**Abstract**

**Objective/Background:** The majority of multiple sclerosis patients experience impaired walking ability, which impacts quality of life. Timed 25-foot walk is commonly used to gauge gait impairment but results can be broadly variable. Objective biological markers that correlate closely with patients’ disability are needed. Diffusion tensor imaging, quantifying fiber tract integrity, might provide such information. In this project we analyzed relationships between timed 25-foot walk, conventional and diffusion tensor imaging magnetic resonance imaging markers.

**Design/Methods:** A cohort of gait impaired multiple sclerosis patients underwent brain and cervical spinal cord magnetic resonance imaging. Diffusion tensor imaging mean diffusivity and fractional anisotropy were measured on the brain corticospinal tracts and spinal restricted field of vision at C2/3. We analyzed relationships between baseline timed 25-foot walk, conventional and diffusion tensor imaging magnetic resonance imaging markers.

**Results:** Multivariate linear regression analysis showed a statistically significant association between several magnetic resonance imaging and diffusion tensor imaging metrics and timed 25-foot walk: brain mean diffusivity corticospinal tracts ($p = 0.004$), brain corticospinal tracts axial and radial diffusivity ($P = 0.004$ and 0.02), grey matter volume ($p = 0.05$), white matter volume ($p = 0.03$) and normalized brain volume ($P = 0.01$). The linear regression model containing mean diffusivity corticospinal tracts and controlled for gait assistance was the best fit model ($p = 0.004$).

**Conclusions:** Our results suggest an association between diffusion tensor imaging metrics and gait impairment, evidenced by brain mean diffusivity corticospinal tracts and timed 25-foot walk.

**Keywords:** Multiple sclerosis, gait, diffusion tensor imaging, biological marker

Date received: 11 January 2016; accepted: 24 May 2016
Diffusion tensor imaging (DTI) measures diffusional (Brownian) motion of water molecules, yielding information about the integrity of white matter (WM) tracks.\textsuperscript{9} The combination of mean diffusivity (MD) and index of fractional anisotropy (FA), derived from tensor calculations, provides more sensitive information regarding potential interruption of neural pathways and demyelination than can be seen on conventional MRI sequences.\textsuperscript{10,11} The role and utility of DTI in MS has previously been studied in the brain.\textsuperscript{9,12,13} For example, there is a well-established significant correlation between brain DTI and conventional MRI metrics, such as T2 lesion volume, grey matter (GM) and WM volume. Brain DTI metrics have also been shown to correlate with level of disability as measured by the EDSS and MS Functional Composite (MFSC).\textsuperscript{14,15} However, fewer studies have evaluated DTI metrics in the spinal cord, and T25FW has not been widely reported as a separate outcome measure of clinical disability.\textsuperscript{15,16}

To develop initial pilot data in the field of imaging biomarkers of clinical disability this project aimed to identify the relationship between T25FW and both conventional MRI and DTI metrics. We hypothesized that characterization of neural pathways with DTI metrics will be associated more closely with T25FW impairment than will conventional MRI metrics.

Methods
This study was approved by the institutional review board of the Icahn School of Medicine at Mount Sinai and all participants provided informed consent.

Participants
Patients with a diagnosis of relapsing–remitting multiple sclerosis (RRMS), secondary progressive multiple sclerosis (SPMS) and primary progressive multiple sclerosis (PPMS) based on current diagnostic criteria\textsuperscript{17,18} were enrolled at the Corrine Goldsmith Dickinson Center for Multiple Sclerosis. Eligible subjects were all participants in a previously established database of gait impaired patients we intended to treat with dalfampridine. Additional eligibility criteria for this study included treatment with dalfampridine for 3 months or longer, start of dalfampridine treatment within 2 years from the enrolment and at least two T25FW measurements within 2 years before dalfampridine treatment. Of a total of 61 patients included in the database, 41 were excluded from this study because of: (a) dalfampridine treatment duration of less than 3 months (10 patients); (b) insufficient T25FW measurements (seven patients); (c) dalfampridine start more than 2 years before enrolment (21 patients); and (d) patients with uncertain MS diagnosis (three patients). Thirteen of 18 eligible patients agreed to participate, and after providing informed consent underwent MRI of the brain and cervical spine including DTI scans between October 2013 and March 2014.

