 405) showed an sNfL value below 1.3 pg/mL (i.e., the lower limit of quantification) and was excluded. Measurements were performed on coded samples by personnel blinded to clinical and MRI data.

Statistical analysis

All analyses were performed using the R statistical software (R-project.org) and SPSS 22.0 (IBM, Armonk, NY). Statistical analysis of the SET cohort data was performed in the CORe Unit (University of Melbourne) and of the GeneMSA cohort data in the Clinical Trial Unit (University of Basel). sNfL levels were grouped into different categories using age-specific percentiles derived from a normative data set of healthy controls. Supplementary table e-1, links.lww.com/NXI/A234, shows the relationship between sNfL levels and age-specific sNfL percentiles.[13]

The sensitivity, specificity, and area under the receiver operating characteristic curve with their 95% CIs (DeLong method)[24] of different predefined sNfL percentile cutoffs (sNfL deciles) for detection of ≥1, ≥2, or ≥3 active MRI lesions during the preceding year were assessed in the pooled analysis of all available data (figure 1). In other words, we investigated the capacity of sNfL at a given time point to identify the presence or absence of combined unique active lesions on brain MRI, defined as occurrence of current new contrast-enhancing T1 lesions, new or enlarging T2 lesions during the previous year. In addition to the pooled analysis of all available data, in the SET cohort we have separately evaluated the sensitivity and specificity of sNfL percentile cutoffs

Figure 1 Study design



as a marker of MRI activity for each year of follow-up. Next, conditional probabilities of sNfL percentile ranges for detection of ≥1, ≥2, or ≥3 active MRI lesions were assessed in both cohorts.

Finally, we estimated the probability of active MRI lesions among patients with different sNfL levels. sNfL percentile ranges with similar sensitivity and specificity were combined into 3 sNfL subgroups (>90th, 31th–90th, and <31th percentile). The presence of active MRI lesions was treated as a binary-dependent and sNfL percentile category as a binary-independent variable. ORs and 95% CIs were estimated with logistic generalized estimating equation models adjusted for age, sex, time point, disability status (only in the sensitivity analysis), number of relapses during the preceding year, and current treatment status (with therapies categorized as low efficacy [dimethyl fumarate, glatiramer acetate, and interferon-β]; high or moderately high efficacy [fingolimod, mitoxantrone, and natalizumab]; or no disease-modifying treatment [DMT]). The sNfL between the 31th and 90th percentile was chosen as the reference category.

The primary analyses were computed in the discovery cohort (SET) and validated in the validation cohort (GeneMSA). A sensitivity analysis was completed in a subgroup of clinically stable patients with no relapses or disability progression during the preceding year. The Benjamini-Hochberg procedure with $p < 0.05$ was used to control the false discovery rate.

Data availability

Anonymized data not published within this article will be made available by request from any qualified investigator.

Results

Baseline and follow-up clinical characteristics

Table 1 describes demographic, clinical, and MRI characteristics of the patient cohorts at baseline and during the follow-up. Of the 220 patients with RRMS enrolled in the SET cohort, 163 had sNfL (405 samples) and MRI follow-up data available ≥1 time point between baseline and 36 months. Of the 259 patients enrolled in the GeneMSA cohort, 179 patients with RRMS had sNfL (664 samples) and MRI follow-up data available at baseline and minimum of 1 additional time point. In the SET cohort, 376 (92.8%) samples were assessed during IFN-β1a treatment. Of the 120 (67%) patients in the GeneMSA treated with DMT, the median time on the DMT at baseline was 44.4 months (interquartile range 18.5–66.4 months).

Sensitivity and specificity of sNfL for identification of brain MRI activity

SET cohort

Higher levels of sNfL correlated with greater numbers of active brain MRI lesions (figure 2). sNfL greater than the 90th percentile was measured in 103 (25.4%) samples and identified patients with any active brain MRI lesions during the preceding year with 90% (95% CI = 86%–94%) specificity, patients with ≥2 active lesions with 90% (95% CI = 86%–93%) specificity, and patients with ≥3 active lesions with 88% (95% CI = 85%–92%) specificity. sNfL ≤30th percentile was observed in 133 (32.8%) samples and identified patients with active MRI lesions with 81% (95% CI = 75%–86%) sensitivity, patients with <2 active lesions with 94% (95% CI = 90%–98%) sensitivity, and patients with <3 active lesions with 99% (95% CI = 99%–100%) sensitivity (table 2). Separate analysis of time points did not show important differences (supplementary table e-2, links.lww.com/NXI/A234).

