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Authors

Benkert, Pascal
Maleska Maceski, Aleksandra
Schaedelin, Sabine
et al.

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Serum Glial Fibrillary Acidic Protein and Neurofilament Light Chain Levels Reflect Different Mechanisms of Disease Progression under B-Cell Depleting Treatment in Multiple Sclerosis

Pascal Benkert, PhD [1,2,3#] Aleksandra Maleska Maceski, MSc,[1,3#]
Sabine Schaedelin, MSc,[1,2,3] Johanna Oechtering, MD [1,3]
Amar Zadic, MSc,[1,3] Juan Francisco Vilchez Gomez, MSc,[1,3] Lester Melie-Garcia, PhD,[1,3,4]
Alessandro Cagol, MD [1,3,4]
Riccardo Galbusera, MD [1,3,4]
Suvitha Subramaniam, MSc,[1,2] Johannes Lorscheider, MD,[1,3]
Edoardo Galli, MD,[1,3] Jannis Mueller, MD [1,3,4] Bettina Fischer-Barnicol, MD,[1,3]
Lutz Achtnichts, MD,[5] Oliver Findling, MD,[5] Patrice H. Lalive, MD,[6,7,8]
Claire Bridel, MD, PhD,[6]
Marjolaine Uginet, MD,[6] Stefanie Müller, MD,[9] Caroline Pot, MD,[10]
Amandine Mathias, PhD,[10] Renaud Du Pasquier, MD,[10] Anke Salmen, MD,[11]
Robert Hoepner, MD,[12] Andrew Chan, MD,[12] Giulio Disanto, MD, PhD,[13]
Chiara Zecca, MD,[13,14] Marcus D'Souza, MD,[1,3] Lars G. Hemkens, MD,[1,2]
Özgür Yaldizli, MD,[1,3] Tobias Derfuss, MD,[1,3] Patrick Roth, MD,[15] Claudio Gobbi, MD,[13,14]
David Brassat, MD,[16,17] Björn Tackenberg, MD,[18,19] Rosetta Pedotti, MD,[19]
Catarina Raposo, PhD,[19] Jorge Oksenberg, PhD,[20] Heinz Wiendl, MD,[21]
Klaus Berger, PhD,[22] Marco Hermesdorf, PhD [22] Fredrik Piehl, MD,[23,24]
David Conen, MD,[25] Andreas Buser, MD,[26] Ludwig Kappos, MD,[1,2,3]
Michael Khalil, MD, PhD [27] Cristina Granziera, MD, PhD,[1,2,3,4] Ahmed Abdelhak, MD [20]
David Leppert, MD [1,2,3] Eline A.J. Willemse, PhD,[1,2,3#] and
Jens Kuhle, MD, PhD, [1,2,3] for the Swiss MS Cohort study (SMSC)

Objective: To investigate the longitudinal dynamics of serum glial fibrillary acidic protein (sGFAP) and serum neurofilament light chain (sNfL) levels in people with multiple sclerosis (pwMS) under B-cell depleting therapy (BCDT) and their capacity to prognosticate future progression independent of relapse activity (PIRA) events.
Methods: A total of 362 pwMS (1,480 samples) starting BCDT in the Swiss Multiple Sclerosis (MS) Cohort were included. sGFAP levels in 2,861 control persons (4,943 samples) provided normative data to calculate adjusted Z scores.
Results: Elevated sGFAP levels (Z score >1) at 1 year were associated with a higher hazard for PIRA (hazard ratio [HR]: 1.80 [95% CI: 1.17–2.78]; $p = 0.0079$) than elevated sNfL levels (HR, 1.45 [0.95–2.24], $p = 0.0886$) in a combined model. Independent of PIRA events, sGFAP levels longitudinally increased by 0.49 Z score units per 10 years follow-up (estimate, 0.49 [0.29, 0.69], $p < 0.0001$). In patients experiencing PIRA, sGFAP Z scores were 0.52 Z score units higher versus stable patients (0.52 [0.22, 0.83], $p = 0.0009$). Different sNfL Z score trajectories were found in pwMS with versus without PIRA (interaction $p = 0.0028$), with an average decrease of 0.92 Z score units per 10 years observed without PIRA (−0.92 [−1.23, −0.60], $p < 0.0001$), whereas levels in patients with PIRA remained high.
Interpretation: Elevated sGFAP and lack of drop in sNfL after BCDT start are associated with increased risk of future PIRA. These findings provide a rationale for combined monitoring of sNfL and sGFAP in pwMS starting BCDT to predict the risk of PIRA, and to use sGFAP as an outcome in clinical trials aiming to impact on MS progressive disease biology.

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Multiple sclerosis (MS) is a disabling disease of the central nervous system with 2 main pathophysiological hallmarks: neuroinflammation and neurodegeneration. Accumulating evidence suggests that acute focal inflammatory activity (relapses and lesion formation) overlaps with brain-diffuse, "non-relapsing" mechanisms, which clinically manifest as continuous worsening of neurological functions or "progression".[1] High efficacy disease modifying therapies in MS, such as anti-CD20 B cell depleting therapies (BCDT), have the capacity to almost completely suppress acute focal inflammatory activity, which contrasts with the small delay of progression.[2–6] This constellation has coined the terms "smouldering MS" and "progression independent of relapse activity" (PIRA).[1,7,8] Therefore, patients with MS (pwMS) under BCDT may provide an optimal population to study the association of biomarkers with underlying progression.

Serum neurofilament light chain (sNfL) is indicative of neuroaxonal injury and has been established in recent years as a biomarker reflecting disease activity in real-time and to correlate with future brain volume loss.[9–14] Moreover, sNfL is a drug response marker as concentrations decrease under disease modifying therapy and can capture

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Address correspondence to Dr Kuhle, Multiple Sclerosis Centre, Neurology, Departments of Head, Spine and Neuromedicine, Biomedicine and Clinical Research and Research Center for Clinical Neuroimmunology and Neuroscience Basel (RC2NB), University Hospital and University of Basel, Petersgraben 4, CH-4031, Basel, Switzerland. E-mail: jens.kuhle@usb.ch

[#]These authors have contributed equally.
Pascal Benkert and Aleksandra Maleska Maceski share first authorship.
Eline A. J. Willemse and Jens Kuhle share last authorship.

