# Supplementary Online Content

Schlaeger R, Papinutto N, Zhu AH, et al. Association between thoracic spinal cord gray matter atrophy and disability in multiple sclerosis. *JAMA Neurol*. Published online June 8, 2015. doi:10.1001/jamaneurol.2015.0993.



This supplementary material has been provided by the authors to give readers additional information about their work.

**eAppendix.** Methods

## Inclusion and exclusion criteria of the participants

Inclusion criteria were: 1) a diagnosis of MS according to International Panel criteria;[1] and 2) age ≥18 years. Exclusion criteria were: 1) ≥one clinical relapse within 4 weeks prior to the visit; 2) the use of glucocorticoids within 4 weeks prior to the MRI exam; 3) a recent history or suspicion of current drug or alcohol abuse; 4) a diagnosis of hepatitis B, C or HIV; 5) participation in ongoing MS trials with unlicensed drugs; and 6) any concurrent illness, disability or clinically significant abnormality (including laboratory tests) that would prevent the subject from safely completing the assessments.

## MRI Acquisition parameters

All participants were scanned at the same 3T Siemens Skyra scanner with a 20-channel neck-head coil and a 32-channel spine coil.

Spinal cord 2D-PSIR: axial acquisition, in-plane resolution = 0.78 x 0.78 $mm^2$, slice thickness = 5mm, matrix 256 x 256, TR = 4000ms, TE = 3.22ms, TI = 400ms, angle 10°, 3 averages. Scan time was 1:50 min per level.

Brain MPRAGE: sagittal acquisition, $1mm^3$ isotropic voxel, TR = 2300ms, TE = 2.98ms, TI = 900ms, angle 9°.

Brain 3D- FLAIR: sagittal acquisition, $1mm^3$ isotropic voxel, TR = 5000ms, TE = 389ms, TI = 1800ms.

Cervical spinal cord T2-weighted images: sagittal acquisition: 0.72 x 0.72 $mm^2$, slice thickness = 1.2 mm, FOV = 230 x 230$mm^2$, TR = 5280ms, TE = 85ms. Axial acquisition: 0.62 x 0.62$mm^2$, slice thickness = 3 mm, TR = 4000ms, TE = 92ms.

Thoracic spinal cord T2-weighted images: sagittal acquisition. 0.68 x 0.68$mm^2$, slice thickness = 2 mm, FOV = 300 x 300$mm^2$, TR = 4290ms, TE = 90ms.

## Analysis of brain MPRAGE, FLAIR and spinal cord T2-images

An MS neuroradiologist (MB), who was masked to both clinical information and the PSIR images, performed the SC lesion count on the sagittal and axial T2-weighted images of the cervical SC and on the sagittal and axial reformatted T2-weighted images of the thoracic SC. Cortical reconstruction and segmentation of the cerebral MPRAGE data sets were performed using the Freesurfer image analysis suite (http://surfer.nmr.mgh.harvard.edu/), and normalized brain GM and WM volumes, and brain T1-lesion load were determined. FLAIR-lesion loads were measured through a semiautomatic procedure utilizing AMIRA 5.4, Mercury Computer Systems, Chelmsford, Mass).

## Statistical analysis

### Receiver Operator Characteristic (ROC) curve and partial linear regression analyses

ROC curves were compiled to assess sensitivity and specificity for the prediction of a progressive disease course given by binary logistic models based on 1) the SC GM areas at each level, 2) the combination of cervical and thoracic cord GM areas, 3) the normalized brain GM volume and 4) the combination of normalized brain GM volume, cervical and thoracic SC GM areas. The respective areas under the curve and their 95% confidence intervals were calculated. Sensitivity and specificity for the prediction of a progressive disease course were determined for the model with the highest AUC at the maximal index of Youden.

In a further analysis we examined value added by T9/T10 SC GM area in correlating with the EDSS while adjusting for C2/C3 SC GM area, SC WM areas, normalized brain GM and WM volumes, age, disease duration, T1-lesion volume, total number of lesions using partial linear regression analysis. The levels of T9/T10 and C2/C3 were selected for this analysis because they had previously been shown to have the highest measurement reliability in the thoracic and cervical cord,[2] and, in addition, demonstrated consistently good image quality in the present cohort. The strength of this net relationship is presented in terms of the slope of a linear regression line relating residual variation of cervical GM area to that in EDSS while accounting for all other variables. Additionally, this net effect is presented using relative importance metrics based on a multivariable regression model given that thoracic GM area is added last to the model.

