RESEARCH ARTICLE OPEN ACCESS

Contribution of Blood Biomarkers to Multiple Sclerosis Diagnosis

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Neurol Neuroimmunol Neuroinflamm 2025;12:e200370. doi:10.1212/NXI.0000000000200370

Abstract

Background and Objectives

Invasive procedures may delay the diagnostic process in multiple sclerosis (MS). We investigated the added value of serum neurofilament light chain (sNfL), glial fibrillary acidic protein (sGFAP), chitinase-3-like 1 (sCHI3L1), and the immune responses to the Epstein-Barr virus–encoded nuclear antigen 1 to current MS diagnostic criteria.

Methods

In this multicentric study, we selected patients from 2 prospective cohorts presenting a clinically isolated syndrome (CIS). Patients were classified as (1) not presenting dissemination in space (DIS) nor dissemination in time (DIT) (noDIS and noDIT); (2) presenting DIS without DIT (DIS and noDIT); and (3) presenting both (DIS and DIT), which were used as a reference. sNfL, sGFAP, and sCHI3L1 levels were measured with single-molecule array immunoassays and EBNA1-specific IgG levels with ELISA. Biomarker levels were compared between groups using linear regression models. Receiver operating characteristic curve analyses and Youden Index were used to determine cutoff values associated with MS diagnosis during follow-up.

Results

We included 181 patients (66.3% females, mean [SD] age of 35.0 [9.7] years). At baseline, 25 (13.8%) were classified as noDIS and noDIT, 62 (34.3%) as DIS and noDIT, and 94 (51.9%) as DIS and DIT. Only sNfL Z-scores discriminated between groups (DIS and DIT vs DIS and noDIT [$p = 0.002$], DIS and DIT vs noDIS and noDIT [$p < 0.001$], and DIS and noDIT vs noDIS and noDIT [$p = 0.026$]). In noDIS and noDIT patients (median interquartile range [IQR] follow-up of 8.1 [5.0–11.7] years), high sNfL Z-scores best predicted MS diagnosis (specificity [SP] and 95% CI of 93.3% [68.1–99.8] and positive predictive value [PPV] of 87.5% [47.3–99.7]). Among DIS and noDIT patients (median [IQR] follow-up of 6.8 [4.0–9.1] years), high sNfL Z-scores best predicted MS diagnosis (SP of 80% [28.4–99.5] and PPV of 97.3% [85.8–99.9]) without considering oligoclonal band (OB) status. In the subset of patients of this group with negative OBs, a combination of high sNfL Z-scores and sGFAP levels predicted MS diagnosis (SP of 100% [39.8–100] and PPV of 100% [54.1–100]).

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The Article Processing Charge was funded by the authors.

Glossary

AUC = area under the curve; **CIS** = clinically isolated syndrome; **DA** = disease activity; **DIS** = dissemination in space; **DIT** = dissemination in time; **DMT** = disease-modifying therapy; **EBNA1** = Epstein-Barr virus–encoded nuclear antigen 1; **EBV** = Epstein-Barr virus; **IQR** = interquartile range; **MS** = multiple sclerosis; **NPV** = negative predictive value; **OB** = oligoclonal band; **PPV** = positive predictive value; **sCHI3L1** = serum chitinase 3-like 1; **SE** = sensitivity; **sGFAP** = serum glial fibrillary acidic protein; **sNfL** = serum neurofilament light chain; **SP** = specificity.

Discussion

These results suggest that sNfL and sGFAP may be incorporated in particular scenarios to diagnose MS in patients with CIS not fulfilling current diagnostic criteria.

Introduction

Multiple sclerosis (MS) is a chronic, inflammatory, and neurodegenerative disorder affecting the CNS.[1] Similar to several medical conditions, MS diagnosis relies on both clinical and paraclinical findings, allowing the initiation of disease-modifying therapies (DMTs) to limit further relapses, disability accumulation, and inflammatory activity in MRI.[2-4]

As neurologic damage derived from disease activity (DA) is often irreversible, it is essential to perform an early and accurate diagnosis. The constant evolution of ancillary tests, particularly MRI and CSF analysis determining the presence of restricted IgG oligoclonal bands (OBs) to this compartment, has led to significant modifications in the diagnostic criteria over time, increasing their accuracy.[5-8] Currently, definite MS diagnosis can be established after a first demyelinating event [clinically isolated syndrome (CIS)] if other alternative diagnoses have been reasonably excluded, demonstrating dissemination in space (DIS) by the presence of typical demyelinating lesions in specific CNS topographies and dissemination in time (DIT) by the presence of gadolinium (Gd) enhancement in 1 or more of such lesions or new and/or enlarging T2 lesions in subsequent MRI scans. Alternatively, when DIT is absent, a definite MS diagnosis can also be established in patients with DIS and the presence of CSF-restricted IgG OBs.[8]

Despite the advantages of incorporating OB determination, several challenges arise. Obtaining a CSF sample is often not possible because an invasive procedure is required; disclosing OB is a complex technique demanding experience and agreed laboratory protocols; other neurologic disorders may exhibit positive OBs as well; prevalence varies across populations; and they are absent in a small proportion of patients.[9-12] In this sense, numerous body fluid biomarkers linked to neuroinflammation and neurodegeneration have been described in recent years, providing insights into MS pathophysiology. These biomarkers can be detected in blood as a surrogate of CSF by ultrasensitive immunoassays and have been thoroughly investigated to anticipate DA.[13-21] However, their contribution to the current MS diagnostic criteria has yet to be explored.

