**Table e1. Correlations between serum/CSF biomarker concentrations and BV, BMI, body weight as well as body height in MS subjects.**
Spearman-rho correlations between serum/CSF GFAP/NfL concentrations (pg/ml) and the individual estimated blood volume (L) were computed (left column). To ensure that the results are not biased, extreme outliers with biomarker levels or body weight lying above the 3rd quartile + 3*interquartile range were removed and the correlational analyses were repeated (right column). For sGFAP n = 3 outliers were identified, for sNfL n = 4, for cGFAP n = 4, for cNfL n = 7. sGFAP/cGFAP = glial fibrillary acidic protein measured in serum/CSF; sNfL/cNfL = neurofilament light chain measured in serum/CSF; CI = confidence intervals.

| | | | 95% CI | | extreme outliers removed | | 95% CI | |
|---|---|---|---|---|---|---|---|---|
| | BV | p-value | lower | upper | BV | p-value | lower | upper |
| serum GFAP | - 0.246 | **0.012** | -0.424 | -0.049 | - 0.220 | **0.028** | -0.404 | -0.019 |
| serum NFL | - 0.223 | **0.023** | -0.405 | -0.026 | - 0.275 | **0.006** | -0.453 | -0.075 |
| CSF GFAP | - 0.020 | 0.840 | -0.218 | 0.180 | - 0.079 | 0.439 | -0.277 | 0.126 |
| CSF NfL | - 0.045 | 0.650 | -0.242 | 0.155 | - 0.075 | 0.468 | -0.277 | 0.133 |
| | BMI | p-value | | | BMI | p-value | | |
| serum GFAP | -0.196 | **0.047** | -0.380 | 0.003 | -0.163 | 0.106 | -0.353 | 0.041 |
| serum NFL | -0.119 | 0.231 | -0.311 | 0.082 | -0.173 | 0.089 | -0.364 | 0.033 |
| CSF GFAP | -0.093 | 0.349 | -0.287 | 0.108 | -0.180 | 0.075 | -0.370 | 0.024 |
| CSF NfL | 0.042 | 0.676 | -0.159 | 0.239 | 0.065 | 0.527 | -0.143 | 0.268 |
| | weight | p-value | | | weight | p-value | | |
| serum GFAP | -0.259 | **0.008** | -0.436 | -0.064 | -0.231 | **0.021** | -0.414 | -0.031 |
| serum NFL | -0.200 | **0.043** | -0.384 | -0.001 | -0.258 | **0.010** | -0.439 | -0.057 |
| CSF GFAP | -0.082 | 0.408 | -0.277 | 0.119 | -0.153 | 0.131 | -0.345 | 0.052 |
| CSF NfL | -0.023 | 0.814 | -0.221 | 0.176 | -0.038 | 0.712 | -0.243 | 0.169 |
| | height | p-value | | | height | p-value | | |
| serum GFAP | -0.181 | 0.068 | -0.367 | 0.019 | -0.171 | 0.089 | -0.360 | 0.032 |
| serum NFL | -0.157 | 0.113 | -0.345 | 0.043 | -0.184 | 0.70 | -0.374 | 0.021 |
| CSF GFAP | 0.096 | 0.337 | -0.106 | 0.289 | 0.082 | 0.421 | -0.123 | 0.280 |
| CSF NfL | -0.008 | 0.936 | -0.207 | 0.191 | -0.094 | 0.363 | -0.294 | 0.115 |

**Table e2. Correlations between serum/CSF concentrations and BV, BMI, body weight as well as body height in the whole cohort.**
Spearman-rho correlations between serum/CSF GFAP/NfL concentrations (pg/ml) and the individual estimated blood volume (L) were computed. sGFAP/cGFAP = glial fibrillary acidic protein measured in serum/CSF; sNfL/cNfL = neurofilament light chain measured in serum/CSF; CI = confidence intervals.

