

# Effect of Estimated Blood Volume and Body Mass Index on GFAP and NfL Levels in the Serum and CSF of Patients With Multiple Sclerosis

Yavor Yalachkov, MD, PhD, Jan Hendrik Schäfer, MD, Jasmin Jakob, Lucie Friedauer, MD, Falk Steffen, MD, Stefan Bittner, MD, PhD, Christian Foerch, MD, PhD, and Martin Alexander Schaller-Paule, MD

## Correspondence

Dr. Yalachkov  
yavor.yalachkov@kgu.de

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## Abstract

### Background and Objectives

To increase the validity of biomarker measures in multiple sclerosis (MS), factors affecting their concentration need to be identified. Here, we test whether the volume of distribution approximated by the patients' estimated blood volume (BV) and body mass index (BMI) affect the serum concentrations of glial fibrillary acidic protein (GFAP). As a control, we also determine the relationship between BV/BMI and GFAP concentrations in CSF. To confirm earlier findings, we test the same hypotheses for neurofilament light chain (NfL).

### Methods

NfL and GFAP concentrations were measured in serum and CSF (sNfL/sGFAP and cNfL/cGFAP) in 157 patients ( $n = 106$  with MS phenotype and  $n = 51$  with other neurologic/somatoform diseases). Using multivariate linear regressions, BV was tested in the MS cohort as a predictor for each of the biomarkers while controlling for age, sex, MS phenotype, Expanded Disability Status Scale score, gadolinium-enhancing lesions, and acute relapse. In addition, overweight/obese patients ( $\text{BMI} \geq 25 \text{ kg/m}^2$ ) were compared with patients with  $\text{BMI} < 25 \text{ kg/m}^2$  using the general linear model. The analyses were repeated including the neurologic/somatoform controls.

### Results

In the MS cohort, BV predicted sGFAP ( $\beta = -0.301$ ,  $p = 0.014$ ). Overweight/obese patients with MS had lower sGFAP concentrations compared with patients with MS and  $\text{BMI} < 25 \text{ kg/m}^2$  ( $F = 4.732$ ,  $p = 0.032$ ). Repeating the analysis after adding patients with other neurologic/somatoform diseases did not change these findings ( $\beta = -0.276$ ,  $p = 0.009$ ;  $F = 7.631$ ,  $p = 0.006$ ). Although sNfL was inversely correlated with BV ( $r = -0.275$ ,  $p = 0.006$ ) and body weight ( $r = -0.258$ ,  $p = 0.010$ ), those results did not remain significant after adjusting for covariates. BV and BMI were not associated with cGFAP or cNfL concentrations.

### Discussion

These findings support the notion that the volume of distribution of sGFAP approximated by BV and BMI is a relevant variable and should therefore be controlled for when measuring sGFAP in MS, while this might not be necessary when measuring cGFAP concentrations.

From the Department of Neurology (Y.Y., J.H.S., L.F., C.F., M.A.S.-P.), University Hospital Frankfurt; and Department of Neurology (J.J., F.S., S.B.), Universitätsmedizin Mainz, Germany. Go to [Neurology.org/NN](https://www.neurology.org/NN) for full disclosures. Funding information is provided at the end of the article.

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## Glossary

**AN** = anorexia nervosa; **BMI** = body mass index; **BV** = blood volume; **cGFAP** = glial fibrillary acidic protein in CSF; **CIS** = clinically isolated syndrome; **cNfL** = neurofilament light chain measured in CSF; **DMT** = disease-modifying therapy; **EDSS** = Expanded Disability Status Scale; **FDR** = false-discovery rate; **GFAP** = glial fibrillary acidic protein; **GLM** = general linear model; **IQR** = interquartile range; **MS** = multiple sclerosis; **PPMS** = primary progressive multiple sclerosis; **RIS** = radiologically isolated syndrome; **RRMS** = relapsing-remitting multiple sclerosis; **sGFAP** = glial fibrillary acidic protein in serum; **sNfL** = neurofilament light chain measured in serum.

Because of their feasibility and cost-effectiveness, serum biomarkers have a significant potential as instruments for the prognosis of disease activity and therapy response in multiple sclerosis (MS).<sup>1</sup> More specifically, markers of ongoing inflammation and neurodegeneration such as neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP) have been identified as particularly promising tools. NfL is a major component of neuronal and axonal cytoskeleton proteins. Its increased levels in serum (sNfL) and CSF (cNfL) indicate neuroaxonal damage<sup>2</sup> and are associated with clinical and MRI measures of disease activity.<sup>3-5</sup> GFAP is an intermediate filament expressed in the CNS in astrocyte cells. Its increased levels measured in serum (sGFAP) and CSF (cGFAP) reflect astrogliosis following neuronal injury<sup>6</sup> and are associated with inflammatory activity,<sup>7</sup> disease severity,<sup>6,8,9</sup> and MRI measures of brain volume.<sup>10</sup>

Before using these biomarkers in clinical trials and daily routine, potential factors affecting their measurement need to be identified to establish standardized analysis procedures.<sup>11</sup> It is well known that NfL and GFAP are not MS specific and may be increased also in other neurologic conditions.<sup>12,13</sup> Non-neurologic factors may affect NfL and GFAP measurements, too. Recently, body mass index (BMI) and estimated blood volume (BV) have been shown to affect sNfL measurements, possibly due to an increase in volume of distribution,<sup>14</sup> and have been accordingly adapted as an important covariate related with this biomarker. Consequently, another recent study used sNfL values corrected for age and BMI to show the suitability of standardized sNfL values to identify individuals with MS at risk for a detrimental disease course and suboptimal therapy response beyond clinical and MRI measures and to assess the suitability of BMI-corrected sNfL values as an end point for group-level comparison of effectiveness across disease-modifying therapies.<sup>15</sup>

This is a highly relevant aspect for the future implementation of MS biomarkers in research and clinical practice because more than 30% of the patients with MS are overweight or obese.<sup>16</sup> Furthermore, overweight and obesity are linked to increased risk for MS<sup>17,18</sup> and a higher rate of second-line disease-modifying treatment.<sup>17</sup> Thus, there is a growing need to determine whether BV and measures of body size such as BMI interact with biomarker measurements in serum and CSF.