In this study, we measured in a cohort of patients presenting a CIS the serum levels of biomarkers known to play a prognostic role in the early phases of MS, such as the neurofilament light chain (sNfL),[22] glial fibrillary acidic protein (sGFAP),[23] chitinase 3-like 1 (sCHI3L1),[24] and the humoral immune responses to the Epstein-Barr virus (EBV)–encoded nuclear antigen 1 (EBNA1),[25] assessing their potential contribution to different scenarios of the current MS diagnostic criteria.[8]

Methods

Participants and Study Design

We retrospectively selected patients presenting a CIS between October 2004 and December 2022. Patients were recruited from 2 neuroimmunology centers: The Multiple Sclerosis Centre of Catalonia (Cemcat), Vall d'Hebron University Hospital (Barcelona), and the Ramón y Cajal Hospital (Madrid). Clinical, demographic, radiologic, biological, and therapeutic information within both cohorts is prospectively obtained using prespecified protocols explained in detail elsewhere.[26,27] The selection of patients for the study was based on the availability of serum samples collected in proximity to the CIS event and both brain and spinal MRI scans at baseline with Gd administration to confirm DIS and DIT. Clinically isolated syndrome was defined as a first clinical episode consistent with a focal or multifocal inflammatory demyelinating event in the CNS, developing acutely or subacutely and lasting at least 24 hours in the absence of fever or infection.[8] Patients with other alternative diagnoses beyond MS confirmed at baseline or during follow-up were excluded.

MRI, CSF, and clinical assessments were performed within 3 months from clinical onset as part of the diagnostic workup in both centers. Demographic and clinical features recorded at

baseline included age, sex, and topography (optic nerve, spinal cord, infratentorial, hemispheric, and multifocal) at CIS. CSF and peripheral blood samples were collected by lumbar puncture and standard venipuncture, respectively, for routine diagnostics. Briefly, CSF samples were centrifuged to remove cells, aliquoted, and conserved at −80°C until used. Blood was allowed to clot spontaneously for 30 minutes, and serum was obtained by centrifugation and stored frozen at −80°C until used. OBs were determined in paired CSF and serum samples by isoelectric focusing combined with immunoblotting.[28,29] Baseline and follow-up MRIs were conducted using 1.5 or 3.0T magnets. Brain imaging sequences included transverse 2-dimensional (2D) dual-echo T2-weighted fast spin-echo, 2D or 3-dimensional (3D) transverse T2-fluid-attenuated inversion recovery, and 2D transverse T1-weighted. All baseline T1-weighted sequences were systematically repeated after the administration of Gd contrast agent (0.2 mmol/kg). Spinal cord imaging sequences included sagittal T2-weighted fast spin-echo, sagittal short tau inversion recovery, and Gd-enhanced sagittal T1-weighted. In addition, axial T2-weighted sequences were acquired on selected spinal cord segments. Two-dimensional sequences were obtained with a contiguous 3 mm section thickness, while 3D sequences were acquired with isotropic voxels measuring 1 × 1 × 1 mm.

Classification of Patients at CIS and Follow-Up

Patients were assessed for DIS and DIT at CIS onset according to the current MS diagnostic criteria.[8] DIS was considered when patients presented at least 1 typical demyelinating lesion in 2 or more topographies (cortical-juxtacortical, periventricular, infratentorial, or spinal cord). DIT was established when patients exhibited 1 or more Gd-enhancing lesions that coexisted with non–Gd-enhancing lesions (regardless of whether the observed Gd-enhancement corresponded to the symptomatic lesion) at baseline MRI. Patients were classified into the following 3 groups: (1) patients not presenting DIS nor DIT (noDIS and noDIT); (2) patients presenting DIS, without DIT (DIS and noDIT); and (3) patients presenting both (DIS and DIT), consequently fulfilling the 2017 McDonald criteria.[8] This last category was used as a reference. The study did not include patients with typical demyelinating lesions in only 1 topography presenting Gd-enhancement (i.e., noDIS and DIT), and all noDIS and noDIT patients exhibited at least 1 typical demyelinating lesion in one of the aforementioned topographies. We explored the added value of biomarkers to the following 3 scenarios of the MS diagnostic criteria: (1) patients with noDIS and noDIT, (2) patients with DIS and noDIT without considering the presence of OBs as a diagnostic criterion, and (3) patients with DIS and noDIT and negative OBs.

Patients were followed until DA, if present, allowed for an MS diagnosis.[8] DA was defined by the presence of 1 or more new relapses and/or 1 or more univocal new T2 or Gd-enhancing lesions using the same criteria as at CIS onset. All relapses were confirmed by experienced neurologists specializing in MS and related conditions.

Biomarker Determinations

Analyses of sNfL and sGFAP levels were performed on the fully automated ultrasensitive Simoa HD-1 Analyzer (Quanterix, Billerica, MA) using the commercially available Simoa Neuro 2-Plex B Advantage Kit (cat# 103520, Quanterix). Samples were run in duplicate at a 1:4 dilution, and appropriate standards and internal controls were included in accordance with the manufacturer's instructions. The intra-assay and interassay coefficients of variation were 4.9% and 9.0% for sNfL, and 6.9% and 13.0% for sGFAP, respectively. Levels of sCHI3L1 were quantified by an *in-house* Simoa-based assay developed at the Cemcat as previously described.[30] Samples were diluted at a 1:100 dilution, and standards and internal controls were run in duplicates in the Simoa HD-1 Analyzer. Intra-assay and interassay coefficients of variation were 4.5% and 6.2%, respectively.

Serum IgG antibodies to EBV-EBNA1 were quantified using a commercial ELISA kit (cat#. DE4246, Demeditec Diagnostics, Kiel, Germany). Samples were run in duplicate previous dilution 1:100 on dilution buffer. Antibody quantification was expressed in arbitrary units per milliliter (U/mL). According to the manufacturer's instructions, seropositivity was considered for concentrations ≥11 U/mL.

