**Supplementary Appendix 2**

**(Imaging)**

**MR Image Acquisition**

All MRI scans analyzed in the study were performed on the same scanner (1.5-Tesla Gyroscan; Philips Medical Systems, Best, the Netherlands) in the Department of Radiology at General University Hospital in Prague with the same protocol. The MRI scanner did not undergo a major hardware upgrade during the study follow-up (in 2001: minor software upgrade; in 2004: minor hardware upgrade of gradients not affecting volumetric analysis). The standardized protocol consisted of two sequences: fluid-attenuated inversion recovery (FLAIR) and T1-weighted 3-dimensional turbo field echo (T1-WI/TFE 3D). Contiguous slices covering the whole-brain were acquired with the following parameters: FLAIR sequence: time to echo (TE) = 140 milliseconds, time to repetition (TR) 11,000 milliseconds, inversion time (TI) 2,600 milliseconds, matrix size 256 × 181, flip angle (FA) 90°, slice thickness (THK) 1.5/0 mm (with no gaps), field of view (FOV) = 256 mm, and T1-WI/TFE 3D (TE/TR: 5/25 milliseconds, FA = 30°, matrix size 256 × 256, THK1.0/0 mm, FOV = 256 mm). All MRI analyses underwent quality control and were reviewed by a trained operator (J.K. in Prague and N.B. in Buffalo) at all critical points of segmentation.

**MR Image Analysis**

Volumetric assessment was performed independently in the Department of Radiology, First Faculty of Medicine and General University Hospital in Prague, Charles University, Czech Republic, and in the Buffalo Neuroimaging Analysis Center, NY, USA. Image analysis was performed in the Department of Radiology, General University Hospital in Prague with the ScanView software. ScanView is a semiautomated software tool for measurement of lesion volume, brain parenchymal fraction, WB, and corpus callosum volumes via segmentation-based techniques.1 T2 lesion volume was measured from the FLAIR sequence. Following standard image processing, T2 lesions were defined to consist of voxels with intensity >140% of the mean of white matter and having a minimum size of 11 voxels (corresponding to a sphere with a 3-mm diameter). Correlation between T2 volumes measured by ScanView and Jim software (http://www.xinapse.com) was high (Spearman´s rho=0.90; p<1x10-10). Detailed processing steps were described elsewhere.1 WB volume was measured from the T1-WI/FFE 3D sequence. Non-normalized, absolute whole-brain volume was measured on T1-WI thresholded at above 4,000 arbitrary units. ScanView provides only nonnormalized, absolute values of whole-brain volumes. Therefore, whole-brain volumes were normalized with respect to the total intracranial volume (ICV). ICV was calculated as the sum of the total brain parenchymal volume and the total intraventricular and subarachnoidal cerebrospinal fluid volume. Normalized compartment volumes were calculated as follows: brain parenchymal fraction = whole-brain volume/ICV. Volumetric assessment of T2 lesions and brain volumes, as was performed using semi-automated ScanView software. For longitudinal changes of the whole-brain volume, we applied the SIENA method.2 Lesion filling on T1-WI 3D images was utilized to reduce the impact of T1 hypointensities on tissue segmentation.3 We found strong correlation between the rates of whole-brain % volume changes analyzed by SIENA in Prague and BNAC (Number of longitudinal measures: 1,221; Pearson´s r=0.97; p<0.0001).

In MRI scans from the ASA and SET cohorts (348 patients; 1,569 MRI scans), we performed validation analysis in the Buffalo Neuroimaging Analysis Center, NY, USA. Deep grey matter and thalamic volume were analyzed by FMRIB´s Integrated Registration and Segmentation Tool (FIRST)4 and white matter, total grey matter, cortical grey matter and lateral ventricle volume by SIENAX software (version 2.6) from FSL,5 with corrections for T1-hypointensity misclassification using an in-house developed method.6

**References:**

1. Uher T, Krasensky J, Vaneckova M, et al. A Novel Semiautomated Pipeline to Measure Brain Atrophy and Lesion Burden in Multiple Sclerosis: A Long-Term Comparative Study. J Neuroimaging 2017;27:620-629.

2. Smith SM, Zhang Y, Jenkinson M, et al. Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. Neuroimage 2002;17:479-489.

3. Gelineau-Morel R, Tomassini V, Jenkinson M, Johansen-Berg H, Matthews PM, Palace J. The effect of hypointense white matter lesions on automated gray matter segmentation in multiple sclerosis. Hum Brain Mapp 2012;33:2802-2814.

4. Patenaude B, Smith SM, Kennedy DN, Jenkinson M. A Bayesian model of shape and appearance for subcortical brain segmentation. Neuroimage 2011;56:907-922.

5. Dwyer MG, Bergsland N, Zivadinov R. Improved longitudinal gray and white matter atrophy assessment via application of a 4-dimensional hidden Markov random field model. Neuroimage 2014;90:207-217.

6. Battaglini M, Jenkinson M, De Stefano N. Evaluating and reducing the impact of white matter lesions on brain volume measurements. Hum Brain Mapp 2012;33:2062-2071.