GeneMSA

Similar to the SET cohort, in the GeneMSA cohort, higher sNfL levels correlated with greater numbers of MRI lesions (figure 3). sNfL greater than the 90th percentile was found in 184 (27.7%) samples and identified patients with any active MRI lesions during the preceding year with 80% (95% CI = 77%–85%) specificity, patients with ≥2 active lesions with 79% (95% CI = 77%–83%) specificity, and patients with ≥3 active lesions with 78% (95% CI = 76%–83%) specificity. sNfL ≤30th percentile was observed in 129 (19.4%) of samples and identified patients with active MRI lesions with 93% (95% CI = 89%–96%) sensitivity, patients with <2 active lesions with 95% (95% CI = 91%–98%) sensitivity, and patients with <3 active lesions with 92% (95% CI = 87%–97%) sensitivity (table 2). Separate analysis of time points did not show important differences (supplementary table e-3, links.lww.com/NXI/A234).

Although there was a trend for slightly lower specificity and higher sensitivity of sNfL levels in the GeneMSA cohort, 95% CIs, especially for sensitivity, overlapped considerably between the 2 cohorts (table 2).

sNfL as a marker of brain MRI activity at the individual level

SET cohort

At the individual level, sNfL greater than the 90th percentile indicated a high probability of past MRI activity: 84 of 103 (81.6%) patients developed ≥1 active lesion, 76 (73.8%) developed ≥2 active lesions, and 66 (64.1%) developed ≥3 active lesions. In multivariable-adjusted analysis, sNfL above the 90th percentile was associated with a greater probability of having ≥3 active lesions compared with the 31–90th percentile (OR = 7.8; 95% CI = 4.1–14.8; $p < 0.0001$). Among the patients with sNfL between the 10th and 30th percentile, 24 (34.8%) developed ≥1 active lesion, 4 (5.8%) developed ≥2 active lesions, and only 1 (1.4%) developed ≥3 active lesions during the preceding year. Even lower radiologic disease activity was observed in patients with sNfL <10th percentile (table 3). In multivariable-adjusted analysis, sNfL ≤30th percentile was associated with a lower probability of having ≥3 active lesions compared with the 31–90th

Table 1 Characteristics of the studied cohorts

Characteristics	Prague	Basel
Total number of patients	163	179
Available sNFL and MRI measures between 0 and 12 mo	149	—
Available sNFL and MRI measures between 12 and 24 mo	131	—
Available sNFL and MRI measures between 24 and 36 mo	125	—
Total available pairs of sNFL and MRI measures	405	664
Females	106 (65.0%)	134 (74.9%)
Age at baseline (y)	28.0 (23–34)[a]	41.3 (34–48)[a]
Disease duration at baseline (y)	0.2 (0.2–0.3)[a]	9.0 (5–17)[a]
EDSS score at baseline	1.5 (1.5–2.0)[a]	2.0 (1.5–3.0)[a]
Follow-up duration (y)	3.0 (3.0–3.0)[a]	6.5 (2.6–9.2)[a]
Clinically definite relapsing-remitting MS		
At baseline	0 (0%)	171 (95.5%)
At last visit	69 (56.1%)	163 (91.1%)
Clinically stable patients within previous year (all time points)[b]	267 (65.9%)	402 (60.5%)
sNFL percentile level (all time points)	59 (19–91)[a]	69 (39–91)[a]
Proportion of scans with active lesions on MRI over follow-up[c]	206 (50.9%)	193 (29.1%)
Treatment at baseline[d]		
No DMT treatment	0 (0%)	59 (33.0%)
Low efficacy DMT	100 (0%)	118 (65.9%)
High or moderately high efficacy DMT	0 (0%)	2 (1.1%)

