## Supplemental Materials

# Increasing serum neurofilament and glial fibrillary acidic protein after discontinuing multiple sclerosis treatment predicts future disease activity

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## eTable 1. Comparison of eligible patients with and without serum samples available

| | Serum available (included) (N=78) | Serum not available (excluded) (N=59) | p-value |
|---|---|---|---|
| Treatment-stop, calendar year, median (min, max) | 2013 (2005, 2019) | 1995 (1980, 2018) | <0.001 |
| Female, No. (%) | 72 (92%) | 46 (78%) | 0.016 |
| Age at first symptoms, median (IQR) | 32.1 (25.2, 41.8) | 27.7 (22.5, 34.2) | 0.003 |
| Age at treatment-stop, median (IQR) | 48.7 (38.8, 55.7) | 40.6 (32.6, 47.6) | <0.001 |
| Disease duration at treatment-stop, years, median (IQR) | 12.9 (7.5, 19.2) | 11.0 (6.0, 16.0) | 0.16 |
| Years from closest attack to treatment-stop, median | -6.5 (-12.6, -4.2) | -10.0 (-16.0, -5.5) | 0.036 |
| EDSS at treatment-stop, median (IQR) | 1.5 (1.0, 2.5) | 4.0 (3.0, 6.5) | <0.001 |

## eTable 2. Multivariate time-to-event outcomes including MRI Lesion Burden (N=76)

| | 6-month CDW | New attack | New MRI activity |
|---|---|---|---|
| Pre-treatment-stop sNfL | 1.37 [0.59, 3.20]; p=0.458 | 1.54 [0.55, 4.13]; p=0.418 | 2.02 [0.84, 4.90]; p=0.119 |
| Post-treatment-stop sNfL | 6.36 [2.27, 17.8]; p<0.001 | 1.48 [0.54, 4.05]; p=0.440 | 3.26 [1.43, 7.47]; p=0.005 |
| Percent-change in sNfL | 1.88 [1.22, 2.89]; p=0.004 | 1.13 [0.69, 1.85]; p=0.633 | 1.36 [0.96, 1.92]; p=0.082 |
| | | | |
| Pre-treatment-stop sGFAP | 1.17 [0.04, 3.43]; p=0.771 | 2.98 [0.87, 10.2]; p=0.082 | 0.99 [0.37, 2.64]; p=0.979 |
| Post-treatment-stop sGFAP | 1.85 [0.53, 6.51]; p=0.337 | 2.73 [0.72, 10.4]; p=0.140 | 2.49 [0.77, 8.07]; p=0.128 |
| Percent-change in sGFAP | 4.21 [0.78, 22.7]; p=0.094 | 0.88 [0.17, 4.52]; p=0.873 | 5.89 [1.39, 24.9]; p=0.016 |

Data shown as hazard ratio [95% confidence interval], and p-value.

Pre- and post-treatment-stop biomarker levels were log-transformed.

Percent-change was calculated from non-transformed levels where reported hazard ratios represent a doubling, or 100% increase.