The same analyses were performed to examine an added value of the cervical GM area at C2/C3 in explaining EDSS variance.
Statistical analysis was performed using IBM SPSS Statistics, Version 21, IBM Cooperation, 2012, JMP Statistics (www.jmp.com), Version 11, 2013 SAS Institute and R, R Foundation, http://www.r-project.org.

## eReferences

**1.** Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 2011; 69: 292–302.

**2.** Papinutto N, Schlaeger R, Panara V, Caverzasi E, Ahn S, Johnson KJ, et al. 2D phase-sensitive inversion recovery imaging to measure in vivo spinal cord gray and white matter areas in clinically feasible acquisition times. J Magn Reson Imaging 2014; doi: 10.1002/jmri.24819.

**3.** Lublin FD, Reingold SC, Cohen JA, Cutter GR, Sørensen PS, Thompson AJ, et al. Defining the clinical course of multiple sclerosis: the 2013 revisions. Neurology. 2014 Jul 15;83(3):278-86.

**eTable 1.** Demographic and Clinical Characteristics of Relapsing and Progressive MS Patients and Control Individuals

| | Relapsing MS[3] (n=99) | Progressive MS[3] (n=43) | Controls (n=20) |
|---|---|---|---|
| Subtype | | SPMS: n=31<br>PPMS: n=12 | |
| Age (mean, SD)<br>*in years* | 48.5 +/- 9.5 | 56.5 +/- 10.2 | 48.6 +/-12.2 |
| Sex (f:m) | 63/36 | 23/20 | 12/8 |
| EDSS<br>(median) | 2.0 | 6 | n/a |
| Disease Duration<br>(mean, SD)<br>*in years* | 15.2 +/- 8.3 | 20.6 +/-10.7 | n/a |

PPMS: primary progressive MS, SPMS: secondary progressive MS. EDSS: Expanded Disability Status Scale. SD: standard deviation.

**eTable 2.** Spearman Rank Correlations Between PSIR Imaging–Derived Cord Measures and Clinical Measures

| Parameter | | EDSS | 9-HPT | T25FW |
|---|---|---|---|---|
| C2/C3 | Cord area | **-0.48** p<0.001 | **-0.30** p<0.001 | **-0.44** p<0.001 |
| | GM area | **-0.64** p<0.001 | **-0.41** p<0.001 | **-0.56** p<0.001 |
| | WM area | **-0.36** p<0.001 | -0.17 p=0.058 | **-0.32** p<0.001 |
| C3/C4 | Cord area | **-0.45** p<0.001 | **-0.36** p<0.001 | **-0.40** p<0.001 |
| | GM area | **-0.63** p<0.001 | **-0.47** p<0.001 | **-0.50** p<0.001 |
| | WM area | **-0.30** p=0.001 | -0.20 p=0.041 | **-0.32** p=0.001 |
| T8/T9 | Cord area | **-0.41** p<0.001 | -0.21 p=0.013 | **-0.43** p<0.001 |
| | GM area | **-0.47** p<0.001 | -0.23 p=0.012 | **-0.37** p<0.001 |
| | WM area | **-0.33** p<0.001 | -0.16 p=0.095 | **-0.40** p<0.001 |
| T9/T10 | Cord area | **-0.40** p<0.001 | -0.18 p=0.055 | **-0.48** p<0.001 |
| | GM area | **-0.48** p<0.001 | -0.13 p=0.209 | **-0.54** p<0.001 |
| | WM area | **-0.37** p<0.001 | -0.11 p=0.303 | **-0.43** p<0.001 |

GM: gray matter, WM: white matter, EDSS: Expanded Disability Status Scale, T25FW: Timed 25 Foot Walk Test, 9-HPT: Nine Hole Peg Test. 2-D PSIR images were acquired at the inter-vertebral disc levels. P-values are 2-sided and uncorrected for multiple comparisons. Tests that survived the Bonferroni correction are bolded.