The timed 25 foot walk
The T25FW data were extracted from the medical records. This measure is obtained during each routine visit, using a standardized test track, in accordance with methods described in the Administration and Scoring Manual published by the National Multiple Sclerosis Society.\textsuperscript{19} Mean T25FW time was calculated using data obtained during at least two separate clinical visits. For this project we used only dalfampridine pre-treatment baseline T25FW data.

MRI acquisition
All brain and cervical spinal cord MRI studies were performed on a 3-Tesla scanner (Magnetom Skyra, Siemens Healthcare, Erlangen, Germany), using a 20-channel head and spine coil. The brain MRI protocol included: (a) axial T2 fluid-attenuated inversion recovery (FLAIR) (TR/TE/TI: 9000/96/2504 ms; field of vision (FOV): 201 × 230; matrix: 280 × 320; yielding 50 contiguous 3-mm-thick slices); (b) sagittal 3D T1 MPRAGE (TR/TE/TI: 2400/2.1/1000 ms; yielding 0.9 × 0.9 × 0.9 mm\textsuperscript{3} isotropic voxels); (c) single-shot echo planar imaging (EPI) sequence for DTI (TR/TE: 11300/91 ms; b-values: 0, 1000 s/mm\textsuperscript{2}, 20 non-collinear directions, pixel size: 1.95 × 1.95 mm\textsuperscript{2} yielding 50 contiguous, axial 3-mm-thick slices). The cervical spine MRI protocol included: (a) sagittal short tau inversion recovery (STIR) (TR/TE: 5840/37 ms; FOV: 236 × 140; matrix: 192 × 227; yielding 15 contiguous 4-mm-thick slices); (b) axial T2 MEDIC (TR/TE: 757/14 ms; FOV: 180 × 180; matrix: 320 × 356; yielding 30 contiguous 3-mm-thick slices); (c) sagittal 3D T1 MPRAGE (TR/TE/TI: 1670/2.1/1000 ms; yielding 1 × 1 × 1 mm\textsuperscript{3} isotropic voxels); (d) axial single-shot EPI for DTI (TR/TE: 4100/75 ms;
b-values: 0, 500 s/mm\(^2\) 20 non-collinear directions, pixel size: \(1.37 \times 1.37\) mm\(^2\); yielding 30 contiguous, axial 3 mm-thick slices).

**MRI analysis**

**Lesion volume assessment.** The T2-hyperintense and T1-hypointense lesion volumes were measured by experienced assessors using a semi-automated segmentation technique based on user-supervised local thresholding (Jim version 3; Xinapse Systems, Northants, England).\(^{20}\) The T2-weighted lesion area was measured by determining lesions’ borders on FLAIR images. The T1-hypointense lesions were defined as lesions with signal intensity between cerebrospinal fluid (CSF) and GM using T1-weighted scans. The total brain T2 and T1 lesion volume was calculated by multiplying lesion area by slice thickness.

**Brain volume assessment.** The normalized brain volume, and normalized GM and WM volumes were measured on lesion-filled T1-weighted image using SIENAX,\(^{21}\) part of FMRIB Software Library (FSL).\(^{22}\) Briefly, SIENAX performs brain extraction, followed by tissue segmentation to separate GM, WM and CSF. A normalization factor is calculated based on the subject’s skull size and is applied to the calculated volumes.

**Cervical spinal cord lesion load, volume and cross-sectional area.** Spinal cord lesions hyperintense to normal-appearing cord tissue were detected and counted on T2-weighted scans by a neuroradiologist. Spine volume (SV) and cervical cord cross-sectional area were measured on the STIR scan from C2 to C3. The calculated mean FA and MD within the top five slices, roughly corresponding to C2–C3. The calculated mean FA and MD within these masks were used for statistical analysis (Figure 2).

**Cervical spinal cord DTI.** As described for the brain, diffusion-weighted images were first corrected for eddy current distortion and head movements by affine registration to the first volume \((b = 0)\). Then, coarse masks of the spine were drawn by hand and the diffusion tensor was fitted at each voxel within the mask by DTIFIT, resulting in MD and FA maps of the spine. Next, we created more conservative masks of the spine, based on the FA map, of the top five slices, roughly corresponding to C2–C3. The calculated mean FA and MD within these masks were used for statistical analysis (Figure 2).