Abbreviations: DMT = disease-modifying treatment; EDSS = Expanded Disability Status Scale; sNFL = serum neurofilament level.
[a] Median and interquartile range.
[b] Clinically stable defined as an absence of clinical relapse and disability worsening (i.e., increase in the EDSS score).
[c] Active lesion was defined as new, newly enlarging, or enhancing lesion on follow-up brain MRI scan compared with brain MRI scan during the preceding year.
[d] Low-efficacy DMT: glatiramer acetate, dimethyl fumarate, and interferons; High or moderately high efficacy DMT: fingolimod, mitoxantrone, and natalizumab.

percentile (OR = 0.05; 95% CI = 0.01–0.3; $p < 0.002$). Similar results were observed in the sensitivity analysis of 267 (65.9%) samples from clinically stable patients (table 4).

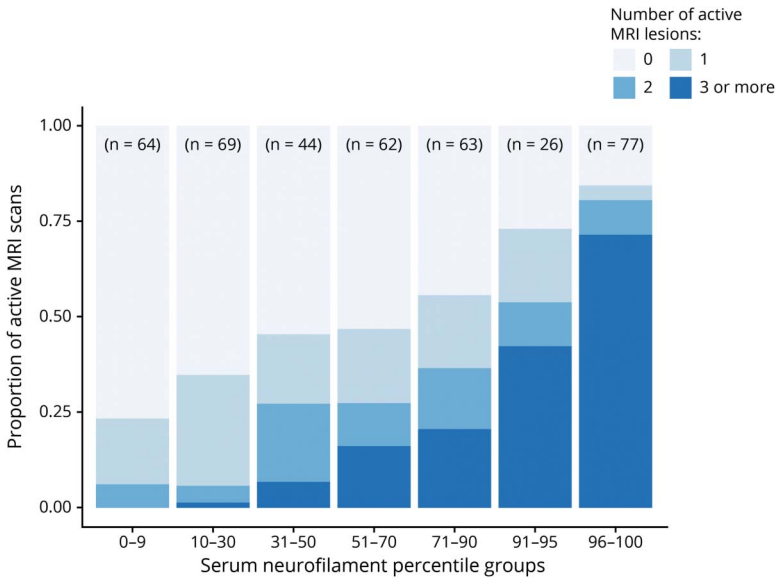
GeneMSA cohort

sNfL above the 90th percentile was reflective of higher MRI activity compared with lower sNfL levels. Eighty-six (48.9%) patients had developed ≥1 active lesion, 69 (39.2%) developed ≥2 active lesions, and 57 (32.4%) developed ≥3 active lesions. In multivariable-adjusted analysis, sNfL greater than the 90th percentile was associated with a greater probability of having ≥3 active lesions on MRI compared with the 31–90th percentile (OR = 5.8; 95% CI = 3.2–10.6; $p < 0.01$). Among the patients with sNfL between the 10th and 30th percentile, only 11 (11.8%) developed ≥1 active lesion, 6 (6.5%) developed ≥2 active lesions, and 6 (6.5%) developed ≥3 active lesions during the preceding year. Again, lower radiologic disease activity was observed in patients with sNfL <10th percentile, where: 3 (8.3%) developed ≥1 active lesion, 1 (2.8%) developed ≥2 active lesions, and 1 (2.8%) developed ≥3 active lesions during the preceding year (table 3). In multivariable-adjusted analysis, sNfL <31th percentile was associated with a lower probability of having ≥1 (OR = 0.3; 95% CI = 0.1–0.7; $p < 0.01$) and ≥2 (OR = 0.3; 95% CI = 0.1–0.9; $p = 0.02$) active lesions compared with the 31–90th percentile. The above observations were confirmed in the sensitivity analysis of 402 (60.5%) measures from clinically stable patients with no relapses or disability progression during the previous year (table 4). A sensitivity analysis in which all models were adjusted also for the EDSS score did not show considerable differences in the estimates (data not shown).

Discussion

In this longitudinal study of RRMS, high serum concentrations of NfL reflected increased recent radiologic disease

Figure 2 Proportion of brain MRI scans with 1, 2, or ≥3 active lesions by serum neurofilament percentile subgroups



The SET cohort. SET = Study of Early interferon-beta1a (IFN-β1a) Treatment

activity defined as occurrence of combined unique new, enlarging, or contrast-enhancing lesions on brain MRI in the previous 12 months. On the contrary, low sNfL levels were associated with negligible MRI activity. Results of the study were validated in an independent MS cohort and in a subgroup of clinically stable patients.