**eTable 3.** Spearman Rank Correlations Between PSIR Imaging–Derived Cord Measures and Their Corresponding Measures at Lower Levels

| Parameter | | C2-C3 | C3-C4 | T8-T9 | T9-T10 |
|---|---|---|---|---|---|
| C2-C3 | Cord area | **1** | **0.91** (p<0.001) | **0.81** (p<0.001) | **0.78** (p<0.001) |
| | GM area | **1** | **0.75** (p<0.001) | **0.53** (p<0.001) | **0.62** (p<0.001) |
| | WM area | **1** | **0.86** (p<0.001) | **0.77** (p<0.001) | **0.75** (p<0.001) |
| C3-C4 | Cord area | | **1** | **0.79** (p<0.001) | **0.77** (p<0.001) |
| | GM area | | **1** | **0.63** (p<0.001) | **0.56** (p<0.001) |
| | WM area | | **1** | **0.77** (p<0.001) | **0.73** (p<0.001) |
| T8-T9 | Cord area | | | **1** | **0.90** (p<0.001) |
| | GM area | | | **1** | **0.58** (p<0.001) |
| | WM area | | | **1** | **0.87** (p<0.001) |

GM: spinal cord gray matter, WM: spinal cord white matter. P-values are 2-sided.

**eTable 4.** Comparison of Cervical PSIR Imaging Derived Measures Between Patients With Relapsing (RMS) and Progressive Disease Courses (PMS) Using Linear Regression With Disease Duration and Sex as Covariates

| Disc level | PSIR measure | Group | Adj. mean | StdErr (mean) | Mean diff. | StdErr (diff.) | P | 95%-Confidence Interval (diff.) | |
|---|---|---|---|---|---|---|---|---|---|
| C2/C3 | Mean GM area ($mm^2$) | RMS<br>PMS | 18.14<br>15.03 | 0.23<br>0.36 | 3.12 | 0.43 | **<0.0001** | 2.27 | 3.97 |
| | Mean WM area ($mm^2$) | RMS<br>PMS | 59.16<br>55.52 | 0.74<br>1.15 | 3.64 | 1.39 | **0.0096** | 0.90 | 6.39 |
| | TCA ($mm^2$) | RMS<br>PMS | 77.18<br>68.52 | 0.93<br>1.39 | 8.66 | 1.69 | **<0.0001** | 5.31 | 12.01 |
| C3/C4 | Mean GM area ($mm^2$) | RMS<br>PMS | 21.19<br>17.10 | 0.30<br>0.47 | 4.09 | 0.56 | **<0.0001** | 2.97 | 5.20 |
| | Mean WM area ($mm^2$) | RMS<br>PMS | 59.51<br>55.22 | 0.88<br>1.40 | 4.29 | 1.68 | **0.0121** | 0.96 | 7.62 |
| | TCA ($mm^2$) | RMS<br>PMS | 80.12<br>71.70 | 1.08<br>1.64 | 8.41 | 1.99 | **<0.0001** | 4.46 | 12.36 |
| T8/T9 | Mean GM area ($mm^2$) | RMS<br>PMS | 10.17<br>9.16 | 0.14<br>0.23 | 1.02 | 0.28 | **0.0003** | 0.47 | 1.56 |
| | Mean WM area ($mm^2$) | RMS<br>PMS | 31.88<br>30.98 | 0.43<br>0.74 | 0.90 | 0.87 | 0.3020 | -0.82 | 2.62 |
| | TCA ($mm^2$) | RMS<br>PMS | 42.26<br>39.00 | 0.53<br>0.81 | 3.26 | 0.98 | **0.0012** | 1.32 | 5.21 |
| T9/T10 | Mean GM area ($mm^2$) | RMS<br>PMS | 11.13<br>9.57 | 0.17<br>0.29 | 1.56 | 0.34 | **<0.0001** | 0.88 | 2.24 |
| | Mean WM area ($mm^2$) | RMS<br>PMS | 32.78<br>31.46 | 0.45<br>0.78 | 1.32 | 0.90 | 0.1479 | -0.48 | 3.12 |
| | TCA ($mm^2$) | RMS<br>PMS | 43.76<br>41.04 | 0.54<br>0.83 | 2.72 | 1.00 | **0.0075** | 0.74 | 4.71 |

Mean values are least square means with adjustment for disease duration and sex. Mean differences refer to the differences between RMS and PMS patients. Adj.: adjusted; StdErr: standard error, Diff.: difference between means, TCA: total cord area, GM: gray matter, WM: white matter area. P-values are 2-sided.