**Statistical analysis**

All statistical analyses were performed using IBM SPSS Statistics 22 (Chicago, Illinois, USA). \(P\) values of less than 0.05 were considered statistically significant. Participants’ demographic data and imaging data were characterized using descriptive statistics. Distribution of data was tested using the Shapiro–Wilk normality test. Association between basic demographic data and T25FW was assessed using Pearson correlation analysis, \(t\)-test and analysis of variance (ANOVA). Multivariate linear regression was used to identify relationships between T25FW and MRI data. We first constructed graphic plots of all independent variables and T25FW to inspect the linearity of relationships visually. We then constructed linear regression models for each imaging metric individually to identify the model with the best fit.

www.sagepub.com/msjetc

Klineova et al.
Results

Baseline demographic characteristics

Sixty-one patients, representing the full cohort of databased dalfampridine-treated patients, were screened for this project. Eighteen eligible patients were approached and 13 agreed to participate. Baseline clinical characteristics are summarized in Table 1. Our sample was composed of 54% women and 69% Caucasians, with a mean age of 53 years. Mean duration of disease was 11 years, 61% patients had a progressive disease course, and gait assistive devices were used by 46% of patients (Table 1).

Gait and imaging assessments

Table 2 illustrates pre-treatment baseline T25FW results in our cohort. Mean T25FW was 8.26 seconds (range 4.75–12.50) with normal distribution of data points. Table 3 summarizes conventional MRI parameters, which included T2 lesion volume (T2LV) and T1 lesion volume (T1LV); GM, WM and normalized brain volume (NBV), cervical cord lesion load and cervical cord volume at C2/3 (CCV). Mean T2LV was 5.51 mL (SD 5.54), mean T1LV was 2.95 mL (SD 3.35). Mean NBV was 1415.49 mL (SD 88.06) and CCV was 164.79 mL (SD 19.51) (Table 3).

Figure 1. The fractional anisotropy (FA) map with overlaying corticospinal tract (CST) mask derived from the JHU white matter tractography atlas. Mean FA values were calculated within each CST.

Figure 2. The spine fractional anisotropy (FA) map with overlaying principal vector map. This was used to identify the main direction of the tracts. The manual C2/C3 mask was used to obtain mean spine FA and mean diffusivity values.
MD and FA were measured in brain CSTs and in the cervical cord at C2/3 region. Axial and radial diffusivity were measured in brain CST. Table 4 further depicts the DTI metrics in our sample. Mean brain CST FA was 0.53 (SD 0.038), mean CST MD was $0.8 \times 10^{-3}$ $\mu$m$^2$/ms (SD 0.06). Mean brain CST axial diffusivity was $1.32 \text{mm}^2$/s $\times 10^{-3}$ (SD 0.06) and mean brain CST radial diffusivity was $0.54 \text{mm}^2$/s $\times 10^{-3}$ (SD 0.06). Mean spinal FA was 0.68 (SD 0.04) and mean MD was $1.08 \times 10^{-3}$ $\mu$m$^2$/ms (SD 0.087) (Table 4).

Association between T25FW and baseline clinical characteristics (age, gender, race, disease duration, type of MS and gait assistance) were assessed using t-test and ANOVA. Only disease type (RRMS, SPMS or PPMS) and type of gait assistance (none, unilateral or bilateral) were significantly associated with T25FW ($p = 0.05$ and 0.003, respectively).

The relationship between T25FW, conventional MRI and DTI metrics was assessed using multivariate linear regression while controlling for gait assistance as a potential confounder. Graphic representations of the univariate association between independent variables (brain and C spine conventional and DTI metrics) and T25FW are depicted in Figures 3–5. Multivariate linear regression analysis showed a statistically significant association between the following MRI and DTI metrics and T25FW: brain MD CST ($p = 0.004$), brain CST axial and radial diffusivity ($p = 0.004$ and 0.02), GM volume ($p = 0.05$), WM volume ($p = 0.03$) and NBV ($p = 0.01$). The linear regression model containing MD CST and controlled for gait assistance was the best fit model ($p = 0.004$) (Table 5).

Discussion

This study evaluated relationships between T25FW and conventional and DTI MRI metrics, thus contributing to existing data exploring the role of advanced imaging techniques in MS.

The novelty of our approach lies in the attempt to identify the relationship between modern neuroimaging techniques and an objective measure of gait impairment, noting that only a few previous studies utilized T25FW in this way. Our analysis of the association between T25FW and basic demographic characteristics showed a statistically significant correlation between MS clinical phenotype, gait assistive device and T25FW. These findings are in agreement with earlier work showing that substantial gait impairment was present in the majority of patients who transitioned into SPMS$^{28}$ and serves as an internal consistency marker for our database.