From the [1]Multiple Sclerosis Centre and Research Center for Clinical Neuroimmunology and Neuroscience Basel (RC2NB), Department of Biomedicine and Clinical Research, University Hospital and University of Basel, Basel, Switzerland; [2]Department of Clinical Research, University Hospital Basel, University of Basel, Basel, Switzerland; [3]Department of Neurology, University Hospital and University of Basel, Basel, Switzerland; [4]Translational Imaging in Neurology (ThINK) Basel, Department of Biomedical Engineering, Faculty of Medicine, University Hospital Basel and University of Basel, Basel, Switzerland; [5]Department of Neurology, Cantonal Hospital Aarau, Aarau, Switzerland; [6]Department of Clinical Neurosciences, Division of Neurology, and Department of Medicine, Translational Biomarker Group, Geneva, Switzerland; [7]Diagnostic Department, Division of Laboratory Medicine, Geneva University Hospital, Geneva, Switzerland; [8]Department of Pathology and Immunology, Faculty of Medicine, University of Geneva, Geneva, Switzerland; [9]Department of Neurology, Cantonal Hospital St. Gallen, St. Gallen, Switzerland; [10]Service of Neurology and Laboratories of Neuroimmunology, Department of Clinical Neurosciences, Lausanne University Hospital (CHUV) and University of Lausanne, Lausanne, Switzerland; [11]Department of Neurology, St Josef-Hospital Bochum, Ruhr-University Bochum, Bochum, Germany; [12]Department of Neurology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland; [13]Multiple Sclerosis Center, Neurocenter of Southern Switzerland, EOC, Lugano, Switzerland; [14]Faculty of Biomedical Sciences, Università della Svizzera Italiana (USI), Lugano, Switzerland; [15]Department of Neurology and Clinical Neuroscience Center, University Hospital Zurich and University of Zurich, Zurich, Switzerland; [16]Novartis Pharma AG, Basel, Switzerland; [17]Department of Neurology, Hôpital de la Pitié-Salpêtrière, AP-HP, Paris, France; [18]Department of Neurology, Philipps-University, Marburg, Germany; [19]F. Hoffmann-La Roche, Basel, Switzerland; [20]Department of Neurology and Weill Institute for Neurosciences, University of California, San Francisco, CA, USA; [21]Department of Neurology with Institute of Translational Neurology, University Hospital Münster, Münster, Germany; [22]Institute of Epidemiology and Social Medicine, University of Münster, Münster, Germany; [23]Department of Clinical Neuroscience, Karolinska Institutet, Center for Molecular Medicine, Karolinska University Hospital, Stockholm, Sweden; [24]Center for Neurology, Academic Specialist Center, Stockholm Health Services, Stockholm, Sweden; [25]Population Health Research Institute, McMaster University, Hamilton, Canada; [26]Regional Blood Transfusion Service, Swiss Red Cross, Basel, Switzerland; and [27]Department of Neurology, Medical University of Graz, Graz, Austria

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disease activity in patients that seem stable on clinical or magnetic resonance imaging (MRI) grounds.[10] sNfL is now increasingly used as the endpoint measure in clinical trialing and for monitoring treatment response in individual patient care.[10,15,16] Important for the evaluation of drug efficacy on PIRA, post hoc clinical trial studies suggested that increased on-treatment sNfL concentrations could predict future brain volume loss and disability worsening.[17–20]

Serum glial fibrillary acidic protein (sGFAP) has been recently described as biomarker associated with disease severity, brain volume loss and future progression in MS[12,21–26]; sGFAP may be as well a drug response marker in progressive MS.[27] GFAP is a major cytoskeletal protein of astrocytes, whose blood concentrations increase on reactive astrogliosis and astrocytic damage.[28,29]

The prognostic power of sGFAP for progression in MS was particularly strong in patients with low focal inflammatory activity, as exemplified by low sNfL levels,[21,25] or in those who remained relapse-free during an 8-year of follow-up.[26] However, the longitudinal dynamics of sGFAP in patients under BCDT have not been evaluated yet and it is unknown whether they differ in patients with versus without PIRA.

The aim of this study was to extend our prior time to event analyses[26] and to describe the longitudinal dynamics of sNfL and sGFAP in pwMS initiating BCDT in the real-world setting of the Swiss MS Cohort (SMSC).

Methods

Study Population

Control Persons. Control persons from 2 cohorts were included: (1) Establishing the links between subclinical arteriosclerosis and depression (BiDirect study; 699 control persons with 1,249 serum samples); and (2) Genetic and phenotypic determinants of blood pressure and other cardiovascular risk factors (GAPP study; 2,162 control persons with 3,694 samples) (Table S1).[10] We studied the associations between sGFAP and the potential confounders age, body mass index (BMI), and sex. In analogy to sNfL,[10] we used this reference database to develop a confounder-adjusted measure of how an individual measurement from a pwMS deviates from control persons (ie, the sGFAP Z score).[30]

People with MS

The SMSC[31,32] (NCT02433028) is a prospective multicentric study performed across 8 Swiss academic medical centers. Demographic, neuroimaging, clinical data, and blood samples are collected every 6 or 12 months and stored at −80°C according to standardized procedures.[33] Standardized clinical assessments with the Neurostatus-Expanded Disability Status Scale (EDSS) score calculations are performed by Neurostatus-eTest certified raters.[34,35]

PIRA was defined as an increase in EDSS of ≥1.5 points from a reference EDSS score of 0, ≥1.0 point from an EDSS score of 1.0–5 or ≥0.5 point from an EDSS score ≥5.5, confirmed at a subsequent visit at least 6 months apart and without relapses between the reference and confirmation visit.[8,26] Progression independent of relapse and MRI activity (PIRMA) was defined as PIRA in absence of new or enlarging T2-weighted (T2w) or contrast enhancing (CEL) T1w lesions between the reference and PIRA event.[36,37]

This study was approved by the ethics committees of all participating centers and all patients gave written informed consent. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines.

MRI

A standardized imaging protocol was applied in SMSC, including a 3 dimensional fluid attenuated inversion recovery (FLAIR) and pre-and post-contrast T1 sequences acquired at a spatial resolution of $1mm^3$. Brain MRI was performed yearly. The evaluated outcomes were T2w hyperintense lesion volume, brain parenchymal fraction (BPF), and number of CEL T1w lesions. T2w lesion volumes were automatically assessed using a deep learning-based approach,[38] followed by manual quality assessment and correction. BPF was assessed as described previously.[39] To assess PIRMA, we performed a longitudinal analysis of all FLAIR and T1w images using LeMan-PV[40,41] to detect new or enlarging T2w hyperintense lesions automatically. The outputs were then manually reviewed by experienced raters.