| | | | 95% CI | |
|---|---|---|---|---|
| | BV | p-value | lower | upper |
| serum GFAP | -0.162 | **0.048** | -0.319 | 0.003 |
| serum NFL | -0.152 | *0.065* | -0.309 | 0.014 |
| CSF GFAP | 0.013 | 0.873 | -0.153 | 0.178 |
| CSF NfL | 0.0004 | 0.996 | -0.165 | 0.166 |
| | BMI | p-value | | |
| serum GFAP | -0.157 | *0.055* | -0.315 | 0.008 |
| serum NFL | -0.069 | 0.402 | -0.232 | 0.097 |
| CSF GFAP | -0.087 | 0.290 | -0.249 | 0.079 |
| CSF NfL | 0.044 | 0.596 | -0.123 | 0.208 |
| | weight | p-value | | |
| serum GFAP | -0.199 | **0.015** | -0.353 | -0.035 |
| serum NFL | -0.145 | *0.079* | -0.303 | 0.021 |
| CSF GFAP | -0.056 | 0.494 | -0.220 | 0.110 |
| CSF NfL | 0.009 | 0.917 | -0.157 | 0.174 |
| | height | p-value | | |
| serum GFAP | -0.105 | 0.204 | -0.265 | 0.062 |
| serum NFL | -0.125 | 0.130 | -0.284 | 0.042 |
| CSF GFAP | 0.086 | 0.294 | -0.080 | 0.248 |
| CSF NfL | -0.009 | 0.916 | -0.174 | 0.157 |

**Table e3.** Linear regressions with serum/CSF GFAP and NfL as dependent variables and BV, age, gender, MS phenotype (RRMS, PPMS, CIS or RIS), EDSS, presence of gadolinium-enhancing T1-weighted MRI lesions and acute relapse at serum/CSF sampling as predictors, using the data of patients with a MS phenotype (n = 106). Before entering the variables, age, BV, EDSS and biomarker concentrations were standardized by dividing them by their standard deviation similarly to other biomarker studies. Serum and CSF parameters were log-transformed. BV = estimated blood volume; sGFAP/cGFAP = glial fibrillary acidic protein measured in serum/CSF; sNfL/cNfL = neurofilament light chain measured in serum/CSF; GE = gadolinium-enhancing T1-weighted MRI lesions. Partial correlation refers to the correlation between the respective independent variable and the dependent variable after the linear effects of the other variables have been removed from both the independent variable and the dependent variable.

| sGFAP | model: R = 0.499, $R^2$ = 0.249, adjusted $R^2$ = 0.192, F change = 4.4, df1 = 7, df2 = 93, p = **0.0003**, q = **0.002** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | 95% Confidence Interval for B | | |
| | B | std. error | ß | t | p-value | lower bound | upper bound | partial correlation |
| constant | -0.243 | 0.496 | | -0.490 | 0.625 | -1.228 | 0.742 | |
| blood volume | -0.297 | 0.119 | -0.301 | -2.492 | **0.014** | -0.534 | -0.060 | -0.250 |
| age | 0.242 | 0.102 | 0.234 | 2.369 | **0.020** | 0.039 | 0.446 | 0.239 |
| gender | -0.118 | 0.261 | -0.054 | -0.453 | 0.652 | -0.638 | 0.401 | -0.047 |
| MS phenotype | 0.240 | 0.093 | 0.281 | 2.590 | **0.011** | 0.056 | 0.424 | 0.259 |
| EDSS | 0.267 | 0.089 | 0.283 | 2.988 | **0.004** | 0.089 | 0.444 | 0.296 |
| GE | -0.106 | 0.150 | -0.064 | -0.704 | 0.483 | -0.403 | 0.192 | -0.073 |
| acute relapse | 0.486 | 0.274 | 0.213 | 1.774 | 0.079 | -0.058 | 1.030 | 0.181 |