We found a statistically significant relationship between gait impairment as measured by the T25FW and measures of brain volume loss,
including grey, white and whole-brain atrophy. We did not find a relationship between T25FW and lesion metrics in the brain or spinal cord, nor with spinal cord volume. These findings are congruent with previous research showing, at most, a weak relationship between clinical disability and conventional MRI metrics in the brain or spinal cord.29,30 This aspect of our results further emphasizes

### Table 3. Conventional MRI metrics.

| Study subject | T2LV (mL) | T1LV (mL) | GM volume (mL) | WM volume (mL) | NBV (mL) | Cervical spine volume at C2–C3 (mL) | C spine lesion load (number) |
|---------------|-----------|-----------|----------------|----------------|---------|-----------------------------------|-----------------------------|
| 1             | 1.53      | 0.30      | 660.33         | 784.26         | 1444.59 | 203.76                            | 4                           |
| 2             | 1.43      | 0.64      | 804.80         | 739.22         | 1544.02 | 163.68                            | 1                           |
| 3             | 13.55     | 9.74      | 721.53         | 667.94         | 1389.47 | 166.02                            | 2                           |
| 4             | 9.21      | 4.23      | 681.29         | 696.54         | 1377.83 | 143.44                            | 1                           |
| 5             | 1.68      | 1.25      | 715.85         | 730.08         | 1445.94 | 163.86                            | 1                           |
| 6             | 6.19      | 4.15      | 648.73         | 669.65         | 1318.37 | 154.35                            | 2                           |
| 7             | 14.22     | 8.51      | 648.77         | 619.54         | 1268.31 | 148.99                            | 3                           |
| 8             | 2.40      | 1.87      | 676.06         | 669.35         | 1345.41 | 193.71                            | 3                           |
| 9             | 1.33      | 0.50      | 656.50         | 675.82         | 1332.32 | 166.74                            | 1                           |
| 10            | 0.45      | 0.23      | 809.30         | 746.77         | 1556.07 | 181.03                            | 1                           |
| 11            | 1.18      | 0.17      | 739.82         | 734.04         | 1473.86 | 150.31                            | 4                           |
| 12            | 15.01     | 6.31      | 661.36         | 747.52         | 1408.89 | 141.62                            | 2                           |
| 13            | 3.41      | 0.52      | 755.63         | 740.62         | 1496.25 | *                                 | *                           |

*Values not calculated.

MRI: magnetic resonance imaging; T1LV: T1 lesion volume; T2LV: T2 lesion volume; GM: grey matter; WM: white matter; NBV: normalized brain volume.