Statistical Analysis

Descriptive data are presented as mean (SD) and median (interquartile range [IQR]) values. Analysis of sNfL levels was performed using age-normalized sNfL Z-scores based on a large reference database of serum samples from controls.[13] The comparisons of baseline serum biomarker levels between patients with CIS classified according to DIS and DIT were assessed using linear regression models. The comparisons of biomarker levels between patients with negative and positive OBs, and between patients with and without DA during follow-up were performed with a Mann-Whitney *U* test. When needed, analysis was adjusted by age. The relationship between the different serum biomarker levels was assessed by partial correlations controlling for age. Receiver operating characteristic (ROC) curve analyses and Youden Index were used to determine the best cutoff values associated with DA during follow-up based on baseline serum biomarker levels and to calculate the respective sensitivity (SE), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV). For this analysis, patients who received DMT after the CIS event and did not present DA during follow-up were excluded, given the strong influence of treatment on said outcome. A sensitivity analysis was also performed, excluding patients who received treatment after the CIS and developed DA. Statistical significance was set at $p < 0.05$.

Standard Protocol Approvals, Registrations, and Patient Consents

Databases have been developed according to national and international standards on ethical aspects (Declaration of

Helsinki and Tokyo), and these data may be used in accordance with the regulations in force regarding the protection of personal data (EU) 2016/679; April 27 of 2016 (General Data Protection Regulation). The study was approved by the Clinical Research Ethics Committee at Vall d'Hebron University Hospital and Ramón y Cajal Hospital. All patients signed written informed consent according to the Declaration of Helsinki.

Data Availability

The data that support the findings of this study are available from the corresponding author on reasonable request.

Results

Demographic and Clinical Characteristics

We included a total of 181 patients with a CIS in this study. The mean (SD) age of patients was 35.0 (9.7) years, and 120 (66.3%) were women. The most frequent CIS clinical topography was the spinal cord (34.3%), followed by the optic nerve (28.2%), infratentorial (27.1%), hemispheric (8.3%), and multifocal (2.2%). OBs were positive in 143 (79%) patients. Twenty-five (13.8%) patients were classified as noDIS and noDIT, 62 (34.3%) as DIS and noDIT, and 94 (51.9%) as DIS and DIT. The median (IQR) time between CIS and sample collection for biomarker determination was 1.5 (0.6–2.3) months, and between sample collection and diagnostic MRI, 1.2 (0.1–2.5) months. The baseline demographic and clinical information of the whole cohort of patients is summarized in Table. Clinical characteristics of patients with CIS stratified by the participating center are detailed in eTable 1.

Table Demographic and Clinical Characteristics of Patients With CIS

Characteristics	Whole cohort
N	181
Age (y)[a]	35.0 (9.7)
Female/male (n [% women])	120/61 (66.3)
CIS topography [n (%)]	
Spinal cord	62 (34.3)
Optic nerve	51 (28.2)
Infratentorial	49 (27.1)
Hemispheric	15 (8.3)
Multifocal	4 (2.2)
IgG oligoclonal bands (n [% positive])	143 (79.0)
Patient classification at CIS (n [%])	
noDIS and noDIT	25 (13.8)
DIS and noDIT	62 (34.3)
DIS and DIT	94 (51.9)
Time between blood samples and CIS (mo)[b]	1.5 (0.6–2.3)
Time between blood sample and diagnostic MRI (mo)[b]	1.2 (0.1–2.5)

Abbreviations: CIS = clinically isolated syndrome; DIS = dissemination in space; DIT = dissemination in time; mo = month(s).
Data are expressed as mean (SD) unless otherwise stated.
[a] Refers to age at the time of the CIS event.
[b] Data are expressed as median (interquartile range). noDIS and noDIT: patients not presenting dissemination in space nor dissemination in time. DIS and noDIT: patients presenting dissemination in space without dissemination in time. DIS and DIT: patients presenting both dissemination in space and dissemination in time.

Distribution of Baseline Serum Biomarker Levels According to Patient Classification at CIS

Stratification based on the presence or absence of OBs revealed significantly higher sNfL Z-scores and EBNA1-specific IgG levels in patients with positive OBs ($p < 0.001$ and $p = 0.002$, respectively). By contrast, sGFAP and sCHI3L1 levels were comparable between patients with positive and negative OBs (eFigure 1). No significant correlations were observed among baseline serum biomarker levels (eTable 2).

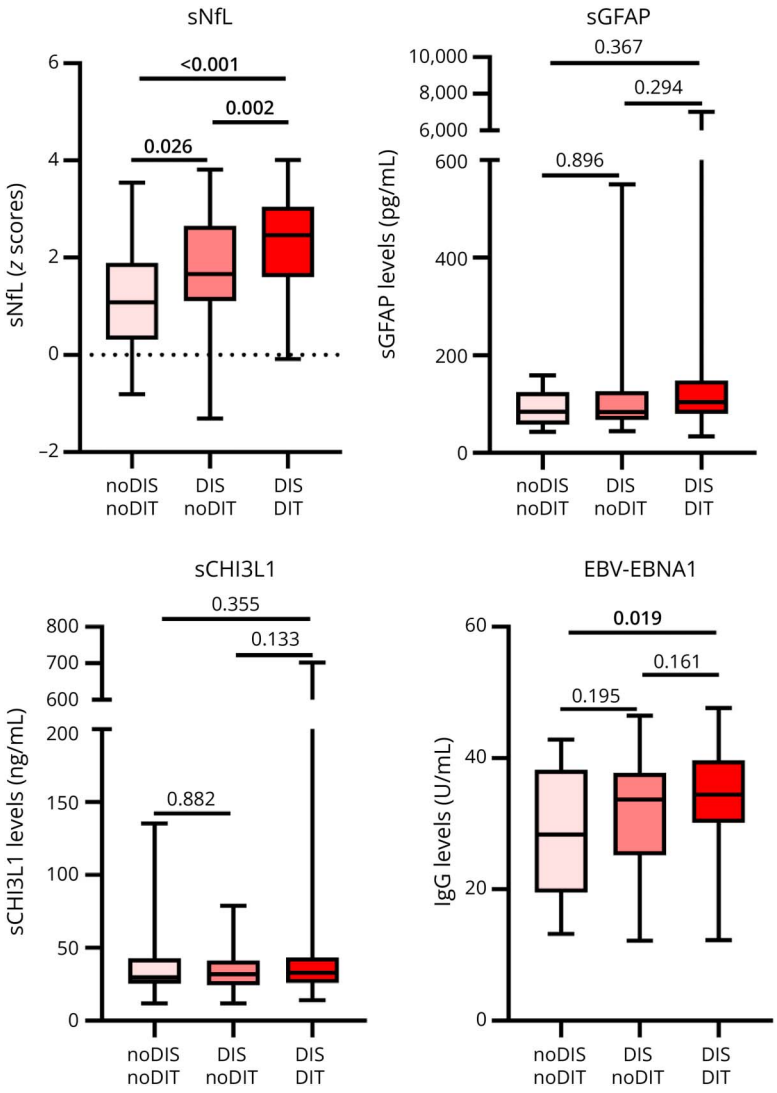
Baseline sNfL Z-scores discriminated between patients with CIS classified according to DIS and DIT because they were significantly more elevated in patients with DIS and DIT compared with patients with DIS and noDIT (differences of the mean [95% CI] 0.53 [0.19–0.87]; $p = 0.002$) and noDIS and noDIT (1.09 [0.63–1.56]; $p < 0.001$). sNfL Z-scores were also higher in patients with DIS and noDIT vs noDIS and noDIT (0.56 [0.04–1.08]; $p = 0.026$). Regarding the remaining biomarkers, only EBNA1-specific IgG levels were higher in patients with DIS and DIT than noDIS and noDIT (4.62 [0.69–8.55]; $p = 0.019$), whereas no significant differences were observed for sGFAP and sCHI3L1 levels across the patient categories (Figure 1).