To our best knowledge, the effects of BV/BMI on GFAP measurements in serum and CSF have not been studied in MS

yet. We hypothesized that higher BV values would be associated with lower levels of GFAP concentration in the serum of patients with MS due to a greater volume of distribution. To confirm earlier findings,<sup>14</sup> we tested the same hypothesis for NfL, too, because in another smaller study, BMI did not correlate significantly with sNfL levels.<sup>19</sup> On the contrary, we did not expect GFAP and NfL measurements in CSF to correlate with BV because individual brain/CSF ratio is dependent on age but not on body size.<sup>20</sup> Furthermore, we explored whether clinically relevant BMI-dependent conditions such as overweight/obesity would have an effect on the measured serum/CSF biomarker concentrations.

## Methods

### Standard Protocol Approvals, Registrations, and Patient Consents

The study was performed in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans and was approved by the local ethics committee at the University Hospital Frankfurt. Written informed consent was obtained from all patients.

### Study Design and Participants

Patients were recruited between October 2017 and December 2020 at the Department of Neurology, University Hospital Frankfurt. MS phenotype patients were referred to the Department of Neurology due to suspected MS or, if they already had an established MS diagnosis, due to a novel clinical/imaging finding. To be included in the study, patients had to be at least 18 years old, able to consent to participation, and have a clinically indicated lumbar puncture scheduled. Following groups were built depending on the results of the diagnostic workup: relapsing-remitting multiple sclerosis (RRMS), primary progressive multiple sclerosis (PPMS), clinically isolated syndrome (CIS), and radiologically isolated syndrome (RIS). The 2017 revision of the McDonald criteria were applied.<sup>21</sup> To explore whether our findings are limited to MS, we recruited also neurologic disease controls (e.g., neurodegenerative diseases, CNS tumors, and polyneuropathies) and somatoform disease controls. Somatoform disease controls were patients whose full diagnostic workup did not reveal an underlying organic disease. Both control groups were referred to the Department of Neurology due to novel clinical or imaging findings for further diagnostic workup.

All patients were neurologically examined and received laboratory tests, a lumbar puncture, and MRI. Serum and CSF samples for biomarker analysis were collected during the clinically scheduled sample collection and before any corticosteroid therapy. The Kurtzke<sup>22</sup> Expanded Disability Status Scale (EDSS) was used to estimate the degree of physical disability. Similarly to earlier studies,<sup>14,23</sup> we recorded self-reported body weight and body height, calculated BMI, and estimated the individual BV using the Nadler equation. The presence of contrast-enhancing T1-weighted lesions was evaluated using MRI data and radiologic reports. Only 3 patients with MS were being treated with disease-modifying therapy (DMT) at the time of study enrollment. One patient had been treated with natalizumab and switched to dimethyl fumarate 4 weeks before enrollment. One patient was treated with ocrelizumab. One patient was treated with fingolimod. All other patients with MS did not have any DMT and had not been treated with DMT before.

### Serum and CSF Measurements

For serum and CSF measurements, we used the single molecule array HD-1 analyzer (Quanterix, Boston, MA) using the Neurology 4-Plex A Advantage Kit (Quanterix, Boston, MA). Sample acquisition and protocol of measurements have been reported in previous works of our group.<sup>24-26</sup>

### Statistical Analysis

Baseline characteristics were computed separately for the patients with MS phenotype and the whole sample. In addition, patients were classified according to their BMI status (BMI  $\geq 25$  vs BMI  $< 25$  kg/m<sup>2</sup>). BMI  $\geq 25$  kg/m<sup>2</sup> was selected as a cutoff because it is the World Health Organization's standard definition for being overweight, which means that patients in this group were either overweight or obese.<sup>27</sup> Baseline characteristics were computed for both groups (BMI  $\geq 25$  vs BMI  $< 25$  kg/m<sup>2</sup>). To test for baseline differences, BMI groups were compared against each other with regard to their baseline characteristics using 1-way analysis of variance and Pearson  $\chi^2$  tests.

### Association Between BV, BMI Status, and Serum/CSF Biomarker Concentrations in Patients With MS

First, we explored the association between serum/CSF biomarker concentrations and BV, BMI, body weight, and body height in the MS sample (n = 106). To this end, Spearman-rho correlations were computed. To ensure that the results are not biased by extreme outliers, biomarker levels or body weight lying above the 3rd quartile +3\*interquartile range were removed, and the correlational analyses were repeated.

For the primary analysis focusing on patients with MS phenotype, we computed 1 multivariable linear regression with each biomarker (sGFAP, sNfL, cGFAP, or cNfL) as outcome. BV as well as age, sex, MS phenotype (RRMS, PPMS, CIS, or RIS), EDSS score, presence of gadolinium-enhancing T1-weighted MRI lesions (GE), and acute relapse at serum/CSF

sampling were used as predictors. Partial correlations were reported demonstrating the associations between BV and serum/CSF markers after accounting for the effect of the other variables. *p* Values for the linear regression models were corrected for false-discovery rate (FDR) with the Benjamini-Hochberg procedure. The significance level was set at  $q < 0.05$ .