### Table 4. Non-conventional MRI metrics.

| Non-conventional metrics | Brain CST FA | Brain CST MD (μm²/ms) | Axial diffusivity CST (mm²/s × 10⁻³) | Radial diffusivity CST (mm²/s × 10⁻³) | Cervical spine CST |
|--------------------------|--------------|-----------------------|-------------------------------------|--------------------------------------|-------------------|
| Study subject | Right | Left | Mean | Right | Left | Mean | Left | Right | Left | Right | Mean | Left | Right | FA | MD (μm²/ms) |
| 1             | 0.576   | 0.554 | 0.565 | 0.743   | 0.727 | 0.735 | 1.234 | 1.287 | 0.474 | 0.471 | 0.670 | 1.146 |
| 2             | 0.600   | 0.576 | 0.588 | 0.713   | 0.729 | 0.721 | 1.258 | 1.262 | 0.464 | 0.438 | 0.719 | 1.084 |
| 3             | 0.588   | 0.577 | 0.582 | 0.783   | 0.786 | 0.7845| 1.370 | 1.340 | 0.495 | 0.504 | 0.693 | 1.116 |
| 4             | 0.588   | 0.494 | 0.541 | 0.884   | 0.934 | 0.909 | 1.476 | 1.369 | 0.664 | 0.641 | 0.694 | 1.016 |
| 5             | 0.570   | 0.497 | 0.533 | 0.776   | 0.852 | 0.814 | 1.361 | 1.337 | 0.598 | 0.496 | 0.616 | 1.177 |
| 6             | 0.523   | 0.529 | 0.526 | 0.862   | 0.798 | 0.83 | 1.324 | 1.415 | 0.535 | 0.585 | 0.770 | 0.887 |
| 7             | 0.541   | 0.542 | 0.542 | 0.849   | 0.829 | 0.839 | 1.382 | 1.423 | 0.553 | 0.563 | 0.705 | 1.04 |
| 8             | 0.546   | 0.516 | 0.531 | 0.772   | 0.752 | 0.762 | 1.230 | 1.283 | 0.513 | 0.517 | 0.702 | 1.154 |
| 9             | 0.429   | 0.483 | 0.456 | 0.927   | 0.828 | 0.8775| 1.313 | 1.392 | 0.585 | 0.695 | 0.623 | 1.172 |
| 10            | 0.542   | 0.522 | 0.532 | 0.739   | 0.757 | 0.748 | 1.238 | 1.239 | 0.516 | 0.489 | 0.665 | 1.013 |
| 11            | 0.531   | 0.560 | 0.545 | 0.759   | 0.739 | 0.749 | 1.265 | 1.265 | 0.476 | 0.505 | 0.650 | 1.082 |
| 12            | 0.459   | 0.495 | 0.477 | 0.878   | 0.806 | 0.842 | 1.298 | 1.353 | 0.560 | 0.640 | 0.706 | 0.983 |
| 13            | 0.492   | 0.487 | 0.490 | 0.795   | 0.833 | 0.814 | 1.310 | 1.247 | 0.595 | 0.569 | *     | *     |

*Values not calculated.

MRI: magnetic resonance imaging; CST: corticospinal tract; FA: fractional anisotropy; MD: mean diffusivity.
Figure 3. Univariate association between T25FW and brain DTI metrics.

Figure 4. Univariate association between T25FW and conventional brain MRI metrics.
the need for novel MRI metrics to understand better the pathological substrate of clinical disability in MS.

Using DTI metrics, we demonstrated a significant association between tract integrity of the brain CST (measured by CST MD) and gait impairment (measured by T25FW) while controlling for use of an assistive device. Our findings are in agreement with a previously observed relationship between brain CST MD and corticospinal tract impairment using T25FW as a clinical measure. Comparison of associations between conventional MRI and DTI metrics and gait dysfunction in our study suggested DTI metrics, specifically brain CST MD, have the stronger association with gait impairment. This could be explained by the higher sensitivity of DTI metrics for axonal damage and consequent disability. Recent work by Tovar-Moll and colleagues showed a significant association between brain CST FA and motor and sensory functional systems of the EDSS and T25FW. In their analyses of the univariate relationship between DTI metrics and EDSS and T25FW, respectively, FA showed higher statistical significance. After controlling for chronic T1 lesion volume, only the association between CST FA and EDSS, but not T25FW, remained statistically significant. Using a different methodological approach, we were able to demonstrate an association between brain CST MD and T25FW. However unlike another previous study of cervical spine DTI metrics and measures of clinical disability by Naismith and colleagues, we did not demonstrate a relationship between T25FW and cervical cord DTI indices.

The results of our study should be interpreted with caution and several limitations must be recognized. Project participants were part of an established database of gait impaired patients we intended to treat with dalfampridine, and as such had to meet inclusion criteria for this database as well as for the current imaging sub-study. Application of these criteria resulted in a small sample size with consequences on the generalizability of our analyses. However, this was a pilot project and we intend to use these data

Figure 5. Univariate association between T25FW and cervical spine DTI and conventional MRI metrics.
to develop a larger sample for further investigation, including novel imaging predictors of dalfampridine response. Known technical difficulties related to spinal DTI are due to small cord size and increased cord mobility resulting in motion artifacts. Present anatomical distortions on DTI maps, often seen in column-specific tract region of interest, can also prevent accurate localization of anatomical tracts. To address these technical limitations, we measured mean FA and MD indices using a single fixed and easily measured area of interest at C2/C3, as this region has maximal yield for DTI analysis. Using multiple regions of interest at different cord levels could provide more data and the possibility of finding additional correlations. A similar technique with use of an area of interest at the C3/C4 level was previously reported; however, the sample size in that study was substantially higher (124 MS patients).