Serum GFAP and NfL Measurements

Serum GFAP and NfL were measured using the Neurology 2-plex B assay (Quanterix, Billerica, MA) according to manufacturer's instructions on the single molecule array HD-X platform. Samples were measured in duplicate with repeated measurement of a few samples that exceeded an intra-assay coefficient of variation (CV) of 20%. Two internal quality control serum samples were included in each run with mean CV of 11.7 and 12.0% for sGFAP (concentrations: 75.8 and 136.3pg/ml, respectively) and 5.9 and 4.6% for sNfL (concentrations: 8.6 and 17.4pg/ml, respectively). Because assay comparison between the Nf-Light kit and Neurology 2-plex B assay based on 480 samples from an in-house cohort showed excellent congruency (Pearson's $r = 0.964$),[26] sNfL Z scores were calculated from current Neurology 2-Plex B results. Three additional serum samples were included in each run as internal calibrators to normalize sNfL results[42] to the sNfL reference database (Table S2).[10]

Statistical Methods

Demographic and clinical characteristics were described as counts and percentages as well as median and interquartile ranges (IQR), as appropriate. To study factors associated with sGFAP levels in control persons, we used linear mixed effects models with sGFAP as the dependent variable (log-transformed) and age, BMI, and sex as predictors. A random intercept was used per individual. As an approximation, age and BMI were modelled linearly for better interpretation of the effects. Further, interaction terms were investigated, and potential non-linear associations were modelled using spline terms with various degrees of freedom. Model selection was performed based on the Akaike information criterion (AIC). Estimates were back transformed and represent percentage change in the geometric mean of the biomarker level per unit change in the independent variable. Age-, BMI-, and sex-specific sGFAP references curves were then generated using a generalized additive model for location, scale and shape (GAMLSS) based on a Box-Cox t distribution with sGFAP as the dependent variable and the 3 covariates.[26] Based on this model, Z scores were calculated that express the deviation of an individual value from concentrations in control persons while accounting for these physiological differences. A Z score 0 corresponds to the mean in the reference population accounting for these 3 variables, whereas a "Z score: 1" corresponds to 1 standard deviation above the reference population value (=84.1st percentile).

Demographic and clinical characteristics were compared between patients with PIRA and non-PIRA using univariate tests: *t* test (for continuous variables) or chi-square test (for categorical variables) for normally distributed variables; Wilcoxon rank-sum test (for continuous variables) or Fisher's exact tests (for categorical variables) were used for non-normally distributed variables.

Biomarker Z scores in all samples under BCDT in relapsing MS (RMS) and progressive MS (PMS) (primary [PPMS] and secondary [SPMS] progressive MS combined) were visualized using boxplots and compared to values in control persons (ie, Z score: 0) using the Wilcoxon rank sum test. To study extremes, proportions of pathological values (Z score >1) were calculated per MS subgroup and compared using chi-squared test.

The association between biomarker Z scores and time to a first PIRA event in all pwMS (and RMS and PMS separately) was investigated using Kaplan–Meier curves and by univariable and multivariable Cox regression models using dichotomized (cut-off of 1) or continuous Z scores. In this analysis, we included all patients who had initiated BCDT (ocrelizumab [OCR], or rituximab [RTX]) with at least 3 documented visits to determine potential PIRA and an index serum sample under BCDT available 8 to 24 months after start of BCDT (Fig S1). The current patient number extends the "cohort 2" from Meier et al[26] by 110 individuals (plus 43.7%). We performed a sensitivity analysis on the association between biomarker Z scores and time to a first PIRMA event in patients with available MRI information between reference visit and PIRA event.

Mixed effects models were used to identify factors longitudinally associated with biomarker levels under BCDT. For these analyses, PIRA events and biomarker values were evaluated in patients with ≥4 years of follow-up on BCDT. PIRA events and biomarker values were evaluated within the entire follow-up period up to a maximum of 6 years. Serum NfL and GFAP Z scores were individual dependent variables, and the following terms were used as predictors: age at BCDT start, EDSS at BCDT start, recent relapse (<90 days before sampling), and time under BCDT. In addition, we used a binary variable indicating whether the patient had experienced a PIRA event during the maximum of 6 years of follow-up. To investigate whether sGFAP or/and sNfL showed different slopes under BCDT in patients developing PIRA or not, we tested for an interaction between PIRA and follow-up time; the interaction was only included in the final model when statistically significant in a log-likelihood test. We performed sensitivity analyses (1) adjusting for T2w lesion volume, number of CEL and BPF and (2) investigating the occurrence of PIRMA instead of PIRA.

To investigate whether sGFAP levels can be used to enrich populations in trials that aim to investigate effects on disease progression outcomes with patients at higher risk of disease progression (thereby reducing sample size), we performed sample size calculations for a hypothetical clinical trial targeting progression in MS, based on the PIRA event rates in the BCDT treated pwMS. PIRA event rates were calculated as parametric ground hazards assuming the hazard for PIRA follows an exponential distribution in the overall population, and in the subsets with "high" (>1) Z scores for sGFAP, sNfL, and their combination. Assuming a power of 0.80 and an alpha error of 0.05, we calculated the required number of PIRA events and the respective sample size based on a 1:1 randomization ratio and hypothetical hazard ratios (HR) for PIRA events of 0.7, 0.6, and 0.5 for an investigational drug combined with BCDT versus BCDT monotherapy.

p-Values ≤0.05 were considered statistically significant. Analyses were performed in R version 4.3.1.

Results

Reference Values of sGFAP and Derivation of Z Scores from a Healthy Control Persons Cohort

We assessed the associations of age, sex, and BMI with sGFAP concentrations in 4,943 samples from 2,861 healthy control persons (Table S1). The variance in

sGFAP concentrations in control persons was best described (AIC: 2392) by a multivariate mixed model including sex and non-linear terms for age and BMI (splines with 3 degrees of freedom; Fig S2). When approximated with linear terms (AIC: 2445), sGFAP concentrations increased by 2.0% per year (estimate: 1.020 (95% confidence interval [CI]: 1.018–1.021), $p < 0.0001$) and decreased by 1.3% per BMI point (0.987 [0.984–0.990], $p < 0.0001$). Furthermore, sGFAP concentrations were 13.6% (1.136 [1.105–1.168], $p < 0.0001$) higher in women compared to men across all ages.