In the next step, we assessed the effect of overweight/obesity on serum and CSF biomarker concentrations. Four different general linear model (GLM) univariate analyses were used with each of the biomarkers of interest as a dependent variable. The group factor BMI  $\geq 25$  vs BMI  $< 25$  kg/m<sup>2</sup> was used as an independent variable. Age, sex, diagnosis (RRMS, PPMS, CIS, or RIS), EDSS score, GE, and acute relapse at serum/CSF sampling were modeled as covariates. Before entering the variables in the above-mentioned linear regression and general linear models, age, BV, EDSS score, and biomarker concentrations were standardized by dividing them by their SD similarly to other biomarker studies.<sup>28</sup> Log transformation was applied to all serum and CSF parameters. The significance level was set at  $p < 0.05$ . Cohen *d* and *f*<sup>2</sup> effect sizes were computed for the direct sGFAP group comparison within the general linear model (BMI  $< 25$  vs BMI  $\geq 25$  kg/m<sup>2</sup>, using the corresponding *F* value) and for the linear regression (using the corresponding *R*<sup>2</sup> value) in the patients with MS.<sup>29,30</sup>

### Association Between BV, BMI Status, and Serum/CSF Biomarker Concentrations in the Whole Cohort

To explore the generalizability of the results from the MS sample, n = 51 controls with other neurologic or with somatoform disorders were added to the whole sample (total n = 157). Associations between serum/CSF biomarker concentrations and BV, BMI, body weight, and body height were explored using Spearman-rho correlations with the significance level set at  $p < 0.05$ . In the next step, 4 different multivariable linear regressions with sGFAP, sNfL, cGFAP, or cNfL as outcomes were computed. For each model, we entered BV as well as diagnosis (neurologic [RRMS, PPMS, CIS, RIS, and other neurologic disease] vs somatoform disease), age, and biological sex as predictors. Partial correlations were reported demonstrating the associations between BV and serum/CSF markers after accounting for the effect of the other variables. Next, 4 different GLM univariate analyses were used with each of the biomarkers of interest as a dependent variable. The group factor BMI  $\geq 25$  vs BMI  $< 25$  kg/m<sup>2</sup> was used as an independent variable. Age, biological sex, and diagnosis were modeled as covariates. Before entering the variables in the above-mentioned linear regression and general linear models, age, BV, EDSS score, and biomarker concentrations were standardized by dividing them by their SD similarly to other biomarker studies.<sup>28</sup> Serum and CSF parameters were log transformed. *p* Values for the linear regression models were corrected for FDR with the Benjamini-Hochberg procedure, and the significance level was set at  $q < 0.05$ . Significance levels for the general linear models were set at  $p < 0.05$ .

## Power Analysis

Achieved power was computed post hoc using G\*Power<sup>31,32</sup> separately for the analysis focusing on patients with MS only and for the analysis encompassing the whole sample.

## Data Availability

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

## Results

### Baseline Characteristics

Baseline characteristics of the MS phenotype sample (n = 106) and the whole sample (n = 157) are reported in Tables 1 and 2. In each table, the baseline characteristics are also presented separately for the subgroups with BMI <25 and BMI ≥25 kg/m<sup>2</sup>. The MS phenotype sample consisted of 69 patients with RRMS, 9 patients with PPMS, 21 patients with CIS, and 7 patients with RIS. Of note, 58.5% of those 106 patients had BMI <25 kg/m<sup>2</sup>, and 41.5% were classified as overweight/obese due to BMI ≥25 kg/m<sup>2</sup>. Not surprisingly, the overweight/obese group had a higher BMI (mean 29.76 kg/m<sup>2</sup> ± SD 5.57 vs 21.76 kg/m<sup>2</sup> ± SD 2.10) particularly because of their heavier weight (mean 85.52 kg ± SD 17.04 vs 62.82 kg ± SD 9.22) while they did not differ from patients with BMI <25 kg/m<sup>2</sup> with regard to their body height (mean 169.59 cm ± SD 9.97 vs 169.56 cm ± SD 7.63). Overweight/obese patients had lower sGFAP concentrations than the BMI <25 group (median 72.36 pg/mL, interquartile range [IQR] range 51.25 vs median 90.36 pg/mL, IQR range 58.42, *p* = 0.048). There were no other significant group differences for the 2 MS subgroups BMI ≥25 vs BMI <25 kg/m<sup>2</sup> (Table 1). BMI-defined subgroups of the whole cohort (n = 157) differed also with regard to their sGFAP levels (median 75.01 pg/mL, IQR range 49.78 vs median 87.47 pg/mL, IQR range 55.82, *p* = 0.013) and also with regard to their female/male ratio (38.1% of the overweight/obese patients were male vs 21.3% of the patients with BMI <25, *p* = 0.021).

### Exploratory Correlations

Serum GFAP was inversely correlated with BV (*r* = -0.246, *p* = 0.012, 95% CI: -0.424 to -0.049), BMI (*r* = -0.196, *p* = 0.047, 95% CI: -0.380 to 0.003), and body weight (*r* = -0.259, *p* = 0.008, 95% CI: -0.436 to -0.064). Serum NfL was inversely associated with BV (*r* = -0.223, *p* = 0.023, 95% CI: -0.405 to -0.026) and body weight (*r* = -0.200, *p* = 0.043, 95% CI: -0.384 to -0.001). No significant correlations were detected between cGFAP/cNfL and BMI, BV, body weight, or body height. After removing extreme outliers (n = 1 for body weight, n = 2 for sGFAP, n = 4 for sNfL, n = 4 for cGFAP, and n = 7 for cGFAP) and repeating the exploratory correlational analysis, the correlations between sGFAP and BV (*r* = -0.220, *p* = 0.028, 95% CI: -0.404 to -0.019), sGFAP and body weight (*r* = -0.231, *p* = 0.021, 95% CI: -0.414 to -0.031), sNfL and BV (*r* = -0.275, *p* = 0.006, 95% CI: -0.453 to -0.075), and sNfL and body weight (*r* = -0.258, *p* = 0.010, 95% CI = -0.439 to -0.057) remained significant. Figure and eTable 1 (links.lww.com/NXI/A751) summarize the results from the correlational analysis.