Contribution of Serum Biomarkers to MS Diagnosis in CIS Patients With noDIS and noDIT

Twenty-five (13.8%) patients were included in this group. The mean (SD) age of patients was 35.8 (10.2) years, and 12 (48.0%) were women. The optic nerve was the most frequent clinical topography (56.0%), and 8 (32.0%) patients presented positive OBs. The median (IQR) follow-up after the CIS event was 8.1 (5.0–11.7) years. Ten (40.0%) patients developed DA during follow-up, allowing for an MS diagnosis, and the median (IQR) time to conversion was 2.0 (0.9–5.0) years. None of these patients received treatment between the CIS and MS diagnosis or between the CIS and the last visit if they did not develop DA. Baseline sNfL Z-scores were significantly higher in patients later diagnosed with MS than those without DA during follow-up ($p = 0.03$). No significant differences were observed for the remaining baseline biomarkers (eTable 3).

Baseline sNfL Z-scores performed best in discriminating between patients who developed DA during follow-up. The area under the ROC curve ([AUC] [95% CI]) was 77% (54%–

Figure 1 Distribution of Serum Biomarker Levels in Patients With CIS Classified According to DIS and DIT



Boxplots showing serum levels of neurofilament light chain (sNfL) represented as Z-scores, levels of glial fibrillary acidic protein (sGFAP) in pg/mL, levels of chitinase 3-like 1 (sCHI3L1) in ng/mL, and IgG levels (U/mL) to Epstein-Barr virus–encoded nuclear antigen 1 (EBV-EBNA1) in patients not presenting dissemination in space nor dissemination in time (noDISnoDIT), patients presenting dissemination in space without dissemination in time (DISnoDIT), and patients presenting dissemination in space and dissemination in time (DISDIT). A y-axis segmentation was performed to represent better high and low sGFAP and sCHI3L1 levels. After adjusting for age: for sNfL Z-scores, $p = 0.001$ and $p < 0.001$ for DISDIT vs DISnoDIT and noDISnoDIT, respectively, and $p = 0.030$ for DISnoDIT vs noDISnoDIT; for EBV-EBNA1, $p = 0.020$ for DISDIT vs noDISnoDIT. Significant p values are shown in bold. CIS = clinically isolated syndrome; DIS = dissemination in space; DIT = dissemination in time.

99%) for a sNfL Z-score of 1.64, resulting in the best cutoff to differentiate between active and nonactive patients during follow-up, with SE (95% CI) of 70% (34.8–93.3), SP of 93.3% (68.1–99.8), and PPV and NPV of 87.5% (47.3–99.7) and 82.4% (56.6–96.2), respectively. Analysis restricted to patients presenting with optic neuritis, the most frequent clinical topography, revealed an AUC of 87.5% (66%–100%) for a sNfL Z-score value of 1.64, resulting again in the best cutoff to discriminate between both groups, with SE of 75% (34.9–96.8), SP of 100% (54.1–100), and PPV and NPV of 100% (54.1–100) and 75.0% (34.9–96.8), respectively (Figure 2A).