| sNfL | model: R = 0.268, $R^2$ = 0.072, adjusted $R^2$ = 0.002, F change = 1.03, df1 = 7, df2 = 93, p = 0.415, q = 0.474 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | 95% Confidence Interval for B | | |
| | B | std. error | ß | t | p-value | lower bound | upper bound | partial correlation |
| constant | -0.424 | 0.484 | | -0.876 | 0.383 | -1.385 | 0.537 | |
| blood volume | -0.128 | 0.116 | -0.148 | -1.100 | 0.274 | -0.359 | 0.103 | -0.113 |
| age | 0.104 | 0.100 | 0.115 | 1.045 | 0.299 | -0.094 | 0.302 | 0.108 |
| gender | 0.094 | 0.255 | 0.049 | 0.368 | 0.714 | -0.412 | 0.600 | 0.038 |
| MS phenotype | 0.038 | 0.090 | 0.050 | 0.416 | 0.678 | -0.142 | 0.217 | 0.043 |
| EDSS | 0.100 | 0.087 | 0.121 | 1.145 | 0.255 | -0.073 | 0.273 | 0.118 |
| GE | 0.133 | 0.146 | 0.093 | 0.911 | 0.365 | -0.157 | 0.423 | 0.094 |
| acute relapse | 0.224 | 0.267 | 0.112 | 0.840 | 0.403 | -0.306 | 0.755 | 0.087 |

| cGFAP | model: R = 0.390, $R^2$ = 0.152, adjusted $R^2$ = 0.088, F change = 2.376, df1 = 7, df2 = 93, p = **0.028**, q = **0.037** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | 95% Confidence Interval for B | | |
| | B | std. error | ß | t | p-value | lower bound | upper bound | partial correlation |
| constant | 1.061 | 0.533 | | 1.990 | 0.049 | 0.002 | 2.120 | |
| blood volume | -0.102 | 0.128 | -0.102 | -0.798 | 0.427 | -0.356 | 0.152 | -0.082 |
| age | 0.234 | 0.111 | 0.222 | 2.110 | **0.038** | 0.014 | 0.454 | 0.214 |
| gender | -0.547 | 0.282 | -0.247 | -1.940 | 0.055 | -1.106 | 0.013 | -0.197 |
| MS phenotype | 0.037 | 0.095 | 0.046 | 0.395 | 0.694 | -0.151 | 0.226 | 0.041 |
| EDSS | 0.091 | 0.097 | 0.095 | 0.938 | 0.351 | -0.101 | 0.283 | 0.097 |
| GE | -0.348 | 0.162 | -0.209 | -2.153 | **0.034** | -0.669 | -0.027 | -0.218 |
| acute relapse | 0.084 | 0.300 | 0.036 | 0.281 | 0.779 | -0.512 | 0.681 | 0.029 |

| cNfL | model: R = 0.160, $R^2$ = 0.025, adjusted $R^2$ = -0.048, F change = 0.347, df1 = 7, df2 = 93, p = 0.930, q = 0.93 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | 95% Confidence Interval for B | | |
| | B | std. error | ß | t | p-value | lower bound | upper bound | partial correlation |
| constant | 0.026 | 0.549 | | 0.047 | 0.963 | -1.065 | 1.116 | |
| blood volume | -0.004 | 0.132 | -0.005 | -0.033 | 0.974 | -0.266 | 0.257 | -0.003 |
| age | 0.049 | 0.114 | 0.048 | 0.429 | 0.669 | -0.178 | 0.276 | 0.044 |
| gender | -0.065 | 0.290 | -0.031 | -0.225 | 0.822 | -0.642 | 0.511 | -0.023 |
| MS phenotype | -0.052 | 0.098 | -0.066 | -0.528 | 0.599 | -0.246 | 0.142 | -0.055 |
| EDSS | -0.026 | 0.100 | -0.029 | -0.263 | 0.793 | -0.224 | 0.172 | -0.027 |
| GE | 0.018 | 0.167 | 0.011 | 0.109 | 0.913 | -0.312 | 0.349 | 0.011 |
| acute relapse | 0.285 | 0.309 | 0.128 | 0.923 | 0.358 | -0.329 | 0.900 | 0.095 |

**Table e4.** Linear regressions with serum/CSF GFAP and NfL as dependent variables and BV as well as diagnosis (neurological vs. somatoform disorder), age and gender as predictors using the data of all subjects (n = 157). Before entering the variables, age, BV and biomarker concentrations were standardized by dividing them by their standard deviation similarly to other biomarker studies. Serum and CSF parameters were log-transformed. BV = estimated blood volume; sGFAP/cGFAP = glial fibrillary acidic protein measured in serum/CSF; sNfL/cNfL = neurofilament light chain measured in serum/CSF. Partial correlation refers to the correlation between the respective independent variable and the dependent variable after the linear effects of the other variables have been removed from both the independent variable and the dependent variable.