When repeating the same exploratory correlational analysis for the whole cohort (n = 157), sGFAP correlated significantly with BV (*r* = -0.162, *p* = 0.048, 95% CI: -0.319 to 0.003) and body weight (*r* = -0.199, *p* = 0.015, 95% CI: -0.353 to -0.035). Correlations between sGFAP and BMI (*r* = -0.157, *p* = 0.055), sNfL and BV (*r* = -0.157, *p* = 0.055), and sNfL and body weight (*r* = -0.145, *p* = 0.079) were close to significance, but their *p* values remained >0.05 (eTable 2, links.lww.com/NXI/A751).

### Primary Analysis: Using BV to Predict Serum/CSF Biomarker Concentrations in MS

Primary analysis based on multivariable linear regressions with the patients with MS (n = 106) revealed significant models for sGFAP (*p* = 0.0003, *q* = 0.002) and cGFAP (*p* = 0.028, *q* = 0.037), see eTable 3 (links.lww.com/NXI/A751). BV ( $\beta$  = -0.301, *p* = 0.014), age ( $\beta$  = 0.234, *p* = 0.020), MS phenotype ( $\beta$  = 0.281, *p* = 0.011), and EDSS score ( $\beta$  = 0.283, *p* = 0.004) were significant predictors of sGFAP. Age ( $\beta$  = 0.222, *p* = 0.038) and GE ( $\beta$  = -0.209, *p* = 0.034) predicted cGFAP significantly. Linear regression models for sNfL and cNfL did not reach significance. eTable 3 (links.lww.com/NXI/A751) summarizes the results from these linear regression analyses. Cohen *f*<sup>2</sup> effect size for the sGFAP linear regression in the patients with MS was *f*<sup>2</sup> = 0.33.

Extending linear regression analysis to the whole cohort (n = 157) revealed significant models for all 4 biomarkers (eTable 4, links.lww.com/NXI/A751). Significant predictors of sGFAP were BV ( $\beta$  = -0.276, *p* = 0.009) and sex ( $\beta$  = 0.226, *p* = 0.005). For sNfL, age ( $\beta$  = 0.168, *p* = 0.036) and sex ( $\beta$  = 0.245, *p* = 0.002) were significant predictors. Sex ( $\beta$  = 0.299, *p* < 0.001) contributed significantly to the prediction of cGFAP and age ( $\beta$  = 0.304, *p* < 0.001) to the prediction of cNfL. eTable 4 summarizes the results from these linear regression analyses.

### Secondary Analysis: Serum/CSF Biomarker Concentration Differences Between BMI-Defined Groups

Secondary analysis based on general linear models with BMI status as a group variable and age, biological sex, MS phenotype, EDSS score, GE, and acute relapse as covariates delivered significant results only for sGFAP (*p* = 0.001) and cGFAP (*p* = 0.035) when applied to the MS sample. BMI status (*F* = 4.732, *p* = 0.032), age (*F* = 6.497, *p* = 0.012), MS phenotype (*F* = 6.947, *p* = 0.010), and EDSS score (*F* = 7.576, *p* = 0.007) contributed significantly to the prediction of the sGFAP with the BMI ≥25 kg/m<sup>2</sup> group showing lower sGFAP levels than the BMI <25 kg/m<sup>2</sup> group. Age (*F* = 4.632, *p* = 0.034) and GE (*F* = 4.671, *p* = 0.033) predicted cGFAP significantly. eTable 5 (links.lww.com/NXI/A751) summarizes the results from this GLM analysis. Cohen *d* effect size for the sGFAP group difference (BMI <25 vs BMI ≥25 kg/m<sup>2</sup>) in patients with MS was *d* = 0.433.

Extending the GLM analysis to the whole cohort (n = 157) delivered significant models for all 4 biomarkers. BMI status