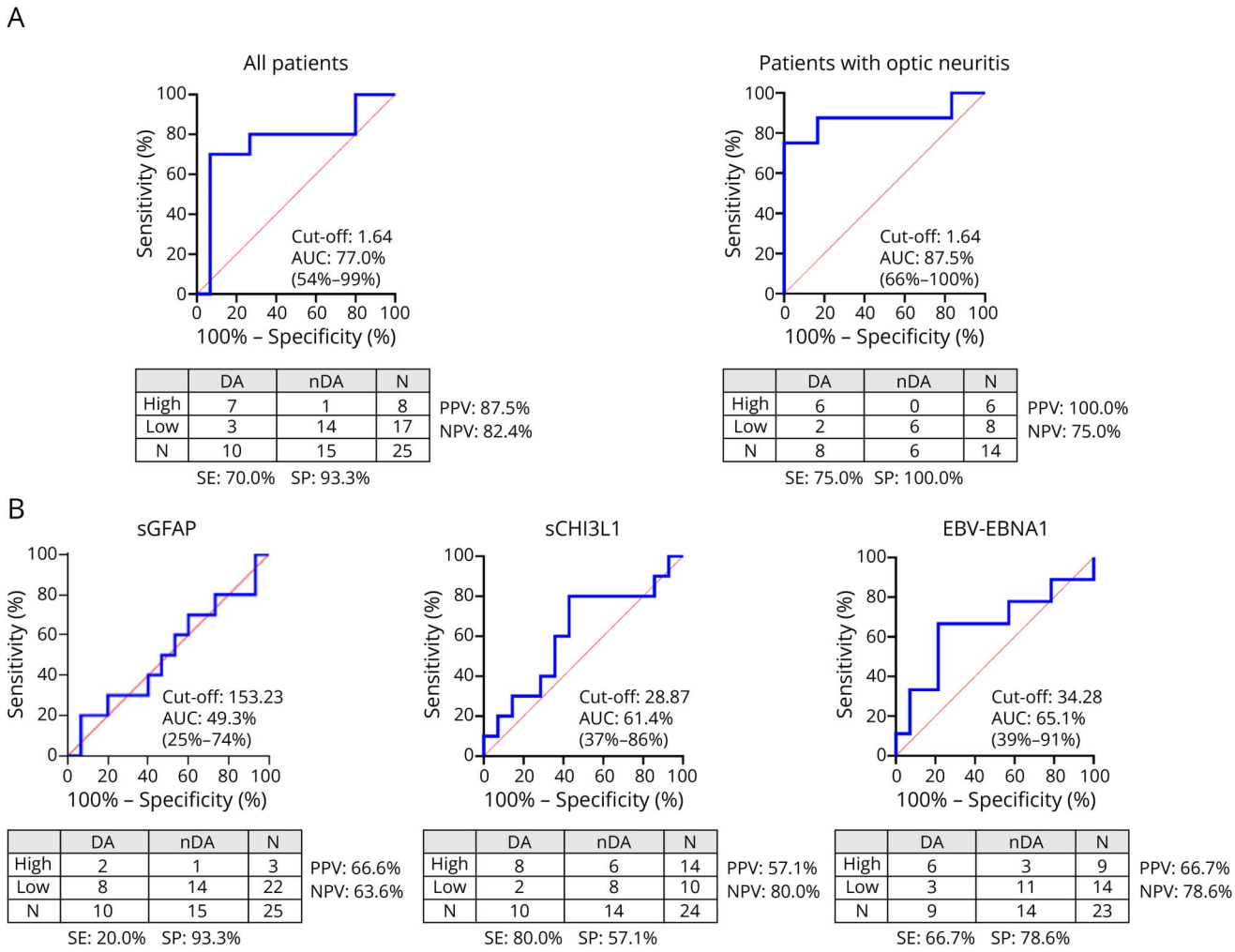
The performance of baseline sCHI3L1 and EBNA1-specific IgG levels was lower compared with sNfL Z-scores, although slightly higher for EBNA1 IgG levels, with an AUC of 65.1% and 61.4% for EBV-EBNA1 and sCHI3L1, respectively. By contrast, sGFAP levels showed no predictive capacity (AUC of 49.3%) to differentiate between active and nonactive patients (Figure 2B).

Contribution of Serum Biomarkers to MS Diagnosis in CIS Patients With DIS and noDIT

The added value of baseline serum biomarker levels to MS diagnosis in patients in this group was explored in 2 different scenarios: first, in all patients regardless of the OB status, and second, only in patients with negative OBs.

Of the initial 62 (34.3%) patients in this category, 10 (16.1%) patients who did not develop DA during follow-up received DMT after the CIS event and were excluded from the analysis. Among the 52 (83.9%) remaining patients, the mean (SD) age was 33.9 (8.5) years and 36 (69.2%) were women. The spinal cord was the most frequent clinical topography (38.5%). Thirty-eight (73.1%) patients presented positive OBs. The median (IQR) follow-up after the CIS was 6.8

Figure 2 Performance of Baseline Serum Biomarker Levels to Discriminate Between MS Converters and Nonconverters in CIS Patients With noDIS and noDIT



	DA	nDA	N
High	7	1	8
Low	3	14	17
N	10	15	25

PPV: 87.5%
NPV: 82.4%
SE: 70.0% SP: 93.3%

	DA	nDA	N
High	6	0	6
Low	2	6	8
N	8	6	14

PPV: 100.0%
NPV: 75.0%
SE: 75.0% SP: 100.0%

	DA	nDA	N
High	2	1	3
Low	8	14	22
N	10	15	25

PPV: 66.6%
NPV: 63.6%
SE: 20.0% SP: 93.3%

	DA	nDA	N
High	8	6	14
Low	2	8	10
N	10	14	24

PPV: 57.1%
NPV: 80.0%
SE: 80.0% SP: 57.1%

	DA	nDA	N
High	6	3	9
Low	3	11	14
N	9	14	23

PPV: 66.7%
NPV: 78.6%
SE: 66.7% SP: 78.6%

ROC curves of baseline serum biomarker levels show the area under the ROC curve (AUC) with 95% CIs, sensitivity (SE), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV). (A) ROC curves for sNfL Z-scores in all patients and patients presenting with optic neuritis. (B) ROC curves for sGFAP, sCHI3L1, and EBV-EBNA1. CIS = clinically isolated syndrome; DA = disease activity; EBV-EBNA1 = Epstein-Barr virus–encoded nuclear antigen 1; MS = multiple sclerosis; nDA = no disease activity; sCHI3L1 = levels of chitinase 3-like 1; sGFAP = glial fibrillary acidic protein; sNfL = serum neurofilament light chain.