The atlas methodology used in our project also confers certain analysis limitations. Recognizing that regional atrophy can cause partial volume effects that might affect the results, we tried to minimize this impact by using a very conservative threshold to create the individual tracts (80% probability). While using tractography to construct individual tracts could have been preferable to the atlas methodology, the available diffusion data with 20 directions are unsuitable for a tractography approach, as a minimum of 25 directions is required to resolve fibers successfully.

In conclusion, our study adds to existing data focusing on the clinical utility of DTI in MS and constitutes a platform for our ongoing research in dalfampridine response predictors. We specifically analyzed brain DTI markers using gait impairment as the target outcome. We plan to utilize these results to investigate brain and spinal cord DTI metrics further, focusing on brain CST MD as a main candidate biomarker to predict dalfampridine response.

### Declaration of conflicting interests
S Klineova and R Farber have received compensation for advisory board work with Teva. BN Delman has received compensation for consulting and advisory board work with Bayer HealthCare. LN Tanenbaum has received compensation for consulting with Siemens. M Inglese has received research grant support from NIH, NMSS, Novartis Pharmaceuticals and Teva Neuroscience. FD Lublin has received compensation for consulting and advisory board work with Bayer HealthCare Pharmaceuticals, Biogen Idec, EMD Serono, Inc., Novartis, Teva Neuroscience, Actelion, Sanofi/Genzyme, Acorda, Questcor/Malinckrodt, Roche/Genentech, Celgene, MedImmune, Osmotica, Xenoport, Receptos, Forward Pharma, BBB Technologies, Akros, TG Therapeutics and Abbvie, and sources of funding for research from Biogen Idec, Novartis Pharmaceuticals Corp, Teva Neuroscience, Inc., Genzyme, Sanofi, Celgene, Transparency Life Sciences, NIH and NMSS. S Krieger has received compensation for consulting and advisory board work with Acorda Therapeutics, Bayer HealthCare, Biogen Idec, EMD Serono, Genentech, Genzyme, Questcor, and Teva, and has given non-promotional lectures with Biogen Idec and Genzyme. C Farrell, C Saiote and J Friedman have nothing to disclose.

### Funding
The imaging portion of this project was supported by grant UL1TR000067 from the National Center for Research Resources, National Institutes of Health.

### References
1. Remelius JG, Jones SL, House JD, et al. Gait impairments in persons with multiple sclerosis across preferred and fixed walking speeds. *Arch Phys Med Rehabil* 2012; 93(9): 1637–1642.
2. Dalgas U, Stenager E, Jakobsen J, et al. Resistance training improves muscle strength and functional capacity in multiple sclerosis. *Neurology* 2009; 73(18): 1478–1484.

3. Kjolhede T, Vissing K, de Place L, et al. Neuromuscular adaptations to long-term progressive resistance training translates to improved functional capacity for people with multiple sclerosis and is maintained at follow-up. *Mult Scler* 2015; 21(5): 599–611.

4. Dunn J and Blight A. Dalfampridine: a brief review of its mechanism of action and efficacy as a treatment to improve walking in patients with multiple sclerosis. *Curr Med Res Opin* 2011; 27(7): 1415–1423.

5. Larson RD, Larson DJ, Baumgartner TB, et al. Brain 1.5 T and 3 T MRI study. *J Neurol Sci* 2009; 279(1): 92–105.

6. Kieseier BC and Pozzilli C. Assessing walking disability in multiple sclerosis. *Mult Scler* 2012; 18(7): 914–924.

7. Klineova S, Friedman J, Farrell C, et al. Dalfampridine real world utilization and efficacy assessment. *Neurology* 2014; 82(10 Suppl P7.244): 1526–632X.

8. Filippi M and Rocca MA. The role of magnetic resonance imaging in the study of multiple sclerosis: diagnosis, prognosis and understanding disease pathophysiology. *Acta Neurol Belg* 2011; 111(2): 89–98.

9. Rovaris M, Gass A, Bammer R, et al. Diffusion MRI in multiple sclerosis. *Neurology* 2005; 65(10): 1526–1532.

10. Ge Y, Law M, Johnson G, et al. Preferential occult injury of corpus callosum in multiple sclerosis measured by diffusion tensor imaging. *J Magn Reson Imaging* 2004; 20(1): 1–7.

11. Basser PJ, Pajevic S, Pierpaoli C, et al. In vivo fiber tractography using DT-MRI data. *Magn Reson Med* 2000; 44(4): 625–632.