**eTable 5.** Analysis of Added Value of Thoracic to Cervical Spinal Cord GM Area (and Vice Versa) in Explaining the Observed Variability in EDSS Based on the Partial Regression Modeling and Analysis of Relative Importance. The bivariable model involves both thoracic and spinal cord GM areas. Multivariable models in addition to including cervical and thoracic spinal cord GM areas also include the following variables: spinal cord WM areas at cervical and thoracic levels, normalized brain GM volume, normalized brain WM volume, brain T1 lesion volume, number of spinal cord lesions, age and disease duration.

| **Spinal cord GM area** | **Bivariable analysis** | | **Multivariable analysis** (including also spinal cord WM areas at cervical and thoracic levels, normalized brain GM volume, normalized brain WM volume, brain T1 lesion volume, number of spinal cord lesions, age and disease duration.) | | |
|---|---|---|---|---|---|
| | **Slope (SE)** | **p-value** | **Slope (SE)** | **p-value** | **Relative importance** of variable when added last in the model (95% Confidence Interval) |
| C**2**/C**3** | **-0.31** (0.01) | <0.001 | **-0.35** (0.01) | <0.001 | **0.68** (0.17, 0.79) |
| T**9**/T**10** | **-0.22** (0.11) | 0.047 | **-0.22** (0.01) | 0.047 | **0.12** (0.001, 0.16) |

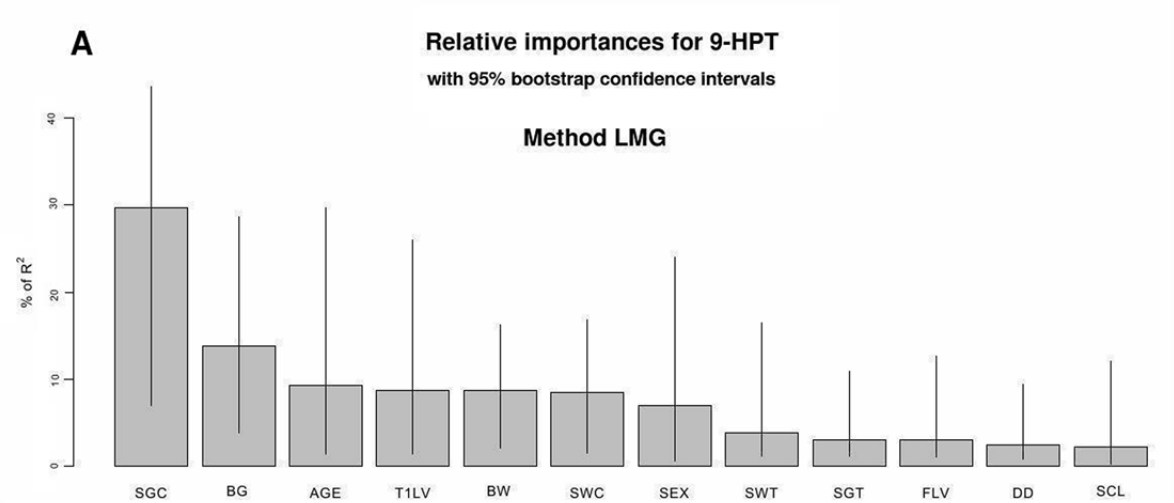
GM: gray matter. WM: white matter. SE: standard error.

**eTable 6.** Receiver Operating Characteristic (ROC) Curves for the Prediction of a Progressive Versus Relapsing Disease Course by Binary Logistic Models, with the spinal cord GM areas at each level as single predictors, the combination of cervical and thoracic spinal cord GM areas, and the combination of normalized brain GM volume with cervical and thoracic spinal cord GM areas as combined predictors.