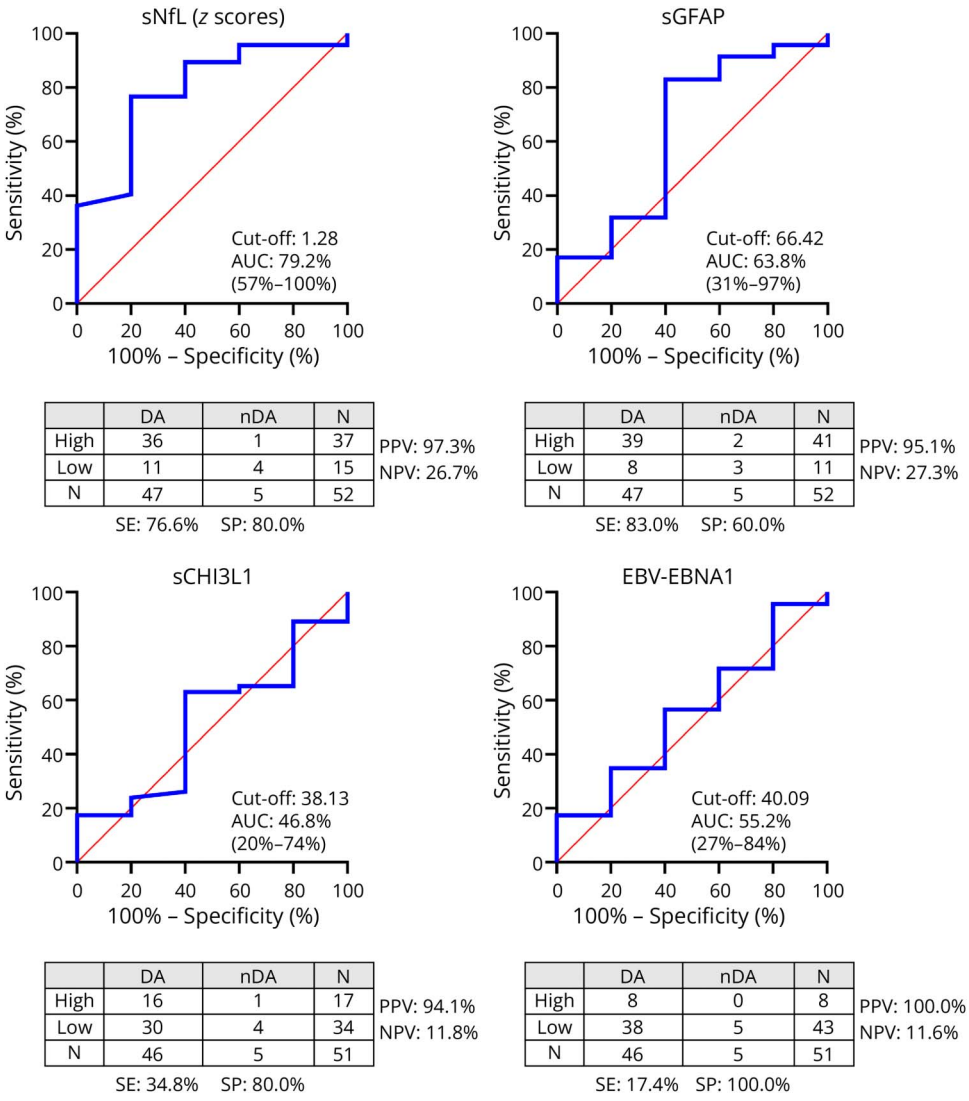
(4.0–9.1) years. Forty-seven (90.4%) patients developed DA during follow-up after a median (IQR) time of 1.1 (0.4–2.9) years, of whom 12 (25.5%) patients received DMT after the CIS. Only baseline sNfL Z-scores were significantly higher in patients with DA during follow-up ($p = 0.03$) (eTable 4).

Without considering the OB status, baseline sNfL Z-scores showed again the best performance to discriminate between patients with DIS and noDIT who developed or not DA during follow-up, with an AUC of 79.2% (57%–100%) and a sNfL Z-score of 1.28 as the best cutoff with SE of 76.6% (62.0–87.7), SP of 80% (28.4–99.5), and PPV and NPV of 97.3% (85.8–99.9) and 26.7% (7.8–55.1), respectively (Figure 3). A sensitivity analysis excluding 11 (21.1%) patients who received treatment after the CIS and developed DA resulted in a similar capacity to discriminate between patients with and without DA (AUC of 78.0% [57–98%]).

Baseline sGFAP levels showed lower performance than sNfL Z-scores to differentiate between both groups, with an AUC of 63.8% (31%–97%). A sGFAP value of 66.42 pg/mL resulted in the best cutoff, with SE of 83% (69.2–92.4), SP of 60% (14.7–94.7), PPV of 95.1% (83.5–99.4), and NPV of 27.3% (6.0–61.0). The combination of sNfL Z-scores and sGFAP levels equal to or higher than the cutoffs mentioned above increased SP to 100% (47.8–100) and PPV to 100% (88.4–100) (eTable 5). The potential for baseline sCHI3L1 and EBNA1-specific IgG levels to discriminate between patients with and without DA during follow-up was also lower, with AUC of 46.8% and 55.2%, respectively (Figure 3).

We finally explored the contribution of baseline biomarker levels to MS diagnosis in 14 (22.6%) patients with CIS in this group with negative OBs. The mean (SD) age was 36.4 (6.8) years, and 10 (71.4%) were women. The optic nerve,

Figure 3 Performance of Baseline Serum Biomarker Levels to Discriminate Between MS Converters and Nonconverters in CIS Patients With DIS and noDIT Regardless of the Oligoclonal Band Status



	DA	nDA	N
High	36	1	37
Low	11	4	15
N	47	5	52

PPV: 97.3%
NPV: 26.7%
SE: 76.6% SP: 80.0%

	DA	nDA	N
High	39	2	41
Low	8	3	11
N	47	5	52

PPV: 95.1%
NPV: 27.3%
SE: 83.0% SP: 60.0%

	DA	nDA	N
High	16	1	17
Low	30	4	34
N	46	5	51

PPV: 94.1%
NPV: 11.8%
SE: 34.8% SP: 80.0%

	DA	nDA	N
High	8	0	8
Low	38	5	43
N	46	5	51

PPV: 100.0%
NPV: 11.6%
SE: 17.4% SP: 100.0%

ROC curves of baseline serum biomarker levels show the area under the ROC curve (AUC) with 95% CIs, sensitivity (SE), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV). CIS = clinically isolated syndrome; DA = disease activity; DIS = dissemination in space; MS = multiple sclerosis; nDA = no disease activity.

infratentorial, and spinal cord topographies were similarly distributed. The median (IQR) follow-up after the CIS event was 6.5 (1.9–8.1) years. Ten (71.4%) patients presented DA during follow-up after a median (IQR) time of 1.4 (0.8–2.3) years, of whom only 1 patient (10.0%) received treatment after the CIS. The remaining 4 patients who did not develop DA during follow-up were not treated after the CIS. Baseline biomarker levels did not significantly differ between patients with and without DA during follow-up (eTable 6).