| **sGFAP** | model: R = 0.320, $R^2$ = 0.102, adjusted $R^2$ = 0.077, F change = 4.107, df1 = 4, df2 = 144, p = **0.004**, q = **0.006** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | 95% Confidence Interval for B | |
| | B | Std. error | ß | t | p-value | lower bound | upper bound | partial correlation |
| constant | 0.215 | 0.508 | | 0.423 | 0.673 | -0.789 | 1.218 | |
| blood volume | -0.275 | 0.103 | -0.276 | -2.654 | **0.009** | -0.479 | -0.070 | -0.216 |
| age | 0.352 | 0.303 | 0.092 | 1.161 | 0.248 | -0.247 | 0.952 | 0.096 |
| gender | 0.223 | 0.078 | 0.226 | 2.855 | **0.005** | 0.069 | 0.377 | 0.231 |
| diagnosis | -0.316 | 0.230 | -0.144 | -1.374 | 0.172 | -0.770 | 0.139 | -0.114 |

| **sNfL** | model: R = 0.322, $R^2$ = 0.104, adjusted $R^2$ = 0.079, F change = 4.176, df1 = 7, df2 = 144, p = **0.003**, q = **0.006** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | 95% Confidence Interval for B | |
| | B | Std. error | ß | t | p-value | lower bound | upper bound | partial correlation |
| constant | -0.290 | 0.507 | | -0.572 | 0.568 | -1.293 | 0.712 | |
| blood volume | -0.169 | 0.103 | -0.170 | -1.633 | 0.105 | -0.373 | 0.035 | -0.135 |
| age | 0.641 | 0.303 | 0.168 | 2.114 | **0.036** | 0.042 | 1.240 | 0.174 |
| gender | 0.242 | 0.078 | 0.245 | 3.097 | **0.002** | 0.087 | 0.396 | 0.250 |
| diagnosis | -0.178 | 0.230 | -0.081 | -0.773 | 0.441 | -0.632 | 0.276 | -0.064 |

| **cGFAP** | model: R = 0.353, $R^2$ = 0.124, adjusted $R^2$ = 0.100, F change = 2.376, df1 = 7, df2 = 144, p = **0.001**, q = **0.003** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | 95% Confidence Interval for B | |
| | B | Std. error | ß | t | p-value | lower bound | upper bound | partial correlation |
| constant | 0.414 | 0.494 | | 0.838 | 0.404 | -0.563 | 1.391 | |
| blood volume | -0.043 | 0.101 | -0.042 | -0.425 | 0.672 | -0.243 | 0.157 | -0.035 |
| age | 0.291 | 0.278 | 0.082 | 1.045 | 0.298 | -0.259 | 0.841 | 0.087 |
| gender | 0.308 | 0.080 | 0.299 | 3.828 | **< 0.001** | 0.149 | 0.467 | 0.304 |
| diagnosis | -0.390 | 0.225 | -0.175 | -1.736 | 0.085 | -0.834 | 0.054 | -0.143 |

| **cNfL** | model: R = 0.355, $R^2$ = 0.126, adjusted $R^2$ = -0.102, F change = 5.181, df1 = 7, df2 = 144, p = **0.001**, q = **0.003** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | 95% Confidence Interval for B | |
| | B | Std. error | ß | t | p-value | lower bound | upper bound | partial correlation |
| constant | -0.642 | 0.494 | | -1.300 | 0.196 | -1.619 | 0.334 | |
| blood volume | -0.056 | 0.101 | -0.056 | -0.557 | 0.578 | -0.257 | 0.144 | -0.046 |
| age | 1.073 | 0.278 | 0.304 | 3.862 | **< 0.001** | 0.524 | 1.623 | 0.306 |
| gender | 0.153 | 0.080 | 0.148 | 1.902 | 0.059 | -0.006 | 0.311 | 0.157 |
| diagnosis | -0.194 | 0.225 | -0.087 | -0.864 | 0.389 | -0.638 | 0.250 | -0.072 |