Patient Characteristics (Table 1)

We studied a cohort of 362 pwMS receiving BCDT (71.5% OCR and 28.5% RTX); 75.7% presented with RMS, whereas 24.4% had PMS (12.2% each had SPMS or PPMS). Median follow-up time after BCDT start was 4.8 years; 26.2% of pwMS experienced a PIRA event during this period (16.8% of the RMS patients and 55.7% of the PMS patients). Patients experiencing PIRA were older, had higher EDSS scores at baseline, had experienced relapses less frequently before starting BCDT treatment, and were more likely PMS than RMS. sGFAP and sNfL levels were higher in those experiencing future PIRA. During follow-up, 95.1% patients continued BCDT (18.0% switched from RTX to OCR), whereas 4.9% switched to another type of MS drug.

sGFAP and sNfL Levels in pwMS versus Controls and RMS versus PMS

We observed elevated Z scores of sGFAP and sNfL in samples of pwMS (n = 2,212, including longitudinal samples) compared to HC (Z score 0): for sGFAP the median Z score was 0.36 in RMS and 0.81 in PMS; for sNfL the median Z score was 0.33 in RMS and 0.67 in PMS (all $p < 0.0001$ versus HC (Z score 0); Fig 1). Both sGFAP and sNfL levels were elevated in PMS compared to RMS (both $p < 0.0001$). Similarly, comparing the proportions of samples with sGFAP Z scores >1, 41.4% in PMS versus 24.8% in RMS were increased, whereas for sNfL this was the case for 37.6% in PMS versus 22.3% in RMS (all $p < 0.0001$; Fig 1).

Capacity of sGFAP and sNfL Levels to Prognosticate PIRA

By dichotomization of sGFAP Z scores between >1 ("high") versus ≤1 ("low") for index samples collected at a median of 1 year after BCDT start, "high" values were prognostic for future PIRA (HR: 2.05 [95% CI: 1.37–3.08], $p = 0.0005$; Fig 2; Table S3). In the first 3 years with BCDT, 29.5% of the patients with "high" sGFAP levels were estimated to experience a PIRA event (Kaplan–Meier estimate 0.295 [95% CI: 0.200–0.379]), compared to 14.0% in the "low" sGFAP group (0.140 [0.095–0.182]). Accordingly, "high" versus "low" sNfL Z scores were associated with a higher risk of PIRA (HR: 1.76 [CI: 1.18–2.64], $p = 0.0058$). However, this association lost significance in a Cox model with both markers for sNfL, but not for sGFAP (HR sGFAP Z score: 1.80 [1.17–2.78], $p = 0.0079$; HR sNfL Z score: 1.45 [0.95–2.24], $p = 0.0886$). Multivariable models confirmed these findings (Table S3). In addition, higher age and higher EDSS score were associated with increased PIRA hazard. In pwMS with both "high" sGFAP and sNfL Z scores, the risk of PIRA was increased more than 2-fold (HR: 2.66 [IQR: 1.65–4.31], $p < 0.0001$), compared to those with both biomarkers being "low". Having one "high" in combination with a "low" biomarker did not significantly increase the risk of PIRA (Fig S3).

In separate models for sGFAP (HR: 2.10 [1.19–3.71], $p = 0.0105$) and sNfL (HR: 2.26 [1.28–3.99], $p = 0.0051$) the prognostic value in PMS remained, but was not seen in RMS (HR: 1.33 [0.71–2.50], $p = 0.3699$ and 1.13 [0.62–2.08], $p = 0.6890$, respectively).

The PIRMA sensitivity analysis was performed in 332 of 362 patients as MRI information was available for 65 of 95 PIRA events: 54 of 65 PIRA events were confirmed as PIRMA (83%). "High" sGFAP values were prognostic for future PIRMA (HR: 2.91 [95% CI: 1.70–4.96], $p < 0.0001$; Fig S4). Accordingly, "high" versus "low" sNfL Z scores were associated with a higher risk of PIRMA (HR: 2.04 [CI: 1.20–3.49], $p = 0.0088$), again this association lost significance in a Cox model with both markers for sNfL, but not for sGFAP (HR sGFAP Z score: 2.53 [1.43–4.47], $p = 0.0014$; HR sNfL Z score: 1.51 [0.85–2.66], $p = 0.1590$).

Longitudinal Dynamics of sGFAP and sNfL under BCDT in Relation to PIRA

We studied associations of disease variables with biomarker dynamics in 72 pwMS developing PIRA and 186 pwMS without PIRA after BCDT start.

sGFAP and sNfL Z scores were lower in older patients, correlated with higher EDSS scores at BCDT start, and were higher in pwMS with a recent history of relapse and experiencing PIRA versus those without PIRA (Table S4; Fig 3).

Serum GFAP steadily increased over time by 0.49 [95% CI: 0.29–0.69] Z score units/10 years ($p < 0.0001$) with similar slopes in those with and without PIRA ($p_{\text{interaction PIRA*follow-up time}} = 0.44$), but Z scores were 0.52 [0.22–0.83] units higher in pwMS experiencing a PIRA event during follow-up versus those who did not ($p = 0.0009$).

TABLE. Patient Characteristics at Start of B-Cell Depleting Therapy Stratified for Disease Stage and the Occurrence of PIRA