**Table 1** Demographic, Clinical, Laboratory, and Imaging Characteristics of the Multiple Sclerosis Sample

	Total MS n = 106	BMI <25 kg/m <sup>2</sup> n = 62 (58.5%)	BMI ≥25 kg/m <sup>2</sup> n = 44 (41.5%)	p Value BMI <25 vs BMI ≥25 kg/m <sup>2</sup>
Weight (kg), mean (SD)	72.25 (17.16)	62.82 (9.22)	85.52 (17.04)	<0.001 <sup>a</sup>
Height (cm), mean (SD)	169.58 (8.63)	169.56 (7.63)	169.59 (9.97)	0.988 <sup>a</sup>
BMI, mean (SD)	25.08 (5.56)	21.76 (2.10)	29.76 (5.57)	< 0.001 <sup>a</sup>
Estimated blood volume (L), mean (SD)	4.42 (0.82)	4.08 (0.62)	4.90 (0.83)	< 0.001 <sup>a</sup>
Age (y), mean (SD)	35.59 (10.64)	35.56 (10.04)	35.64 (11.56)	0.973 <sup>a</sup>
Female, n (%)	80 (75.5)	51 (82.3)	29 (65.9)	0.054 <sup>b</sup>
Male, n (%)	26 (24.5)	11 (17.7)	15 (34.1)	
RRMS, n (%)	69 (65.1)	38 (61.3)	31 (70.5)	0.393 <sup>b</sup>
PPMS, n (%)	9 (8.5)	15 (24.2)	6 (13.6)	
CIS, n (%)	21 (19.8)	4 (6.5)	5 (11.4)	
RIS, n (%)	7 (6.6)	5 (8.1)	2 (4.5)	
EDSS score, mean (SD)	1.96 (1.3)	1.85 (1.25)	2.11 (1.38)	0.311 <sup>a</sup>
Gadolinium-enhancing T1-weighted lesions in MRI, n (%)				0.154 <sup>b</sup>
Yes	56 (52.8)	29 (46.8)	27 (61.4)	
No	45 (42.5)	31 (50.0)	14 (31.8)	
MRI not available	5 (4.7)	2 (3.2)	3 (6.8)	
With acute relapse, n (%)	81 (76.4)	47 (75.8)	34 (77.3)	0.861 <sup>b</sup>
Blood-CSF barrier dysfunction, n (%)	23 (21.7)	11 (17.7)	12 (27.3)	0.241 <sup>b</sup>
CSF white blood cell count (/mm <sup>3</sup> ), mean (SD)	9.95 (12.92)	8.73 (13.83)	11.68 (11.43)	0.247 <sup>a</sup>
CSF red blood cell count (/mm <sup>3</sup> ), mean (SD)	6.42 (41.30)	0.74 (3.29)	14.43 (63.54)	0.093 <sup>a</sup>
CSF/blood albumin quotient, mean (SD)	5.28 (2.35)	5.11 (2.00)	5.50 (2.79)	0.406 <sup>a</sup>
Serum GFAP (pg/mL), mean ± SD (median, IQR range)	96.95 ± 54.93 (82.89, 55.41)	104.63 ± 62.07 (90.36, 58.42)	86.24 ± 41.41 (72.36, 51.25)	<b>0.048<sup>a</sup></b>
Serum NFL (pg/mL), mean ± SD (median, IQR range)	16.38 ± 16.05 (11.83, 9.89)	17.17 ± 17.88 (12.88, 11.90)	15.29 ± 13.19 (10.14, 9.43)	0.419 <sup>a</sup>
CSF GFAP (pg/mL), mean ± SD (median, IQR range)	18,998.53 ± 14,941.51 (15,077.87, 13,218.02)	18,138.83 ± 11,986.30 (15,327.08, 15,342.99)	20,247.13 ± 18,505.46 (14,284.78, 13,646.67)	0.861 <sup>a</sup>
CSF NFL (pg/mL), mean ± SD (median, IQR range)	3,142.53 ± 4,290.66 (1,682.87, 2,515.05)	3,139.66 ± 4,721.11 (1,673.03, 1789.29)	3,146.69 ± 3,630.71 (2087.97, 3,225.57)	0.611 <sup>a</sup>

Abbreviations: CIS = clinically isolated syndrome; EDSS = Expanded Disability Status Scale; IgG = immunoglobulin G; IQR = interquartile range; PPMS = primary progressive multiple sclerosis; RIS = radiologically isolated syndrome; RRMS = relapsing-remitting multiple sclerosis; sGFAP/cGFAP = glial fibrillary acidic protein measured in serum/CSF; sNFL/cNFL = neurofilament light chain measured in serum/CSF.

Sample characteristics are shown for the whole MS sample (total n = 106) and the 2 BMI-defined MS groups. Patients were grouped according to their body mass index, BMI (BMI ≥25 kg/m<sup>2</sup>, i.e., overweight or obese, n = 62 vs BMI <25 kg/m<sup>2</sup>, n = 44). The groups were compared with regard to their demographic, clinical, laboratory, and imaging characteristics using 1-way analysis of variance and Pearson  $\chi^2$  tests. GFAP and NFL parameters were log transformed for the ANOVA analysis. The significance level was set at  $p < 0.05$ . Significant  $p$  values are formatted in bold.

<sup>a</sup> ANOVA.

<sup>b</sup> Pearson  $\chi^2$  tests.

was again a significant predictor for sGFAP only ( $F = 7.631$ ,  $p = 0.006$ ) along with age ( $F = 8.794$ ,  $p = 0.004$ ) but not for sNFL, cGFAP, and cNFL. Again, the BMI ≥25 group showed

lower sGFAP levels than the BMI <25 group. eTable 6 ([links.lww.com/NXI/A751](https://www.lww.com/NXI/A751)) summarizes the results from this GLM analysis.