**Table e5.** Four different general linear model (GLM) univariate analyses with each of the biomarkers of interest as a dependent variable (sNfL, cNfL, sGFAP, cGFAP) were computed for the MS subjects (n = 106). The group factor "BMI ≥ 25 vs. BMI < 25" was employed as an independent variable. Variables known to affect NfL and GFAP concentration such as age, biological gender, MS disease phenotype, EDSS, presence of gadolinium-enhancing (GE) T1-weighted MRI lesions and acute relapse at serum/CSF sampling were modelled as covariates. Before entering the analysis, age, EDSS and biomarker concentrations were standardized by dividing them by their standard deviation similarly to other biomarker studies. Serum and CSF parameters were log-transformed. sGFAP/cGFAP = glial fibrillary acidic protein measured in serum/CSF; sNfL/cNfL = neurofilament light chain measured in serum/CSF.

| | Type III Sum of Squares | F | p-value |
|---|---|---|---|
| **sGFAP** | | | |
| corrected model | 21.181 | 4.137 | **0.001** |
| intercept | 2.879 | 3.936 | 0.050 |
| BMI < 25 vs. ≥ 25 | 3.462 | 4.732 | **0.032** |
| age | 4.753 | 6.497 | **0.012** |
| gender | 0.786 | 1.074 | 0.303 |
| MS phenotype | 5.082 | 6.947 | **0.010** |
| EDSS | 5.542 | 7.576 | **0.007** |
| GE | 0.210 | 0.287 | 0.593 |
| acute relapse | 2.103 | 2.876 | 0.093 |
| | | | |
| **sNfL** | | | |
| corrected model | 4.508 | 0.934 | 0.484 |
| intercept | 1.961 | 2.844 | 0.095 |
| BMI < 25 vs. ≥ 25 | 0.393 | 0.570 | 0.452 |
| age | 0.877 | 1.272 | 0.262 |
| gender | 1.035 | 1.501 | 0.224 |
| MS phenotype | 0.133 | 0.193 | 0.661 |
| EDSS | 0.732 | 1.061 | 0.306 |
| GE | 0.600 | 0.871 | 0.353 |
| acute relapse | 0.455 | 0.660 | 0.419 |
| | | | |
| **cGFAP** | | | |
| corrected model | 13.518 | 2.273 | **0.035** |
| intercept | 2.925 | 3.444 | 0.067 |
| BMI < 25 vs. ≥ 25 | 0.019 | 0.022 | 0.882 |
| age | 3.934 | 4.632 | **0.034** |
| gender | 2.883 | 3.395 | 0.069 |
| MS phenotype | 0.118 | 0.139 | 0.710 |
| EDSS | 0.592 | 0.698 | 0.406 |
| GE | 3.967 | 4.671 | **0.033** |
| acute relapse | 0.063 | 0.075 | 0.785 |
| | | | |
| **cNfL** | | | |
| corrected model | 2.324 | 0.372 | 0.917 |
| intercept | 0.000 | 0.000 | 0.988 |
| BMI < 25 vs. ≥ 25 | 0.149 | 0.166 | 0.684 |
| age | 0.158 | 0.177 | 0.675 |
| gender | 0.028 | 0.031 | 0.861 |
| MS phenotype | 0.252 | 0.282 | 0.597 |
| EDSS | 0.081 | 0.090 | 0.764 |
| GE | 0.001 | 0.001 | 0.969 |
| acute relapse | 0.782 | 0.874 | 0.352 |

**Table e6.** Four different general linear model (GLM) univariate analyses with each of the biomarkers of interest as a dependent variable (sNfL, cNfL, sGFAP, cGFAP) were computed for all subjects (n = 157). The group factor "BMI ≥ 25 vs. BMI < 25" was employed as an independent variable. Age, gender and diagnosis (neurological vs. somatoform disease) were modelled as covariates. Before entering the analysis, age and biomarker concentrations were standardized by dividing them by their standard deviation similarly to other biomarker studies. Serum and CSF parameters were log-transformed. sGFAP/cGFAP = glial fibrillary acidic protein measured in serum/CSF; sNfL/cNfL = neurofilament light chain measured in serum/CSF.