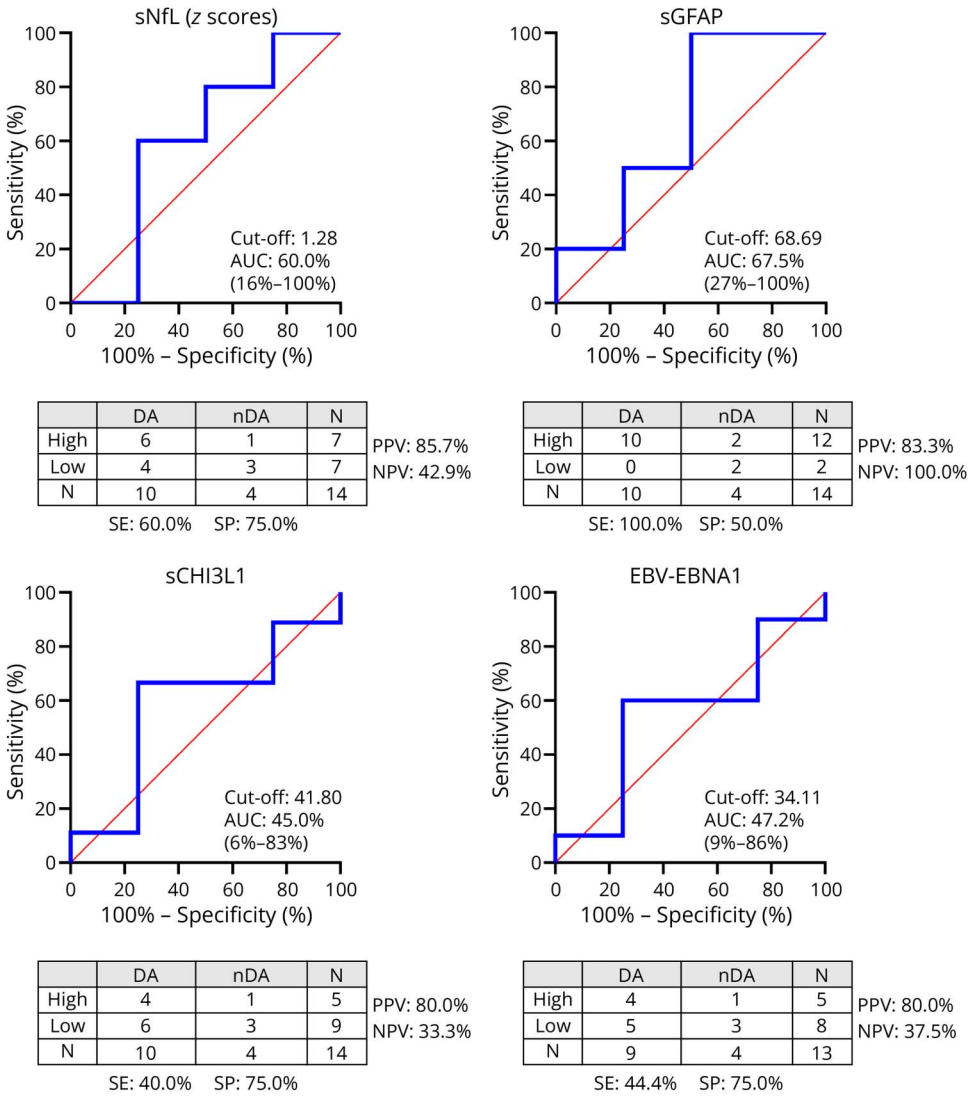
In patients with negative OBs, baseline sGFAP levels performed best to differentiate patients diagnosed with MS during follow-up from those without DA, with an AUC of 67.5% (27%–100%). The predictive accuracy associated with the best cutoff (68.69 pg/mL) exhibited a SE of 100% (69.2–100), NPV of 100% (15.8–100), PPV of 83.3% (51.6–97.9), and SP of 50% (6.8–93.2) (Figure 4). The performance of baseline sNfL Z-scores, sCHI3L1, and EBNA1-specific IgG levels was overall low, with AUC ranging from 60% for sNfL Z-scores to 45% for sCHI3L1. However, the combination of sGFAP levels and sNfL Z-scores equal to or higher than 68.69 pg/mL and 1.28, respectively, increased SP to 100% (39.8–100) and PPV to 100% (54.1–100) (eTable 7).

Discussion

In this study, we measured the serum levels of several biomarkers at CIS and examined their added value during the diagnostic process to predict future conversion to MS.