**Table 2** Demographic, Clinical, Laboratory, and Imaging Characteristics of the Sample

	Total n = 157	BMI <25 kg/m <sup>2</sup> n = 94 (59.9%)	BMI ≥25 kg/m <sup>2</sup> n = 63 (40.1%)	p Value BMI <25 vs BMI ≥25 kg/m <sup>2</sup>
Weight (kg), mean (SD)	73.15 (18.27)	63.11 (10.26)	88.14 (17.34)	<b>&lt;0.001<sup>a</sup></b>
Height (cm), mean (SD)	170.55 (9.11)	170.40 (8.65)	170.76 (9.83)	0.81 <sup>a</sup>
BMI, mean (SD)	25.11 (5.94)	21.62 (2.24)	30.30 (5.96)	<b>&lt;0.001<sup>a</sup></b>
Estimated blood volume (L), Mean (SD)	4.5 (0.87)	4.14 (0.70)	5.04 (0.82)	<b>&lt;0.001<sup>a</sup></b>
Age (y), mean (SD)	37.00 (11.82)	36.39 (10.62)	37.90 (13.46)	0.434 <sup>a</sup>
Female, n (%)	113 (72)	74 (78.7)	39 (61.9)	<b>0.021<sup>b</sup></b>
Male, n (%)	44 (28)	20 (21.3)	24 (38.1)	
Neurologic disorder, n (%)	143 (91)	83 (88.3)	60 (95.2)	0.135 <sup>b</sup>
Somatoform disorder, n (%)	14 (8.9)	11 (11.7)	3 (4.8)	
Blood-CSF barrier dysfunction, n (%)	34 (21.7)	16 (17.2)	18 (28.6)	0.092 <sup>b</sup>
CSF white blood cell count (/mm <sup>3</sup> ), mean (SD)	13.14 (47.47)	15.45 (60.71)	9.70 (11.00)	0.459 <sup>a</sup>
CSF red blood cell count (/mm <sup>3</sup> ), mean (SD)	4.91 (34.19)	1.18 (5.62)	10.48 (53.30)	0.095 <sup>a</sup>
CSF/blood albumin quotient, mean (SD)	6.39 ± 10.41	5.21 (3.31)	8.15 (15.85)	0.083 <sup>a</sup>
Serum GFAP (pg/mL), mean ± SD (median, IQR range)	98.11 ± 61.80 (82.48, 48.96)	107.27 ± 72.09 (87.47, 55.82)	84.89 ± 39.80 (75.01, 49.78)	<b>0.013<sup>a</sup></b>
Serum NFL (pg/mL), mean ± SD (median, IQR range)	21.17 ± 45.85 (11.25, 9.44)	18.99 ± 29.54 (11.89, 10.40)	24.99 ± 62.48 (9.90, 8.81)	0.617 <sup>a</sup>
CSF GFAP (pg/mL), mean ± SD (median, IQR range)	20,059.83 ± 15,948.21 (15,077.87, 15,512.37)	19,619.78 ± 14,173.18 (15,327.08, 16,540.37)	20,750.24 ± 18,504.97 (14,439.87, 12,329.82)	0.925 <sup>a</sup>
CSF NfL (pg/mL), mean ± SD (median, IQR range)	3,107.83 ± 4,648.48 (1,485.35, 2,594.64)	2,890.68 ± 4,272.44 (1,348.02, 2015.94)	3,448.51 ± 5204.89 (1781.04, 3,149.94)	0.506 <sup>a</sup>

Abbreviations: IgG = immunoglobulin G; IQR = interquartile range; sGFAP/cGFAP = glial fibrillary acidic protein measured in serum/CSF; sNfL/cNfL = neurofilament light chain measured in serum/CSF.

Sample characteristics are shown for the whole sample (total n = 157) and for the 2 BMI-defined groups. Patients were grouped according to their body mass index, BMI (BMI ≥25 kg/m<sup>2</sup>, i.e., overweight/obese, n = 63 vs BMI <25 kg/m<sup>2</sup>, n = 94). The 2 groups were compared with regard to their demographic, clinical, laboratory, and imaging characteristics using 1-way analysis of variance and Pearson  $\chi^2$  tests. GFAP and NFL parameters were log transformed for the ANOVA analysis. The significance level was set at  $p < 0.05$ . Significant  $p$  values are formatted in bold.

<sup>a</sup> ANOVA.

<sup>b</sup> Pearson  $\chi^2$  tests.

## Power Analysis

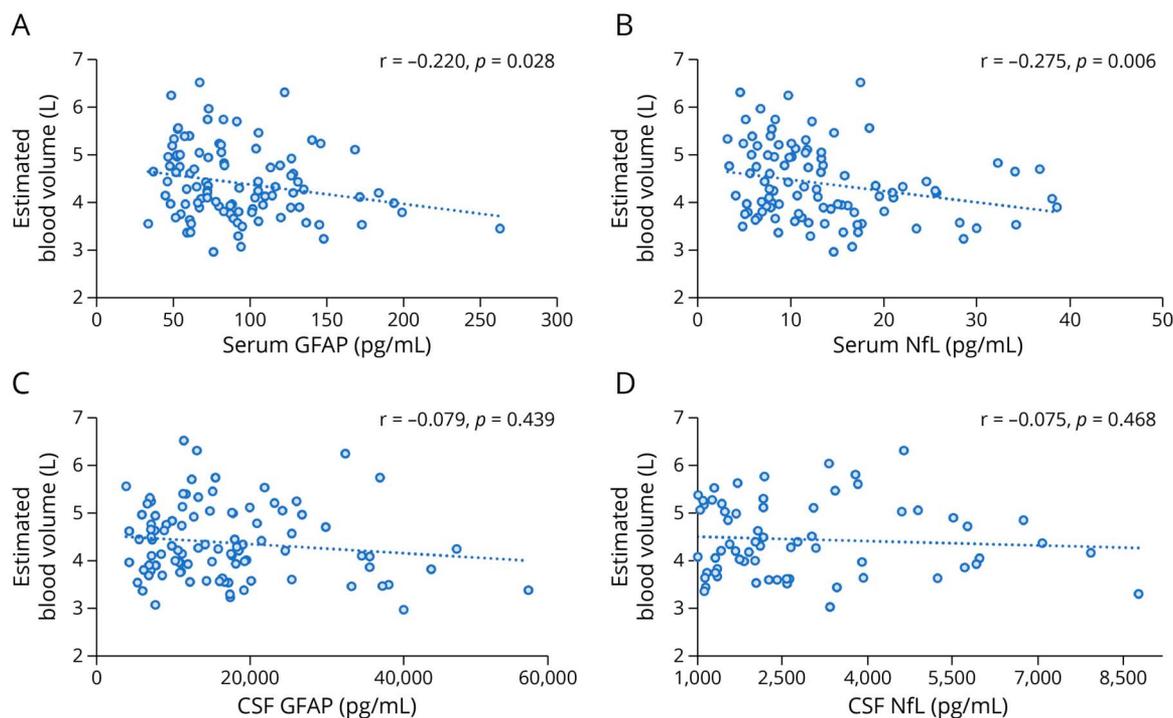
Post hoc analysis of the observed power for the MS phenotype sample (n = 106) demonstrated that the reached power was >0.80 for sGFAP (1- $\beta$  = 0.996) and cGFAP (1- $\beta$  = 0.889) but not for sNfL (1- $\beta$  = 0.482) or cNfL (1- $\beta$  = 0.166). When applied to the results from the whole sample (n = 157), the calculated observed power was >0.80 in all 4 cases (eTable 7, [links.lww.com/NXI/A751](https://links.lww.com/NXI/A751)).