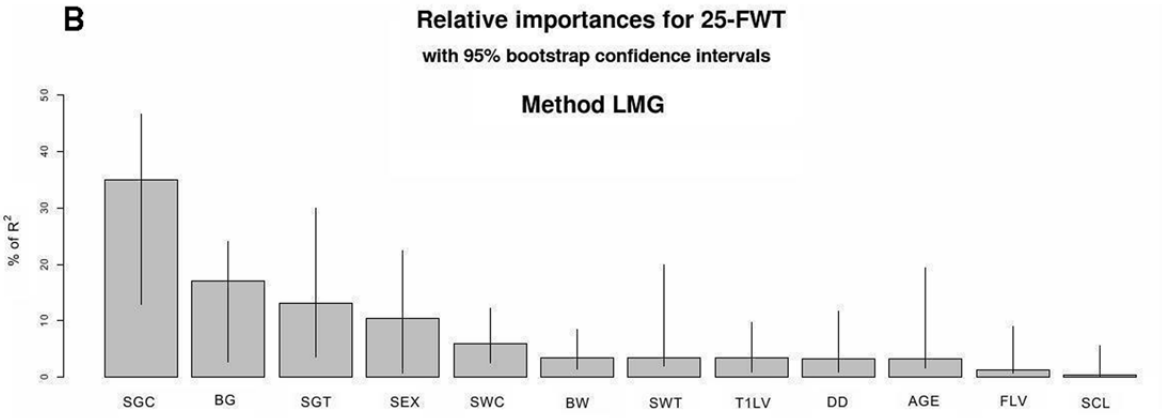
| Parameter | Area under the curve | Standard error (non-parametric) | 95% Confidence interval | |
|---|---|---|---|---|
| C2/C3 GM area | 0.835 | 0.049 | 0.739 | 0.931 |
| C3/C4 GM area | 0.832 | 0.058 | 0.719 | 0.945 |
| T8/T9 GM area | 0.717 | 0.061 | 0.596 | 0.837 |
| T9/T10 GM area | 0.778 | 0.060 | 0.661 | 0.896 |
| C2/C3 and T8/T9 GM areas | 0.838 | 0.047 | 0.745 | 0.930 |
| C2/C3 and T9/T10 GM areas | 0.842 | 0.047 | 0.749 | 0.934 |
| C3/C4 and T8/T9 GM areas | 0.827 | 0.058 | 0.713 | 0.941 |
| C3/C4 and T9/T10 GM areas | 0.839 | 0.055 | 0.730 | 0.947 |
| Normalized brain GM volume | 0.679 | 0.066 | 0.550 | 0.808 |
| T9/T10 GM area and normalized brain GM volume | 0.793 | 0.061 | 0.674 | 0.912 |
| C2/C3, T9/T10 GM areas and normalized brain GM volume | 0.874 | 0.040 | 0.795 | 0.953 |

GM: Gray matter

**eFigure 1.** Relative contributions of the variables (cervical spinal cord gray matter [SGC] and white matter [SWC] areas at C2/C3, thoracic spinal cord gray matter [SGT] and white matter [SWT] at T9/T10, normalized brain gray matter volume [BG] and normalized brain white matter volume [BW], the number of spinal cord T2 lesions [SCL], brain T1 lesion volume [T1LV], brain fluid-attenuated inversion recovery lesion volume [FLV], age, sex, and disease duration [DD]) to the 9 Hole Peg Test (9-HPT) (A) and Timed 25 Foot Walk Test (25-FWT) (B) using a linear model.




$R^2$ = 29.48%, metrics are normalized to sum 100%




$R^2$ = 30.07%, metrics are normalized to sum 100%

**eFigure 2.** Receiver Operating Characteristic (ROC) Curves for the Prediction of a Progressive Versus Relapsing Disease Course by Binary Logistic Models, with the spinal cord GM areas at each level as single predictors (A), and the combination of cervical and thoracic GM areas (B) as combined predictors. The areas under the curve were 0.84, 0.83, 0.72 and 0.79 for the prediction based on the spinal cord GM areas at the levels C2/C3, C3/C4, T8/T9 and T9/T10 as single predictors, respectively. The areas under the curve for the combined prediction models based on cervical and thoracic GM areas were as follows: C2/C3 and T9/T10: 0.84; C2/C3 and T8/T9: 0.84; C3/C4 and T9/T10: 0.84; and C3/C4 and T8/T9: 0.83.