Near the CIS event, sNfL Z-scores discriminated well between patients stratified according to different diagnostic

Figure 4 Performance of Baseline Serum Biomarker Levels to Discriminate Between MS Converters and Nonconverters in CIS Patients With DIS and noDIT With Negative Oligoclonal Bands



	DA	nDA	N
High	6	1	7
Low	4	3	7
N	10	4	14

PPV: 85.7%
NPV: 42.9%
SE: 60.0% SP: 75.0%

	DA	nDA	N
High	10	2	12
Low	0	2	2
N	10	4	14

PPV: 83.3%
NPV: 100.0%
SE: 100.0% SP: 50.0%

	DA	nDA	N
High	4	1	5
Low	6	3	9
N	10	4	14

PPV: 80.0%
NPV: 33.3%
SE: 40.0% SP: 75.0%

	DA	nDA	N
High	4	1	5
Low	5	3	8
N	9	4	13

PPV: 80.0%
NPV: 37.5%
SE: 44.4% SP: 75.0%

ROC curves of baseline serum biomarker levels show the area under the ROC curve (AUC) with 95% CIs, sensitivity (SE), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV). CIS = clinically isolated syndrome; DA = disease activity; DIS = dissemination in space; DIT = dissemination in time; MS = multiple sclerosis; nDA = no disease activity.

scenarios. Patients fulfilling DIS and DIT showed significantly higher sNfL Z-scores than the remaining groups, most likely reflecting the presence of Gd-enhancing lesions, known to increase sNfL levels.[31] Similarly, sNfL Z-scores were higher in patients presenting positive OBs, in line with a probably more substantial inflammatory component in this subgroup.[32] Although in our study, sNfL Z-scores and EBNA1-specific IgG responses did not correlate, immune responses to the latter were also elevated in patients with DIS and DIT and patients with positive OBs. Higher EBNA1-specific IgG responses have already been reported in MS patients with Gd-enhancing lesions and positive OBs,[33,34] findings that possibly reflect a more inflammatory environment in patients with elevated humoral immune responses to EBV-EBNA1 and reinforce the link between EBV and intrathecal B-cell activation.[35]

Among patients not fulfilling DIS nor DIT, 40% were finally diagnosed with MS after a median follow-up of 8 years. sNfL Z-scores at baseline outperformed the rest of the biomarkers for predicting this outcome. Noteworthy, a cutoff sNfL Z-score value of 1.64 at the time of the CIS can be used in clinical practice to identify patients with noDIS and noDIT who will be diagnosed with MS during follow-up with a PPV higher than 87%. It should be highlighted that the same cutoff value applied to patients with CIS presenting optic neuritis, the most frequent clinical topography, had a PPV of 100% to identify those patients who will later develop DA. The performance of baseline levels of sCHI3L1, sGFAP, and EBNA1-specific IgG titers was overall poorer compared with sNfL Z-scores, findings that do not support their use in clinical practice in this subgroup of patients.

Patients fulfilling DIS but not DIT during the first demyelinating event are particularly challenging in clinical practice. Although a formal MS diagnosis is not established, increasing evidence has emerged showing that these patients will finally convert to MS, and clinicians are frequently prone to start DMT.[36] In this population, we observed that not only almost all patients (90%) fulfilled current diagnostic criteria after a median follow-up of 6.8 years, but they did so in a short period (median of 1.1 years), questioning the DIT criterion. Without considering the information provided by the OBs, sNfL Z-scores at baseline performed best for predicting MS diagnosis, followed by sGFAP levels, with PPV higher than 95% for both biomarkers. The presence of high sNFL Z-scores and high sGFAP levels improved the accuracy of predicting further DA with SP and PPV of 100%, findings that may support an added value of this biomarker to make an MS diagnosis in patients presenting DIS without DIT when information on OBs is not known.

With the current MS diagnostic criteria,[8] patients presenting DIS without DIT and negative OBs are not diagnosed with MS at the time of the CIS event. In our study, 71.4% of patients in this subgroup were diagnosed with MS after a median follow-up of 6.5 years. Surprisingly, sGFAP levels had an acceptable performance in identifying patients who later presented an MS diagnosis. In addition, all patients with high sGFAP levels and sNfL Z-scores at baseline developed DA. These findings support the determination of both biomarkers to predict DA accurately,[16] but in our study, they identify patients with CIS who will convert to MS during follow-up.

The main strength of this multicentric study is the inclusion of a well-characterized cohort of patients thoroughly assessed clinically and radiologically at CIS. In addition, patients presented an extensive median follow-up, allowing us to observe the disease behavior over a considerable period for all subgroups. Furthermore, various serum biomarkers related to different facets of MS pathophysiology and prognosis were explored, assessing each one's potential contribution to MS diagnosis. Finally, all MRI procedures and serum determinations were performed close to the first demyelinating event, allowing for an accurate interpretation of results. The main limitation of the study is the relatively small number of patients after stratification for diagnostic scenarios, particularly for the noDIS and noDIT and DIS and noDIT with negative OB groups. Another significant limitation is the small number of DIS and noDIT patients who did not convert to MS and were left for analysis after excluding patients receiving DMT. This reduced the statistical power to associate biomarker levels with the outcome satisfactorily. Furthermore, this study did not examine patients exhibiting DIT without DIS, representing a fair proportion of patients with CIS. Exploring this population would have covered all the possible diagnostic scenarios at CIS onset.

Although further studies in independent cohorts are needed, our results suggest that sNfL Z-scores can aid MS diagnosis at CIS onset. With the advent of new diagnostic criteria, this work bridges current criteria and future directions by emphasizing 3 essential aspects: the role of novel diagnostic tools alongside traditional measures, the minimally invasive nature of these tests as alternatives to invasive procedures, and the potential for serum biomarkers to serve as surrogates for DIT, serving as a reasonable alternative to OB determination. In this regard, we propose that patients with noDIS and noDIT with high baseline sNfL Z-scores can be diagnosed with MS at disease onset. In addition, CIS patients with DIS without DIT and no available OBs can be diagnosed with MS if they show high sNfL Z-scores, with added predictive value from elevated sGFAP in specific scenarios.

Author Contributions

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Study Funding

The authors report no targeted funding.

Disclosure

The authors report no relevant disclosures. Go to Neurology.org/N for full disclosures.

Publication History

Received by *Neurology: Neuroimmunology & Neuroinflammation* September 5, 2024. Accepted in final form December 16, 2024. Submitted and externally peer reviewed. The handling editor was Deputy Editor Scott S. Zamvil, MD, PhD, FAAN.

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