## Discussion

Growing interest in the research of biomarkers for MS disease activity has led to the need for standardized analysis procedures and identification of variables affecting biomarker

concentrations. Because serum measurements of biomarkers are minimally invasive and can be thus repeated over regular periods of time, they are particularly suitable for monitoring of disease activity and therapy response. However, particularly high or low volumes of distribution (e.g., due to a greater body size and thus higher blood volume, BV) might affect biomarker concentrations in a relevant manner. Furthermore, because more than one-third of patients with MS are overweight or obese<sup>16</sup> and a link between obesity and MS risk as well as response to disease-modifying therapy has been established,<sup>17,18</sup> it is essential to explore whether BMI and its associated factors (body weight and body height) should be considered when measuring biomarkers of MS. Here, we show that even after controlling for other demographic and clinical variables, the estimated BV is inversely associated with

**Figure** Serum GFAP ( $r = -0.220, p = 0.028$ ) and Serum NfL ( $r = -0.275, p = 0.006$ ) Were Inversely Correlated With the Estimated Blood Volume



A, B. Serum GFAP ( $r = -0.220, p = 0.028$ ) and serum NfL ( $r = -0.275, p = 0.006$ ) were inversely correlated with the estimated blood volume (BV). C, D. No significant correlations were detected between BV and CSF GFAP/NfL. To ensure that the results are not biased, extreme outliers were removed. GFAP = glial fibrillary protein; NfL = neurofilament light chain.

sGFAP levels and sGFAP concentrations are lower in overweight/obese patients with MS compared with patients with MS with BMI  $<25 \text{ kg/m}^2$ . More specifically, our model suggests that the effect size of BV on sGFAP measurements might be at least as important as the ones of age and EDSS score ( $\beta_{\text{BV}} = -0.301, \beta_{\text{EDSS}} = 0.283, \beta_{\text{age}} = 0.234$ , eTable 3, links.lww.com/NXI/A751). Similarly, Cohen  $d$  and  $f^2$  values indicated a small to medium effect sizes of BMI/BV on sGFAP concentrations in patients with MS. These findings support the notion that the volume of distribution of the respective biomarker as approximated by BV is a relevant variable and should therefore be controlled for when measuring sGFAP.

Of interest, our results suggest that clinically meaningful BMI categories (e.g., overweight/obesity) are also an important factor in biomarker measurements. Overweight/obese patients with MS had lower sGFAP concentrations (median =  $86.24 \text{ pg/mL}$ ) compared with patients with BMI  $<25 \text{ kg/m}^2$  (median =  $104.63 \text{ pg/mL}$ ) after adjusting for relevant demographic and clinical variables. Obesity has been suggested to contribute to dysfunctions of the blood-brain barrier (BBBd).<sup>33</sup> However, in our analysis, the BMI-defined groups did not differ regarding presence of gadolinium-enhancing lesions on MRI, presence of BBBd as seen in CSF analysis, and CSF/blood albumin quotient values (Tables 1 and 2). Moreover, we were not able to demonstrate significant group differences for CSF biomarker concentrations, most likely due to the fact that the brain/CSF

volume ratio is related more strongly to age than to body size.<sup>20</sup> Furthermore, CSF volume might be decreased in obese individuals as suggested by estimations based on thoracic and lumbosacral MRIs.<sup>34</sup> Thus, although serum GFAP measurements should be corrected for BV/BMI, CSF measurements of GFAP may not necessarily need that correction.

The association between sGFAP levels and BV as well as the effects of the BMI status on GFAP concentration in serum remained significant after adding controls with either other neurologic or somatoform diseases (eTables 2, 4, and 6, links.lww.com/NXI/A751). Although the sample size of the control groups was not large enough to allow separate analysis focusing exclusively on patients without MS, our results indicate that increasing the volume of distribution might result in lower sGFAP levels not only in MS but also in patients with other neurologic or without neurologic diseases. Judging by the partial correlations between BV and sGFAP ( $r = -0.250$  for the MS cohort and  $r = -0.216$  for the whole sample), this effect might be more easily detectable in the MS population. However, the difference in the size of the effect might be also due to the fact that the MS cohort was more homogenous (e.g., regarding disease phenotype) compared with the control groups.