Of the patients with activity during the previous 12 months detected by brain MRI, 41%–47% had sNfL levels exceeding the 90th percentile. At the individual level, sNfL greater than the 90th percentile was indicative of high MRI activity: 64.1% and 32.4% of patients developed ≥3 active lesions, 73.8% and 39.2% of patients developed ≥2 active lesions, and 81.6% and 48.9% of patients showed ≥1 active lesion on MRI in the SET and GeneMSA cohorts, respectively. However, a considerable proportion of patients showed high sNfL in the absence of any reported brain MRI activity (18.4% in the discovery cohort and 51.1% in the validation cohort). These instances could represent occasions when the amount of brain inflammatory activity was below the detection threshold of brain MRI (inflammation of normal-appearing white and gray matter),[25,26] where inflammation was localized with in the spinal cord or where the source of sNfL was accelerated global brain tissue loss due to neurodegenerative processes. In both cohorts, sNfL greater than the 90th percentile was associated with approximately 3 times higher probability of having any active MRI lesion compared with the 31–90th percentile. Patients with sNfL >90th percentile had also approximately 4 times higher probability of having ≥2 and 6–8 times higher probability of having ≥3 active MRI lesions compared with the 31–90th percentile. Importantly, 90% (SET) and 80% (GeneMSA) of the patients with no detected MRI activity had sNfL lower than the 90th percentile.

Based on the observations summarized above, we interpret the findings of high sNfL (adjusted for age) as an indicator of a high likelihood of disease activity that should prompt further investigations with detailed imaging of the whole neuraxis.[9] In these instances, MRI may help explain elevated sNfL, but may also add clinically relevant information with regard to the location and extent of lesion activity.[27,28] Moreover, sNfL as a marker is not specific to MS lesion activity but may also reflect neuroaxonal damage due to other reasons.[6] Last but not least, increased sNfL levels were associated with an increased risk of future disease activity.[9,11–14] Therefore, patients with high sNfL levels without concurrent MRI or clinical evidence of disease activity should be followed closely to detect early indicators of treatment failure. Frequent screening for high (or increasing) sNfL levels may assist physicians with clinical decision making by providing information about high probability of recent, ongoing, and future subclinical disease activity.

It should be noted that a high sNfL level was strongly associated with brain MRI activity, but the sensitivity of the high sNfL levels for detection of MRI activity during the preceding year was expectedly lower. This suggests that MRI activity was reported also in instances with sNfL lower than the 90th percentile. This can be explained by the time course of sNfL levels following neuroaxonal injury. sNfL levels peak at 1 month and return to baseline at 6 months after neuroaxonal injury. Therefore, sNfL levels reflect only recent or ongoing MRI activity and are not sensitive to neuroaxonal injury occurring before more that 6–9 months.[7,14,29]

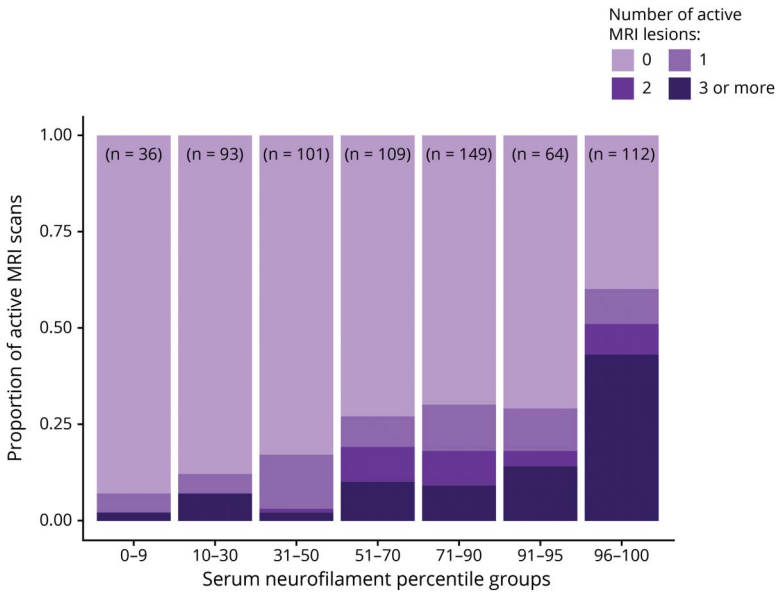
From the clinical perspective, a low sNfL level represented a very informative finding. sNfL ≤30th percentile was