	Total	RMS	PMS	Non-PIRA	PIRA	*p*
Patients (n)	362	274 (75.7)	88 (24.3)	267 (73.8)	95 (26.2)	-
Sex (women)	237 (65.5)	191 (69.7)	46 (52.3)	182 (68.2)	55 (57.9)	0.092
Age, y	43.0 [33.1, 52.1]	39.2 [30.5, 47.7]	54.7 [45.6, 61.6]	40.9 [31.5, 50,0]	47.1 [38.9, 57.9]	<0.001
Disease subtype						
RMS	274 (75.7)	274 (100)	0 (0)	228 (85.4)	46 (48.4)	<0.001
SPMS	44 (12.2)	0 (0)	44 (50)	18 (6.7)	26 (27.4)	
PPMS	44 (12.2)	0 (0)	44 (50)	21 (7.9)	23 (24.2)	
EDSS	3.0 [2.0, 4.5]	2.5 [1.5, 3.5]	5.5 [4.0, 6.0]	2.5 [2.0, 4.0]	4.0 [3.0, 5.5]	<0.001
Disease duration, y	10.7 [4.3, 18.6]	9.2 [3.9, 16.6]	16.0 [7.7, 22.2]	9.8 [4.0, 17.9]	12.5 [4.9, 20.5]	0.077
Treatment						
RTX	103 (28.5)	60 (21.9)	43 (48.9)	61 (22.8)	42 (44.2)	<0.001
OCR	259 (71.5)	214 (78.1)	45 (51.1)	206 (77.2)	53 (55.8)	
Relapse <4 months before start of BCDT	79 (21.8)	77 (28.1)	2 (2.3)	67 (25.1)	12 (12.6)	0.017
Relapse <1 y before start of BCDT	138 (38.1)	127 (46.4)	11 (12.5)	110 (41.2)	28 (29.5)	0.058
Treatment start to index sample, y[a]	1.0 [0.9, 1.3]	1.0 [0.9, 1.3]	1.0 [0.9, 1.2]	1.0 [0.9, 1.3]	1.0 [0.9, 1.2]	0.898
sGFAP (pg/ml) at index sample[a]	83.7 [58.9, 117.7]	77.0 [55.6, 109.1]	110.0 [77.8, 177.8]	77.5 [56.3, 108.3]	109.1 [70.4, 154.5]	<0.001
sGFAP Z score at index sample[a]	0.2 [−0.8, 1.1]	0.1 [−0.8, 1.0]	0.5 [−0.7, 1.3]	0.1 [−0.9, 1.0]	0.6 [−0.6, 1.3]	0.002
sNfL (pg/ml) at index sample[a]	8.6 [6.2, 12.2]	7.9 [6.0, 11.1]	11.7 [8.8, 17.0]	8.0 [6.1, 11.3]	10.7 [7.5, 15.0]	<0.001
sNfL Z score at index sample[a]	0.5 [−0.3, 1.3]	0.4 [−0.3, 1.1]	0.7 [−0.3, 1.5]	0.4 [−0.3, 1.1]	0.9 [−0.2, 1.5]	0.027
Follow-up duration, y	4.8 [3.6, 5.6]	4.7 [3.5, 5.4]	5.3 [3.9, 6.7]	4.6 [3.4, 5.4]	5.2 [4.1, 6.7]	<0.001
Patients (n) with min. 4 y follow-up[b]	258	189 (73.3)	69 (26.7)	184 (71.3)	74 (28.7)	–
Samples (n)	1,480	1,068 (72.2)	412 (27.8)	1,005 (67.9)	475 (32.1)	–
Relapse under BCDT	58 (16.0)	48 (17.5)	10 (11.4)	48 (18.0)	10 (10.5)	0.124
PIRA event under BCDT	95 (26.2)[c]	46 (16.8)	49 (55.7)	0 (0)	95 (100)	<0.001

Variables are expressed as n (%) or median [IQR]. *p*-values indicate whether patient characteristics differ between Non-PIRA and PIRA.
[a]Index sample was used for time to PIRA/PIRMA analysis.
[b]104 patients had a follow-up <4 years and were excluded from the longitudinal analysis of biomarker dynamics.
[c]65 of 95 patients with PIRA had MRI information available: 54 (83.1%) confirmed as PIRMA.
Abbreviations: BCDT = B-cell depleting therapy; EDSS = Expanded Disability Status Score; IQR = interquartile range; MRI = magnetic resonance imaging; n = number; OCR = ocrelizumab; PIRA = progression independent of relapse activity; PIRMA = progression independent of relapse and MRI activity; PMS = progressive MS; PPMS = primary progressive MS; RMS = relapsing MS; RTX = rituximab; sGFAP = serum glial fibrillary acidic protein; sNfL = serum neurofilament light chain; SPMS = secondary progressive MS; y = years.