Similar effect of BMI and BV on sNfL has been reported in previous studies<sup>14,15</sup> but could not be completely confirmed

in other works,<sup>19</sup> including ours. Although sNfL was inversely correlated with BV and body weight (eTable 1, links.lww.com/NXI/A751), this association did not remain significant in the linear regression models where the effects of other covariates were controlled for. However, sNfL levels might be more robust to BV and BMI variations, which would imply that the effects for this biomarker are seen only in larger, better powered studies. This is in line with our post hoc power calculation, which demonstrated that the achieved power by our sample size was sufficient (i.e., >0.80) for the sGFAP and cGFAP measurements in the MS group but not for sNfL and cNfL, where a larger sample size seems to be necessary (eTable 7, links.lww.com/NXI/A751). This could explain why a larger study<sup>14</sup> (n = 2,586) were able to demonstrate a robust negative correlation between BV/BMI and NfL, while we (n = 106) as well as Bock et al.<sup>19</sup> (n = 40) were not able to demonstrate an association between BV/body size measures and NfL levels in patients with MS.

Of interest, a recent work modeling the trajectories of GFAP levels in blood after mild traumatic brain injury suggested that variations in total blood volume could significantly change the measured levels of the biomarker,<sup>35</sup> which complies with our findings. Longitudinal changes in body weight seem also to affect GFAP and NfL levels, as has been recently shown in patients with anorexia nervosa (AN). Patients with AN were studied in the severely underweight state and again after short-term partial weight restoration.<sup>36</sup> Remarkably, serum levels decreased on short-term partial weight restoration. These results were interpreted as a sign of partial restoration of neuronal and astroglial integrity on weight gain after initial AN-associated cell damage processes.<sup>36</sup> However, because the observed BMI increase ranged from 14% to the impressive 46%, these findings might have also been at least partially influenced by the increased volume of distribution and might thus be interpreted as a consequence of dilution effects for sGFAP. Similarly, Rebelos et al.<sup>37</sup> determined sNfL and sGFAP concentrations in lean and morbidly obese patients and found that the latter had lower absolute concentrations of circulating NfL and GFAP. Six months after bariatric surgery and weight loss, both biomarker levels were increased,<sup>37</sup> which suggests that decreased volume of distribution can result in increased biomarker concentrations. The findings of Hellerhoff et al.<sup>36</sup> and Rebelos et al.<sup>37</sup> demonstrate how weight gain and loss—supposedly via changes in volume of distribution—might affect sNfL and sGFAP levels.

One of the limitations of our study is that BV and BMI measurements were based on self-reported values of body weight and height. Although this is not an ideal substitute for true measurements of the parameters of interest, there is a significant amount of evidence that patients' own reports are fairly precise and reliable, deviating only insignificantly from their true values.<sup>38-40</sup> Furthermore, obesity (i.e., BMI  $\geq$ 30) was found in n = 14 patients in the MS cohort and n = 21 patients in the whole sample. The lack of a larger subgroup of these participants did not allow a separate analysis focusing on

obese patients only, and this might explain some of the negative findings with regard to sNfL. Similarly, our calculations suggest that our study was underpowered for detecting associations between BV and sNfL concentration. A larger study sample seems reasonable when investigating volume of distribution effects on sNfL, whereas sGFAP effects seem to be less sensitive to the used sample size and more easily detected.

Obtaining both serum and CSF samples from non-neurologic controls can be a challenging task. We cannot provide a fully balanced distribution of patients across the groups MS/neurologic diseases vs somatoform diseases, but our results suggest that the BV effects on sGFAP levels are not entirely MS specific.

Although it can be argued that the relevance of our findings is limited to large sample sizes and less essential for the individual patient, recent data strongly suggest that comparing the individual patient's biomarker concentration with the corresponding representative reference values can be used to predict the MS disease activity in individual patients.<sup>15</sup> Establishing such reference values requires, however, knowing the exact relationship between certain clinical or epidemiologic variables and the biomarker levels. Thus, knowing how BV and BMI affect sGFAP concentrations and identifying BV/BMI-adjusted reference values for the corresponding biomarker can be eventually relevant also for the individual patient.

To our best knowledge, this is the first study to show significant effects of BV and BMI on sGFAP measurements in MS and to control for similar effects in CSF, while exploring the same effects in sNfL and cNfL. Future studies investigating sGFAP as a marker for MS disease activity should consider including BV and/or BMI as a relevant factor.

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## Appendix Authors

Name	Location	Contribution
<b>Yavor Yalachkov, MD, PhD</b>	Department of Neurology, University Hospital Frankfurt, Frankfurt am Main, Germany	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data
<b>Jan Hendrik Schäfer, MD</b>	Department of Neurology, University Hospital Frankfurt, Frankfurt am Main, Germany	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data
<b>Jasmin Jakob</b>	Department of Neurology, Universitätsmedizin Mainz, Mainz, Germany	Major role in the acquisition of data
<b>Lucie Friedauer, MD</b>	Department of Neurology, University Hospital Frankfurt, Frankfurt am Main, Germany	Major role in the acquisition of data
<b>Falk Steffen, MD</b>	Department of Neurology, Universitätsmedizin Mainz, Mainz, Germany	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; and analysis or interpretation of data
<b>Stefan Bittner, MD, PhD</b>	Department of Neurology, Universitätsmedizin Mainz, Mainz, Germany	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data
<b>Christian Foerch, MD, PhD</b>	Department of Neurology, University Hospital Frankfurt, Frankfurt am Main, Germany	Drafting/revision of the manuscript for content, including medical writing for content, and study concept or design
<b>Martin Alexander Schaller-Paule, MD</b>	Department of Neurology, University Hospital Frankfurt, Frankfurt am Main, Germany	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data

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## Effect of Estimated Blood Volume and Body Mass Index on GFAP and NfL Levels in the Serum and CSF of Patients With Multiple Sclerosis

Yavor Yalachkov, Jan Hendrik Schäfer, Jasmin Jakob, et al.

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