Table 2 Sensitivity and specificity of serum neurofilament levels for detection of active lesions on brain MRI

sNFL percentile		No. of patients	≥1 Active lesion[a]				≥2 Active lesions[a]				≥3 Active lesions[a]			
			Sensitivity %	95% CI	Specificity %	95% CI	Sensitivity %	95% CI	Specificity %	95% CI	Sensitivity %	95% CI	Specificity %	95% CI
SET	>95th	77 (19.0%)	32	25–38	93	90–97	48	38–54	93	91–97	60	50–70	92	89–95
	>90th	103 (25.4%)	41	34–47	90	86–94	56	47–64	90	86–93	71	61–80	88	85–92
	>70th	166 (41.0%)	57	51–64	76	69–81	73	66–81	75	69–80	85	77–91	72	66–76
	>50th	228 (56.3%)	71	65–78	59	53–66	85	79–91	58	53–64	96	91–99	55	50–61
	>30th	272 (67.2%)	81	75–86	47	41–55	94	90–98	46	41–52	99	98–100	42	37–48
	>10th	339 (83.7%)	93	89–96	25	20–32	97	94–99	22	18–28	100	NA	21	NA
	AUC ROC	—	0.74 (0.69–0.79)				0.83 (0.78–0.87)				0.88 (0.85–0.92)			
GeneMSA	>95th	128 (19.3%)	38	28–41	89	88–93	48	37–53	88	87–92	56	43–63	87	86–91
	>90th	184 (27.7%)	47	38–52	80	77–84	56	46–62	79	77–83	65	53–73	78	76–82
	>70th	328 (49.4%)	69	62–75	59	54–63	77	69–83	57	53–62	79	69–86	55	51–60
	>50th	434 (65.4%)	84	78–89	42	38–47	92	88–96	41	37–45	90	84–96	39	35–42
	>30th	535 (80.6%)	93	89–96	24	21–28	95	90–98	23	19–26	92	87–98	21	18–25
	>10th	625 (94.1%)	98	96–100	8	5–10	99	98–100	7	5–9	99	97–100	7	5–9
	AUC ROC	—	0.71 (0.67–0.75)				0.77 (0.72–0.81)				0.77 (0.72–0.83)			

Abbreviations: AUC ROC = area under the receiver operating characteristic curve; GeneMSA = Genome-Wide Association Study of Multiple Sclerosis; NA = unable to analyze; SET = Study of Early interferon-beta1a (IFN-β1a) Treatment; sNFL = serum neurofilament level.
[a] Active lesion was defined as new, newly enlarging, or enhancing lesion on follow-up brain MRI scan compared with brain MRI scan during the preceding year. All available time points were pooled (repeated measures in the majority of patients).

Figure 3 Proportion of brain MRI scans with 1, 2, or ≥3 active lesions by serum neurofilament percentile subgroups



The GeneMSA cohort. GeneMSA = Genome-Wide Association Study of Multiple Sclerosis.

reflective of negligible MRI activity: approximately 0.8%–5.4% of the patients developed ≥3 active lesions, 5.4%–6.0% developed ≥2 active lesions, and 10.9%–29.3% showed ≥1 active lesion. In both cohorts, sNfL ≤30th percentile was associated with approximately 3 times lower probability of any recent MRI activity compared with the 31–90th percentile. The lower probability of lesion MRI activity in patients with sNfL ≤30th percentile levels was also observed for occurrence of ≥2 and ≥3 active MRI lesions, with the relative odds of a clinically significant brain MRI activity in the SET cohort being extremely low (20 times lower in the ≤30th percentile than the 31–90th percentile). In fact, 81% (SET) and 93% (GeneMSA) of the patients with any brain MRI activity had sNfL levels in excess of the 30th percentile, and 93% (SET) and 98% (GeneMSA) of the patients with any brain MRI activity had sNfL levels in excess of the 10th percentile.