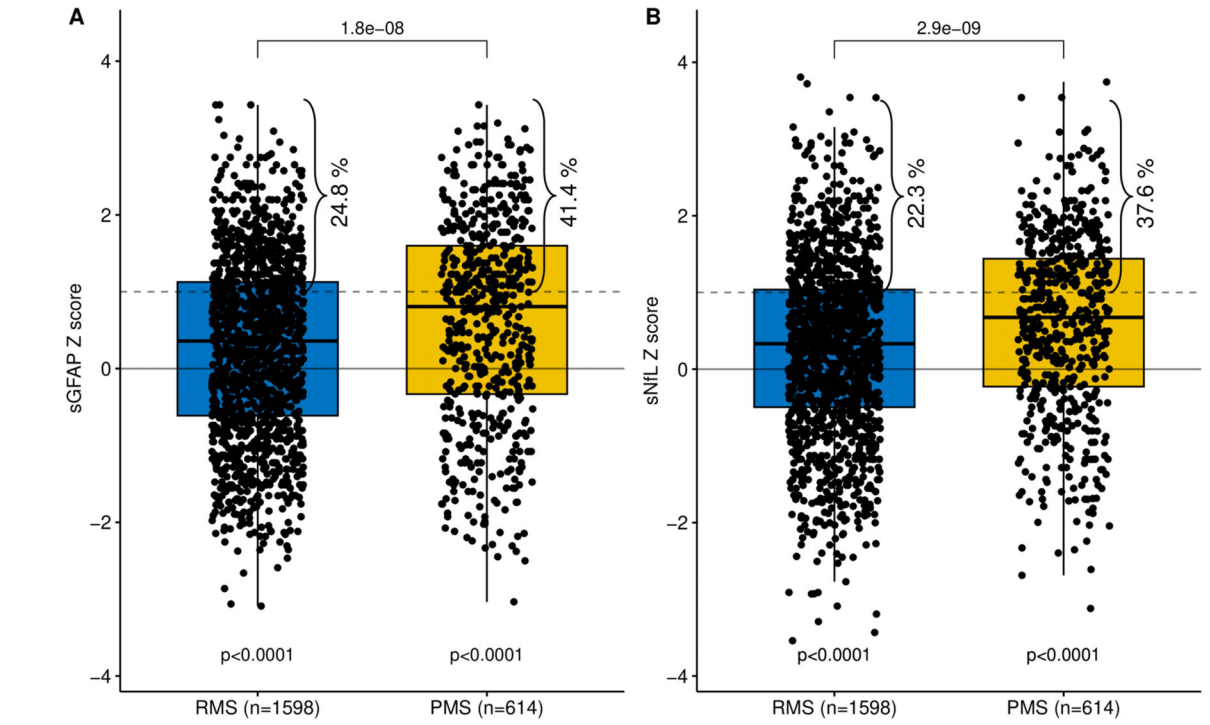

FIGURE 1: sGFAP (A) and sNfL (B) Z scores in samples from RMS and PMS patients under B-cell depleting therapy. In RMS and PMS, sGFAP (A) and sNfL (B) levels were increased compared to control persons (ie, Z = 0, solid line; all $p < 0.0001$ in Wilcoxon rank sum test as indicated below). Both sGFAP and sNfL levels were elevated in PMS compared to RMS (both $p < 0.0001$ above *square brackets*). Furthermore, the proportion of samples with Z score >1 (>84.1st percentile; *dashed line*) was considerably higher (*vertically written percentages*) than expected in control persons (15.9% according to a standard normal distribution): sGFAP: 41.4% in PMS and 24.8% in RMS (A); sNfL: 37.6% in PMS and 22.3% in RMS (B). A total of 2,212 longitudinal samples from all 362 patients were included. MS, multiple sclerosis; n, number; PMS, progressive MS; RMS, relapsing MS; sGFAP, serum glial fibrillary acidic protein; sNfL, serum neurofilament light chain. [Color figure can be viewed at www.annalsofneurology.org]

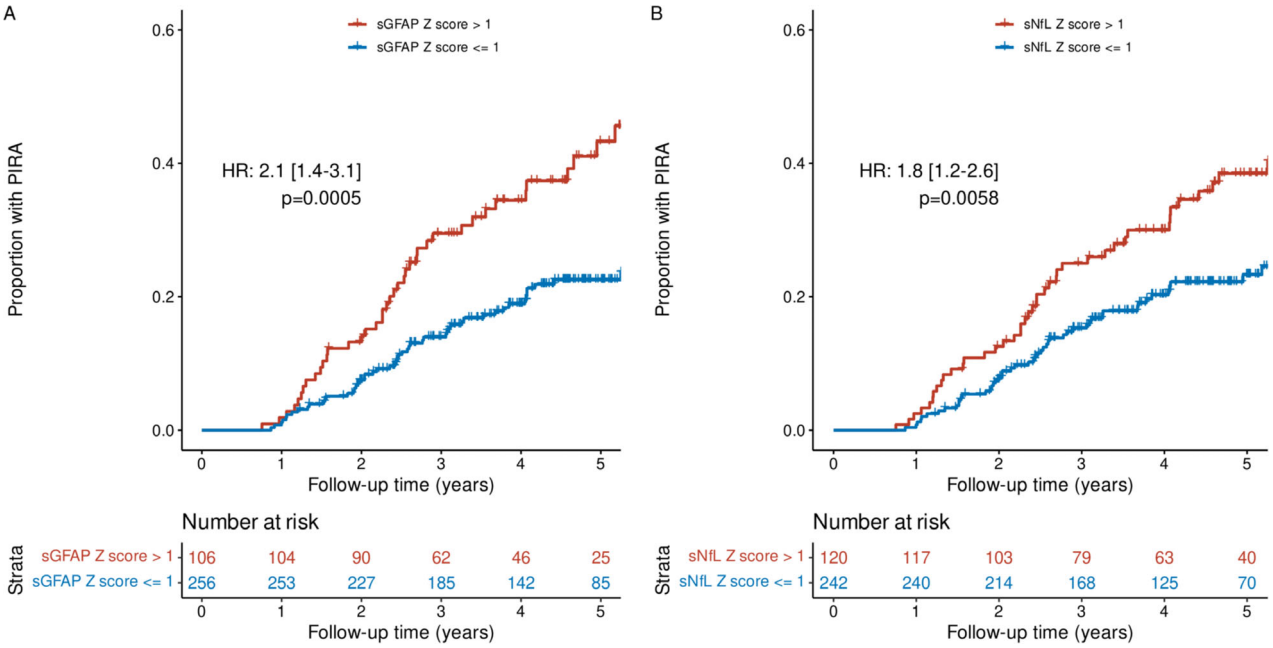

FIGURE 2: Kaplan–Meier curves showing the proportion of patients experiencing future PIRA when having high (Z score > 1) versus low (Z score ≤ 1) biomarker levels of sGFAP (A) and sNfL (B) at index sample. Patients with a sGFAP Z score > 1 (≥84.1st percentile) at index sample (median 1 year after BCDT start) were at 2.1-fold risk of a future PIRA event versus those with sGFAP Z score of ≤1 (HR: 2.1 [1.4–3.1], $p = 0.0005$); accordingly, patients with a sNfL Z score >1 showed 1.8-fold increased risk to develop PIRA compared to patients with a sNfL Z score ≤1 (HR: 1.8 [CI: 1.2–2.6], $p = 0.0058$). HR, hazard ratio; PIRA, progression independent of relapse activity; sGFAP, serum glial fibrillary acidic protein; sNfL, serum neurofilament light chain. [Color figure can be viewed at www.annalsofneurology.org]

The dynamics of sNfL levels over time differed between pwMS with versus without PIRA ($p_{\text{interaction PIRA*follow-up time}} = 0.0028$): sNfL decreased by 0.92 [−1.23 to −0.60] Z score units/10 years ($p < 0.0001$) in pwMS without PIRA while remaining elevated in those with PIRA (Table S4; Fig 3). Notably, only RMS patients without PIRA contributed to the decrease in sNfL on the start of BCDT, whereas a sustained increase in PMS patients was observed, with higher levels in those with versus without PIRA (Table S5; Fig S5).

Sensitivity Analysis Including MRI Metrics

We performed a sensitivity analysis in 236 of 258 pwMS with available MRI metrics (Table S6). Results were congruent with those of the overall cohort as sGFAP steadily increased over time with constantly higher levels in pwMS with versus without PIRA, whereas sNfL strongly decreased after BCDT initiation in pwMS free of PIRA.

Using PIRMA instead of PIRA, resulted in 238 of 258 pwMS with MRI coverage and confirmed the main results (Table S7).