| | Type III Sum of Squares | F | p-value |
|---|---|---|---|
| **sGFAP** | | | |
| corrected model | 14.632 | 3.950 | **0.005** |
| Intercept | 0.005 | 0.005 | 0.942 |
| BMI < 25 vs. ≥ 25 | 7.067 | 7.631 | **0.006** |
| age | 8.145 | 8.794 | **0.004** |
| gender | 0.042 | 0.045 | 0.832 |
| diagnosis | 0.001 | 0.001 | 0.976 |
| **sNfL** | | | |
| corrected model | 10.391 | 2.718 | **0.032** |
| intercept | 0.117 | 0.122 | 0.727 |
| BMI < 25 vs. ≥ 25 | 0.611 | 0.639 | 0.425 |
| age | 9.996 | 10.461 | **0.002** |
| gender | 0.035 | 0.036 | 0.849 |
| diagnosis | 0.922 | 0.965 | 0.328 |
| **cGFAP** | | | |
| corrected model | 17.678 | 4.883 | **0.001** |
| intercept | 2.976 | 3.288 | 0.072 |
| BMI < 25 vs. ≥ 25 | 0.325 | 0.359 | 0.550 |
| age | 12.759 | 14.099 | **< 0.001** |
| gender | 3.793 | 4.192 | **0.042** |
| diagnosis | 0.092 | 0.102 | 0.750 |
| **cNfL** | | | |
| corrected model | 9.997 | 2.608 | **0.038** |
| intercept | 3.164 | 3.302 | 0.071 |
| BMI < 25 vs. ≥ 25 | 0.037 | 0.039 | 0.844 |
| age | 4.822 | 5.032 | **0.026** |
| gender | 1.871 | 1.952 | 0.164 |
| diagnosis | 5.006 | 5.224 | **0.024** |

**Table e7. Power analysis**
Achieved power was computed post hoc using the software G*Power separately for the analysis focusing on MS subjects only and encompassing the whole sample. MS = multiple sclerosis; sGFAP/cGFAP = glial fibrillary acidic protein measured in serum/CSF; sNfL/cNfL = neurofilament light chain measured in serum/CSF.

| sGFAP | n = 106 MS subjects | n = 157, whole sample |
|---|---|---|
| effect size $f^2$ | 0.33 | 0.11 |
| α error probability | 0.05 | 0.05 |
| total sample size | 106 | 157 |
| number of predictors | 7 | 4 |
| noncentrality parameter λ | 35.15 | 17.83 |
| critical F | 2.10 | 2.43 |
| numerator df | 7 | 4 |
| denominator df | 98 | 152 |
| power (1 − β error probability) | 0.996 | 0.933 |

| sNfL | n = 106 MS subjects | n = 157, whole sample |
|---|---|---|
| effect size $f^2$ | 0.08 | 0.12 |
| α error probability | 0.05 | 0.05 |
| total sample size | 106 | 157 |
| number of predictors | 7 | 4 |
| noncentrality parameter λ | 8.22 | 18.22 |
| critical F | 2.10 | 2.43 |
| numerator df | 7 | 4 |
| denominator df | 98 | 152 |
| power (1 − β error probability) | 0.482 | 0.939 |

| cGFAP | n = 106 MS subjects | n = 157, whole sample |
|---|---|---|
| effect size $f^2$ | 0.18 | 0.14 |
| α error probability | 0.05 | 0.05 |
| total sample size | 106 | 157 |
| number of predictors | 7 | 4 |
| noncentrality parameter λ | 19 | 22.22 |
| critical F | 2.10 | 2.43 |
| numerator df | 7 | 4 |
| denominator df | 98 | 152 |
| power (1 − β error probability) | 0.889 | 0.975 |

| cNfL | n = 106 MS subjects | n = 157, whole sample |
|---|---|---|
| effect size $f^2$ | 0.03 | 0.14 |
| α error probability | 0.05 | 0.05 |
| total sample size | 106 | 157 |
| number of predictors | 7 | 4 |
| noncentrality parameter λ | 2.72 | 22.63 |
| critical F | 2.10 | 2.43 |
| numerator df | 7 | 4 |
| denominator df | 98 | 152 |
| power (1 − β error probability) | 0.166 | 0.978 |