Only a very few patients with sNfL ≤30th percentile showed clinically significant amount of MRI activity, defined as ≥3 (or even ≥2) active MRI lesions.[30] In the cohort with clinically stable disease, the proportion of patients with low sNfL levels and MRI activity is even lower. We therefore suggest that assessment of sNfL may substitute the need for annual brain MRI monitoring in clinically stable patients with low sNfL levels (≤30th percentile). This constituted a considerable proportion of the MRI scans from patients with clinically stable disease included in this study: 36.4% in the SET and 23.1% in the GeneMSA cohort. However, one should remember that a small amount of MRI activity (1 active lesion) in the last year can be detected even in patients with such low sNfL levels. This could be mostly attributed to only transient increase of sNfL levels following neuroaxonal injury, which may not correlate with detected MRI activity, if lesion formation occurred more that 6–9 months ago.[7,14,29]

In general, the discovery (SET) cohort showed slightly lower sNfL levels but higher proportion of active MRI scans (51% vs 29%) than the validation (GeneMSA) cohort. In the GeneMSA cohort, we found a lower number of active MRI lesions, which is in agreement with previous research that showed decreasing incidence of MRI activity over disease course.[25] Considering younger age and shorter disease duration of the SET cohort, a higher proportion of active MRI scans in the SET cohort is therefore not surprising.

The 2 studied MS cohorts used different scanning protocols and techniques for detection of active MRI lesions. The cohorts also differed slightly in terms of the disease burden, clinical and radiologic disease activity, and sNfL levels over follow-up. It is therefore reassuring that similar optimal cutoffs for sNfL were identified in the 2 cohorts. In the SET cohort, we observed a trend for marginally higher specificity and lower sensitivity of sNfL than in the GeneMSA cohort, which was consistent throughout the follow-up. The 95% CIs for sensitivity overlapped considerably between the 2 cohorts. One can speculate that these trends were secondary to the differences in disease burden, disease activity, DMT, or differences in MRI protocols. In the preliminary subanalysis of the GeneMSA cohort, we found a trend for slightly higher accuracy of sNfL marker of MRI activity in patients with younger age and short disease duration and lower accuracy of sNfL cutoffs in patients with older age and long disease duration. These trends remain to be investigated in further studies and larger cohorts.

Table 3 Predictive values of serum neurofilament percentiles for detection of active lesions on brain MRI

sNFL percentile range		No. of samples	Patients with <1 active lesion	Patients with ≥1 active lesion	OR (95% CI)	Patients with <2 active lesion	Patients with ≥2 active lesions	OR (95% CI)	Patients with <3 active lesions	Patients with ≥3 active lesions	OR (95% CI)
SET	>90th	103 (25.4%)	19 (18.4%)	84 (81.6%)	3.4 (1.8–6.4)[a]	27 (26.2%)	76 (73.8%)	4.5 (2.5–8.3)[a]	37 (35.9%)	66 (64.1%)	7.8 (4.1–14.8)[a]
	51–90th	125 (30.9%)	61 (48.8%)	64 (51.2%)	Reference group	85 (68.0%)	40 (32.0%)	Reference group	102 (81.6%)	23 (18.4%)	Reference group
	31–50th	44 (10.9%)	24 (54.5%)	20 (45.5%)		32 (72.7%)	12 (27.3%)		41 (93.2%)	3 (6.8%)	
	10–30th	69 (17.0%)	45 (65.2%)	24 (34.8%)	0.4 (0.3–0.7)[a]	65 (94.2%)	4 (5.8%)	0.1 (0.01–0.3)[a]	68 (98.6%)	1 (1.4%)	0.05 (0.01–0.3)[a]
	<10th	64 (15.8%)	49 (76.6%)	15 (23.4%)		60 (93.8%)	4 (6.2%)		64 (100.0%)	0 (0%)	
GeneMSA	>90th	176 (26.5%)	90 (51.1%)	86 (48.9%)	2.6 (1.7–3.9)[a]	27 (26.2%)	76 (73.8%)	3.5 (2.2–5.5)[a]	119 (67.6%)	57 (32.4%)	5.8 (3.2–10.6)[a]
	51–90th	258 (38.9%)	182 (70.5%)	76 (29.5%)	Reference group	85 (68.0%)	40 (32.0%)	Reference group	233 (90.3%)	25 (9.7%)	Reference group
	31–50th	101 (15.2%)	84 (83.2%)	17 (16.8%)		32 (72.7%)	12 (27.3%)		99 (98.0%)	2 (2.0%)	
	10–30th	93 (14.0%)	82 (88.2%)	11 (11.8%)	0.3 (0.1–0.7)[a]	65 (94.2%)	4 (5.8%)	0.3 (0.1–0.9)[b]	87 (93.5%)	6 (6.5%)	0.9 (0.3–2.1)
	<10th	36 (5.4%)	33 (91.7%)	3 (8.3%)		60 (93.8%)	4 (6.2%)		35 (97.2%)	1 (2.8%)	