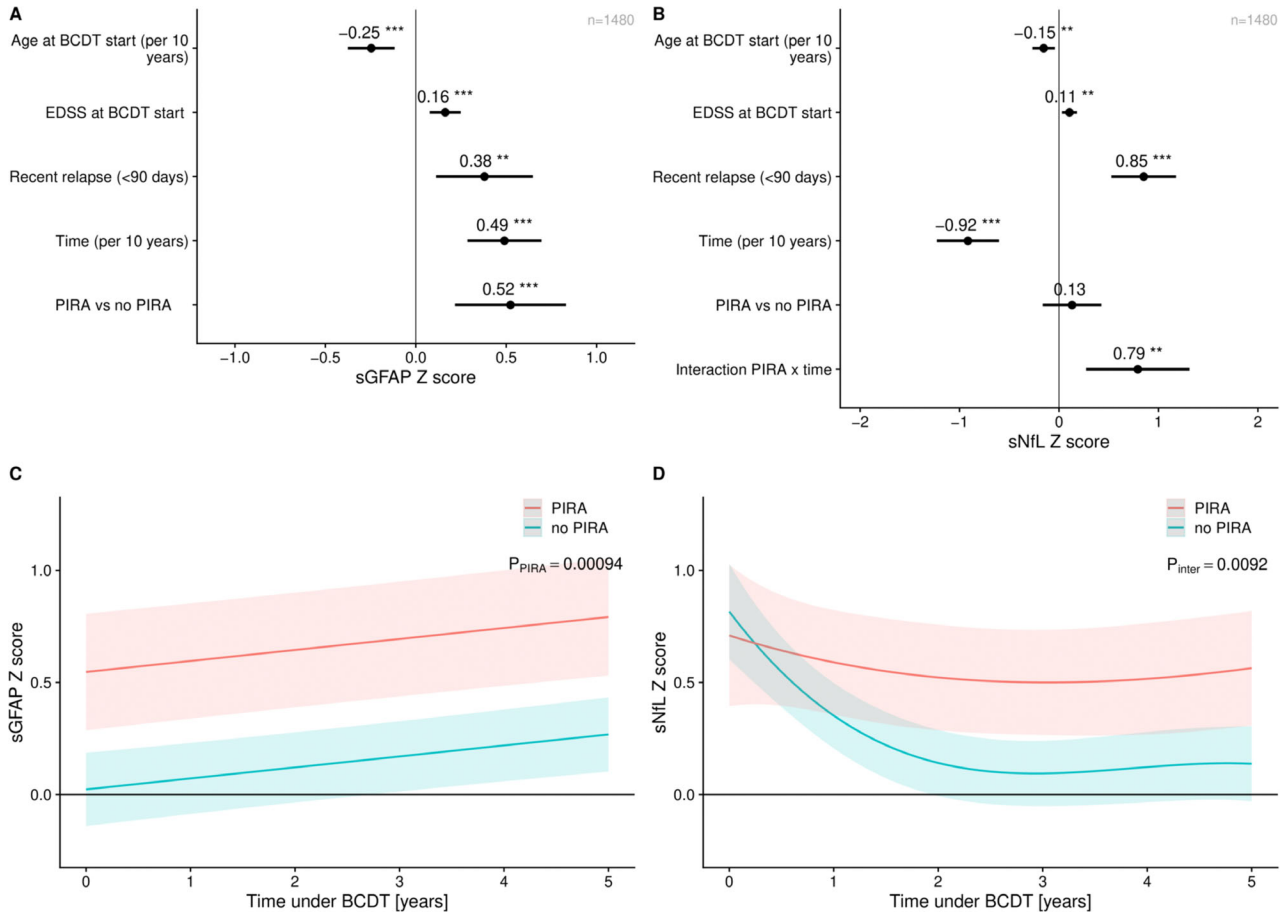

FIGURE 3: Longitudinal dynamics of sGFAP (A,C) and sNfL (B,D) Z scores under BCDT in relation to PIRA. A and B show estimates (*dots*) with 95% CI (*error bars*) from multivariable mixed models with sGFAP and sNfL Z score, respectively, as outcome variable. C and D show marginal effects on predicted biomarker Z scores over time. Statistical significance indicated as **$p < 0.01$ or ***$p < 0.001$. Z score: 0 represents the mean biomarker concentration in control persons. Models are adjusted for age and EDSS at BCDT start and for recent relapse (<90 days before sampling). (A + C): sGFAP Z scores steadily increased over time by 0.49 Z score units/10 years ($p < 0.0001$) in both PIRA and non-PIRA patients, whereas there was no difference in slopes between the 2 groups ($p_{\text{interaction PIRA*follow-up time}} = 0.44$), and therefore, the interaction term was excluded from the statistical model (see Table S2 for model details including 95% CI). However, Z scores were 0.52 units higher in patients developing PIRA during follow-up ($p = 0.0009$). sGFAP levels were lower in older patients and higher with higher EDSS and recent relapse. (B + D): No difference in sNfL Z scores was observed in patients with versus those without PIRA at start of BCDT ($p = 0.38$; Table S2). However, the dynamics of sNfL over time differed between these groups ($p_{\text{interaction PIRA*follow-up time}} = 0.0028$ in model B where time is approximated by a linear term; $p_{\text{interaction PIRA*follow-up time}} = 0.0092$ in model D in which a spline term for time was used): in patients without PIRA, sNfL strongly decreased by 0.92 Z score units/10 years ($p < 0.0001$), whereas in those with PIRA, Z scores remained stable over time. BCDT, B-cell depleting therapy; CI, confidence interval; EDSS, expanded disability disease score; PIRA, progression independent of relapse activity; sGFAP, serum glial fibrillary acidic protein; sNfL, serum neurofilament light chain. [Color figure can be viewed at www.annalsofneurology.org]

Serum GFAP as Tool for Clinical Trial Enrichment

Last, we simulated how patient enrichment based on biomarker profiles for clinical trials with PIRA as primary endpoint may be optimized by adding sGFAP Z score >1 as inclusion criterion (Table S8). We assumed an investigational drug reduces PIRA events by 40% (HR: 0.6; HR of 0.7 and 0.5 also shown in Table S8) as add-on therapy to BCDT, compared to BCDT monotherapy in a 1:1 randomized clinical trial over 2 years. The required sample size in this scenario would be 1,224 participants without enrichment, and 798 (ie, 35% less) by incorporating the "high" sGFAP (Z score >1) criterion. Simulating the same scenario for "high" sNfL, or the combination of "high" sGFAP/"high" sNfL, corresponding reductions in sample size would be 27% and 46%, respectively. An inevitable consequence of enrichment, however, is that only 17.4% (63/362) of the present cohort would fulfill this latter criterion.