12. Cercignani M, Inglese M, Pagani E, et al. Mean diffusivity and fractional anisotropy histograms of patients with multiple sclerosis. *AJNR Am J Neuroradiol* 2001; 22(5): 952–958.

13. Valsasina P, Rocca MA, Agosta F, et al. Mean diffusivity and fractional anisotropy histogram analysis of the cervical cord in MS patients. *Neuroimage* 2005; 26(3): 822–828.

14. Harrison DM, Shiee N, Bazin PL, et al. Tract-specific quantitative MRI better correlates with disability than conventional MRI in multiple sclerosis. *J Neurol* 2013; 260(2): 397–406.

15. Oh J, Saidha S, Chen M, et al. Spinal cord quantitative MRI discriminates between disability levels in multiple sclerosis. *Neurology* 2013; 80(6): 540–547.

16. Naismith RT, Xu J, Klawiter EC, et al. Spinal cord tract diffusion tensor imaging reveals disability substrate in demyelinating disease. *Neurology* 2013; 80(24): 2201–2209.

17. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol* 2011; 69(2): 292–302.

18. Lublin FD, Reingold SC, Cohen JA, et al. Defining the clinical course of multiple sclerosis: the 2013 revisions. *Neurology* 2014; 83(3): 278–286.

19. Fisher JS, Jak AJ, Kniker JE, et al. *Multiple Sclerosis Functional Composite Administration and Scoring Manual*. National Multiple Sclerosis Society, USA, 2001.

20. Inglese M, Madelin G, Oesingmann N, et al. Brain tissue sodium concentration in multiple sclerosis: a sodium imaging study at 3 tesla. *Brain* 2010; 133(Pt 3): 847–857.

21. Smith SM, Zhang Y, Jenkinson M, et al. Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. *Neuroimage* 2002; 17(1): 479–489.

22. Smith SM, Jenkinson M, Woolrich MW, et al. Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage* 2004; 23(Suppl 1): S208–S219.

23. Horsfield MA, Sala S, Neema M, et al. Rapid semi-automatic segmentation of the spinal cord from magnetic resonance images: application in multiple sclerosis. *Neuroimage* 2010; 50(2): 446–455.

24. Healy BC, Arora A, Hayden DL, et al. Approaches to normalization of spinal cord volume: application to multiple sclerosis. *J Neuroimaging* 2012; 22(3): e12–e19.

25. Jenkinson M and Smith S. A global optimisation method for robust affine registration of brain images. *Med Image Anal* 2001; 5(2): 143–156.

26. Jenkinson M, Bannister P, Brady M, et al. Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage* 2002; 17(2): 825–841.

27. Hua K, Zhang J, Wakana S, et al. Tract probability maps in stereotaxic spaces: analyses of white matter anatomy and tract-specific quantification. *Neuroimage* 2008; 39(1): 336–347.

28. Katz Sand I, Krieger S, Farrell C, et al. Diagnostic uncertainty during the transition to secondary progressive multiple sclerosis. *Mult Scler* 2014; 20(12): 1654–1657.

29. Stankiewicz JM, Neema M, Alsop DC, et al. Spinal cord lesions and clinical status in multiple sclerosis: a 1.5 T and 3 T MRI study. *J Neurol Sci* 2009; 279(1–2): 99–105.

30. Tjoa CW, Benedict RH, Weinstock-Guttman B, et al. MRI T2 hypointensity of the dentate nucleus is related...
to ambulatory impairment in multiple sclerosis. J Neurol Sci 2005; 234(1–2): 17–24.

31. Reich DS, Ozturk A, Calabresi PA, et al. Automated vs. conventional tractography in multiple sclerosis: variability and correlation with disability. Neuroimage 2010; 49(4): 3047–3056.

32. Tovar-Moll F, Evangelou IE, Chiu AW, et al. Diffuse and focal corticospinal tract disease and its impact on patient disability in multiple sclerosis. J Neuroimaging 2015; 25(2): 200–206.

33. Losseff NA, Webb SL, O’Riordan JI, et al. Spinal cord atrophy and disability in multiple sclerosis. A new reproducible and sensitive MRI method with potential to monitor disease progression. Brain 1996; 119(Pt 3): 701–708.

34. Jbabdi S, Woolrich MW, Andersson JL, et al. A Bayesian framework for global tractography. Neuroimage 2007; 37(1): 116–129.