Abbreviations: GeneMSA = Genome-Wide Association Study of Multiple Sclerosis; SET = Study of Early interferon-beta1a (IFN-β1a) Treatment; sNFL = serum neurofilament level.
Active lesion was defined as new, newly enlarging, or enhancing lesion on follow-up brain MRI scan compared with brain MRI scan during the preceding year.
All available time points were pooled (repeated measures in the majority of patients).
ORs were estimated by generalized estimating equations adjusted for age, sex, treatment status, time point, and number of relapses during the preceding year.
[a] $p < 0.05$ after Benjamini-Hochberg correction.
[b] $p < 0.05$.

Table 4 Predictive values of serum neurofilament percentiles for detection of active lesions on brain MRI in clinically stable patients with MS

sNFL percentile range		No. of samples	≥1 active lesion[a]			≥2 active lesions[a]			≥3 active lesions[a]		
			Patients with <1 active lesion	Patients with ≥1 active lesion	OR (95% CI)	Patients with <2 active lesion	Patients with ≥2 active lesions	OR (95% CI)	Patients with <3 active lesions	Patients with ≥3 active lesions	OR (95% CI)
SET	>90th	53 (19.9%)	14 (26.4%)	39 (73.6%)	2.3 (1.1–4.8)[b]	20 (37.7%)	33 (62.3%)	3.5 (1.7–7.4)[c]	25 (47.2%)	28 (52.8%)	6.2 (2.7–14.2)[c]
	51-90th	83 (31.1%)	43 (51.8%)	40 (48.2%)	Reference group	61 (73.5%)	22 (26.5%)	Reference group	71 (85.5%)	12 (14.5%)	Reference group
	31–50th	34 (12.7%)	15 (44.1%)	19 (55.9%)		23 (67.6%)	11 (32.4%)		31 (91.2%)	3 (8.8%)	
	10–30th	52 (19.5%)	37 (71.2%)	15 (28.8%)	0.3 (0.2–0.6)[c]	50 (96.2%)	2 (3.8%)	0.1 (0.05–0.3)[c]	51 (98.1%)	1 (1.9%)	0.1 (0.01–0.6)[b]
	<10th	45 (16.9%)	35 (77.8%)	10 (22.2%)		42 (93.3%)	3 (6.7%)		45 (100.0%)	0 (0%)	
GeneMSA	>90th	81 (20.1%)	47 (58.0%)	34 (42.0%)	2.3 (1.2–4.5)[c]	56 (69.1%)	25 (30.9%)	3.0 (1.5–6.0)[c]	62 (76.5%)	19 (23.5%)	6.2 (2.4–15.7)[c]
	51–90th	158 (39.3%)	116 (73.4%)	42 (26.6%)	Reference group	132 (83.5%)	26 (16.5%)	Reference group	148 (93.7%)	10 (6.3%)	Reference group
	31–50th	70 (17.4%)	57 (81.4%)	13 (18.6%)		67 (95.7%)	3 (4.3%)		68 (97.1%)	2 (2.9%)	
	10–30th	66 (16.4%)	59 (89.4%)	7 (10.6%)	0.3 (0.1–0.8)[c]	64 (97.0%)	2 (3.0%)	0.2 (0.0–0.7)[c]	64 (97.0%)	2 (3.0%)	0.5 (0.1–2.1)
	<10th	27 (6.7%)	25 (92.6%)	2 (7.4%)		27 (100%)	NA		27 (100.0%)	NA	