Discussion

Several recent studies have demonstrated the association of both sGFAP and sNfL with progression, and for sNfL with focal inflammatory/relapse activity in MS.[21,25,26,30,43,44] Because BCDT leads to an almost complete suppression of focal inflammatory activity, the focus shifts to how disability worsening (independent of focal inflammatory activity) can be controlled therapeutically and how this can be anticipated with biomarkers. The increase of sGFAP may result from a disturbed astrocyte homeostasis and is, therefore, a plausible biomarker to reflect the scaring of neural tissue (ie, "sclerosis") and its clinical correlate progression in its strict sense (ie, PIRA).[28,29,45] Although most studies have shown the association of elevated sGFAP with PIRA,[21,25,26,46] this was not the case in others.[47,48]

To resolve this conflict we increased power, data density, and length of follow-up compared to our previous work,[26] to characterize longitudinal trajectories of sGFAP and sNfL levels in pwMS starting BCDT. Further, we used a considerably larger, and hence, more robust reference cohort of control persons.

The finding that sGFAP Z scores were constantly higher in pwMS with versus without PIRA provides further evidence supporting sGFAP as a reliable prognosticator for the risk for PIRA. Important from a clinical and practical point of view is that a single measurement at any time point after initiation of therapy may suffice to anticipate the risk of PIRA (Meier et al[26] and present results), as results of an index sample are fully congruent with those of further longitudinal measurements. As our methods for the clinical reference of PIRA notoriously lack accuracy, it is encouraging to find such sensitivity of sGFAP to differentiate for the risk of progression. Equally important, the stable increase over time of sGFAP levels after initiation of BCDT may indicate no or only limited impact of this treatment modality on progressive disease biology mechanisms.[12] However, this is not limited to BCDT, because longitudinal sGFAP levels remained stable or increased over time in a clinical trial in SPMS with natalizumab compared to placebo.[47,48] In contrast, in another SPMS trial after 24 months siponimod led to a modest decrease in sGFAP versus placebo.[27] As this treatment, unlike natalizumab, also reduced confirmed disease progression,[49] decreased cognitive worsening,[50] and reduced brain tissue damage,[51] all of which are features associated with progressive disease, this may indicate that decreasing GFAP levels may reflect clinically relevant differences in effects on mechanisms relating to progressive disease biology across treatment modalities.

The decrease of sNfL after BCDT start in RMS patients without PIRA confirms that this biomarker reflects the modification of disease processes by this therapy. In return, the sustained elevation of sNfL in PMS suggests that neuro-axonal degeneration is also an essential aspect of progressive disease biology that is not equally addressed by BCDT. Although current results support the concept that sGFAP and sNfL reflect largely orthogonal pathomechanisms (ie, smouldering neurodegeneration and acute inflammation, respectively) it is evident that both mechanisms of neural damage represent a spectrum that, to a variable degree, occur interdependently and at all stages of MS.

The absence of association of sGFAP levels with PIRA in some studies, both of which involved natalizumab,[47,48] may be because of drug specific factors, but also trial cohort characteristics. However, in line with our results, sGFAP and sNfL levels differed between pwMS experiencing PIRA compared to those with stable disability status in a recent real world cohort.[46] Accordingly, in phase 3 trials in PMS with siponimod or fingolimod, higher blood NfL levels were prognostic for the risk of future disease progression.[20] Further, a post hoc analysis of open-label extension studies of phase 3 OCR trials showed that patients with high sNfL at week 48 had a higher risk of future disability worsening[18] and suggested that the effect of OCR on progression, but not relapses, correlated with drug exposure, thereby providing a rationale for testing higher and weight-adjusted OCR doses.[52] As a corollary, sNfL is not only prognostic, but also predictive in terms of the dose-dependent efficacy of OCR on progression. Based on present results this similarly could be the case for the differences of sGFAP levels with and without PIRA.

sGFAP has strong potential to accelerate the clinical development of drugs targeting neurodegeneration by allowing for a better targeted patient selection, because pwMS with increased sGFAP will reach the endpoint of disability worsening earlier, resulting in considerably reduced trial duration.

The clinical trial design we simulated demonstrates that sample size can be reduced by 35% when enriching the trial population for high sGFAP. Combining high sGFAP and sNfL had an incremental effect. The substantial reduction of sample size resulting from such pre-selection comes with the possible disadvantage of a more restricted target population and more difficult study recruitment.

This study has the following limitations. First, we had no comparison group to assess a potential treatment effect of BCDT on sGFAP levels, such as pwMS being untreated or exposed to other treatments. Second, the role of astrocytes in the pathogenesis of MS remains only partly understood and is made more complex by the fact that 10 isoforms have been described in the brain.[53] As a marker detected in blood, GFAP likely reflects a mixture of astrogliosis and cellular degeneration from several different subtypes of astrocytes that may reflect both pro- and anti-inflammatory activity.[28,29,45] A deeper qualitative understanding of what "sGFAP" represents, different isoforms or degradation products detected by available assays, differences in kinetics of degradation and correlation analyses between concentrations in blood versus the central nervous system compartment will be needed to fully understand its potential as a biomarker for MS progression.

In summary, comparison of longitudinal trajectories of sGFAP and sNfL in pwMS starting BCDT who develop PIRA versus those who do not confirms sGFAP as a robust marker for risk of PIRA. sNfL was closer related to focal and acute inflammatory processes, although both biomarkers may show changes because of the overlap between the 2 pathomechanisms. Our findings highlight the potential value of sGFAP as a prognostic marker for PIRA in clinical practice and its potential as a tool for patient stratification in clinical trials.

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Author Contributions

P.B., A.M.M., D.L., E.A.J.W. and J.K. contributed to the conception and design of the study. P.B., A.M.M., S.S., J.O., A.Z., J.F.V.G., L.M.G, A.C., R.G., S.Su., J.L., E.G., J.M., B.F.B, L.A., O.F., P.H.L., C.B., M.U., S.M., C.P., A.M., R.D.P., A.S., R.H., A.C., G.D., C.Z., M.D., L.G.H., O.Y., T.D., P.R., C.G., K.B., M.H., D.C., C. Gr., D.L., E.A.J.W. and J.K contributed to the acquisition and analysis of data. P.B., A.M.M., S.S., D.B., B.T., R.P., C.R., J.Ok., H.W., F.P., A.B., L.K., M.K., A.A., D.L., E.A.J.W. and J.K. contributed to drafting the text or preparing the figures.

Potential Conflicts of Interest

Nothing to report.

Data Availability

Written requests for access to the data reported in this paper will be considered by the corresponding author and the Scientific Board of the Swiss MS Cohort study and a decision made about the appropriateness of the use of the data. If the use is appropriate, a data sharing agreement will be put in place before a fully de-identified version of the dataset used for the analysis with individual participant data is made available.

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