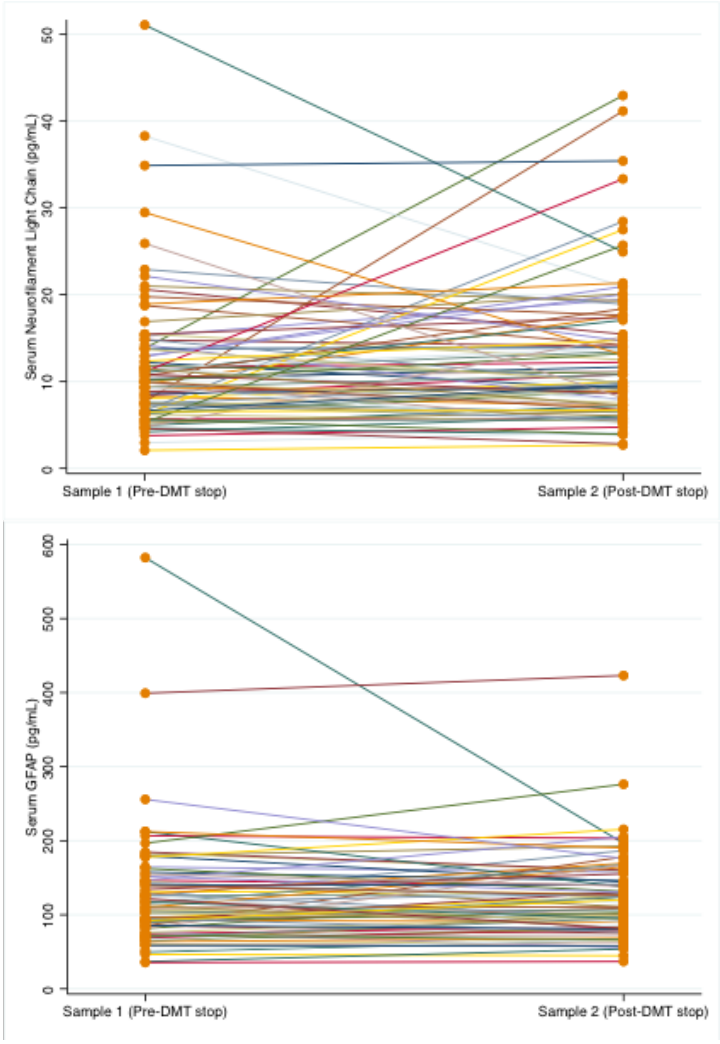
*Adjusted for covariates: age, disease duration, EDSS, and duration from last attack (at treatment-stop date), T2LV at time of discontinuation, as well as batch effect

*CDW = confirmed disability worsening; EDSS = expanded disability status scale; sNfL = serum neurofilament light chain; sGFAP = serum glial fibrillary acidic protein*

**eTable 3. Area Under Receiver Operating Characteristic Curve (AUC) analyses**

| | Liu cutpoint: | Sensitivity | Specificity | AUC |
|---|---|---|---|---|
| **Confirmed Disability Worsening** | | | | |
| Pre NFL (pg/mL) | 9.336 | 0.59 | 0.45 | 0.52 |
| Post NFL (pg/mL) | 11.04 | 0.56 | 0.59 | 0.57 |
| Change in NFL (%) | 1.22 | 0.63 | 0.51 | 0.57 |
| Pre GFAP (pg/mL) | 116.34 | 0.41 | 0.51 | 0.46 |
| Post GFAP (pg/mL) | 99.77 | 0.56 | 0.39 | 0.47 |
| Change in GFAP (%) | -1.81 | 0.63 | 0.51 | 0.57 |
| **New Attack** | | | | |
| Pre NFL (pg/mL) | 10.050 | 0.53 | 0.51 | 0.52 |
| Post NFL (pg/mL) | 7.27 | 0.74 | 0.27 | 0.50 |
| Change in NFL (%) | 19.05 | 0.42 | 0.63 | 0.52 |
| Pre GFAP (pg/mL) | 95.19 | 0.53 | 0.39 | 0.46 |
| Post GFAP (pg/mL) | 88.28 | 0.74 | 0.29 | 0.51 |
| Change in GFAP (%) | 3.20 | 0.63 | 0.58 | 0.60 |
| **MRI Activity** | | | | |
| Pre NFL (pg/mL) | 9.336 | 0.42 | 0.37 | 0.39 |
| Post NFL (pg/mL) | 14.95 | 0.35 | 0.73 | 0.54 |
| Change in NFL (%) | 19.94 | 0.42 | 0.67 | 0.55 |
| Pre GFAP (pg/mL) | 93.86 | 0.54 | 0.37 | 0.45 |
| Post GFAP (pg/mL) | 97.34 | 0.62 | 0.37 | 0.49 |
| Change in GFAP (%) | 2.47 | 0.69 | 0.62 | 0.65 |

Empirical optimal cutpoint determined by Liu test




## eFigure 1. Profile plots.

Each patient's pre-treatment-stop (sample 1) and post-treatment-stop (sample 2) levels of sNfL and sGFAP are shown. Out of 78 patients, 43 (55%) had an increase in sNfL, 41 (53%) had an increase in sGFAP, and 30 (38%) had an increase in both biomarkers (chi-squared P=0.001). There were 7 patients with a 100% increase in sNfL, and 5 patients with a 50% increase in sGFAP, of whom 3 patients had both.