Abbreviations: GeneMSA = Genome-Wide Association Study of Multiple Sclerosis; SET = Study of Early interferon-beta1a (IFN-β1a) Treatment; sNFL = serum neurofilament level.
All available time points were pooled (repeated measures in the majority of patients).
Clinically stable patients were defined as those with an absence of clinical relapse and disability progression during the preceding year.
[a] Active lesion was defined as new, newly enlarging, or enhancing lesion on follow-up brain MRI scan compared with brain MRI scan during the preceding year.
[b] $p < 0.05$.
[c] $p < 0.05$ after Benjamini-Hochberg correction.

The ability to detect active MRI lesions is influenced by a variety of technical, rater- and patient-related factors.[31,32] Therefore, variability in the sensitivity of the scanning protocols was likely. We have mitigated the risk of reporting error for MRI activity by using also a more rigorous requirement of higher number of active MRI lesions as our study outcome. Moreover, 3-dimensional subtracted images after image registration, with thin-slice thickness, were analyzed by semiautomatic techniques (SET) or by experienced MRI raters (GeneMSA). These techniques detect considerably higher number of active lesions compared with imaging used in clinical practice.[31,33] Therefore, in the context of clinical practice, sNfL levels may show a very good sensitivity even in detection of single active MRI lesion. This needs to be investigated in future studies.

Second, although the levels of sNfL were interpreted in the context of its gradual increase with age (through the use of normative reference data), we did not adjust for other potential confounders of sNfL. The differences in sNfL levels between the 2 studied cohort support that in MS, other disease-specific factors may codetermine sNfL. Among these, disease duration, time since disease activity, disability, differences in DMT, and comorbidities would be the likely candidates. Although the SET patients initiated DMT at baseline, potential effect of delayed treatment response resulting in decoupling between sNfL levels at 12 months and MRI activity during the preceding year was not observed. Also, we did not find an effect of disability on the relationship between sNfL levels and MRI activity. Another important question requiring better understanding is the dynamics of rising and decreasing sNfL levels following lesion formation and disease activity. Finally, a large normative database for sNfL in MS patients is needed.

NfL is a promising biomarker of neuroaxonal injury[6–8] strongly associated with measures of disease activity in MS.[9–14] Given that a considerable number of patients with high sNfL levels did not have detectable lesion activity on brain MRI, further studies are needed to clarify the origin of increased sNfL in patients with radiologically stable brain MRI. Based on the results of previous research, we hypothesize that active spinal cord lesions,[9] accelerated loss of brain volume, or subclinical inflammation of normal-appearing white and gray matter may play a role.[25,26] If this hypothesis is correct, sNfL may, in addition, qualify as a sensitive surrogate biomarker of inflammation and axonal loss.[9]

sNfL is a cumulative indicator of neuronal damage over weeks to months.[7,14,29] Future studies are required to investigate the benefit of more frequent sNfL assessment (e.g., 6-monthly), which may represent a sensitive and accessible method for capturing subclinical disease activity in routine follow-up. Finally, assessment of the relationship between sNfL and MRI activity in different subgroups is needed to allow generalization of the results to the prevalent MS population.

This study, using discovery-validation design in 2 large MS centers, demonstrates that low levels of sNfL help identify patients with MS with no clinically relevant radiologic disease activity. In future, sNfL monitoring may potentially supplement the need for annual brain MRI monitoring in a proportion of patients with clinically stable MS and with low sNfL levels. Further research is required to understand whether high sNfL levels in the absence of new or enlarging brain lesions may signal subclinical disease activity in patients, who would otherwise be considered stable.

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Appendix *(continued)*

Appendix *(continued)*

Name	Location	Contribution
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Tomas Kalincik, MD, PhD	CORe, Department of Medicine, The University of Melbourne, Department of Neurology, The Royal Melbourne Hospital, Melbourne, VIC, Australia	Study concept and design, data interpretation, and manuscript preparation

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Monitoring of radiologic disease activity by serum neurofilaments in MS

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CORRECTION

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