Highlights

- A study investigating the relative contribution of damage in several parts of the motor system in relation to physical dysfunction in MS has not yet been performed.

- Depending on the measure, different combinations of neuroimaging measures formed the models for physical dysfunction. Infratentorial damage, spinal cord damage and damage in the corticospinal tract recurrently evolved as strongest statistical predictors.

- Motor dysfunction in long-standing multiple sclerosis has a complex substrate that cannot be ascribed to a single neuroimaging measure.
Chapter 4.3

Unraveling the neuroimaging predictors for motor dysfunction in long-standing multiple sclerosis


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Abstract

Objective
To find the strongest neuroimaging predictors for motor dysfunction using conventional and quantitative imaging measures focusing on the corticospinal tract (CST) in a large cohort of patients with long-standing MS.

Methods
In this cross-sectional study, a wide spectrum of neuroimaging measures at the whole-brain, cervical and CST level were analyzed in 195 MS patients and 54 healthy controls. Motor function was assessed using the Expanded Disability Status Scale (EDSS), Nine-Hole-Peg test (9-HPT), 25 feet Timed-Walk Test (TWT) and Multiple Sclerosis Walking Scale (MSWS). Associations between damage in different parts of the motor system and motor functioning were assessed using stepwise linear regression.

Results
Patients had an average disease duration of 19.98 (±6.99) years and a median EDSS of 4 (range 1.0–8.0). EDSS was associated with number of infratentorial and cervical cord lesions, lesion volume in the CST and mean upper cervical cord area (MUCCA) (adjusted $R^2 = 0.403$). TWT score was associated with number of infratentorial lesions and cerebellar volume (adjusted $R^2 = 0.150$), 9-HPT score with number of infratentorial lesions and thickness of the cortex connected to the CST (adjusted $R^2 = 0.245$), and MSWS with number of infratentorial and cervical lesions, thickness of the cortex connected to the CST and MUCCA (adjusted $R^2 = 0.354$).

Conclusions
Motor dysfunction in MS has a complex substrate that cannot be ascribed to a single neuroimaging finding, but is the consequence of infratentorial and spinal cord damage, as well as damage in the CST.
Introduction

Multiple sclerosis (MS) has been primarily considered an inflammatory demyelinating white matter (WM) disease. However, a substantial neurodegenerative component is recognized, particularly in later disease stages (1). Clinically, a remarkable heterogeneity is seen, frequently including motor deficits that have a tremendous impact on daily functioning and quality of life (2,3). However, conventional imaging measures can not fully explain motor dysfunction. This lack of correlation between clinical status and conventional MRI measures is called the ‘clinico-radiological paradox’ (4).

Recently, diffusion tensor imaging (DTI) has shown to be valuable in minimizing this gap by quantitatively assessing the integrity of brain tissue and investigating specific tracts (5–10). Decreased diffusivity along the corticospinal tract (CST) has been related to specific motor symptoms (7,11). Moreover, some studies reported an association of motor deficits with lesion volume and MD, but not FA, within the CST (8,12). In addition, reduced volumes of the cerebellum and spinal cord play an important role in motor dysfunction in MS (13,14).

So far, an integrated study investigating damage in all these compartments in relation to motor dysfunction has not been performed. Therefore, this study aims to analyze a comprehensive set of neuroimaging measures in different parts of the motor system to quantify the relevance of damage in each part to motor disability compared to controls. To maximize contrast, we investigated a large cohort of patients with long-standing MS, in which clinical and pathological differences will be pronounced (15).

Methods

Standard protocol approvals, registrations and patient consents

The study was approved by the local institutional review board and written informed consent was obtained from all subjects.

Subjects

We included patients diagnosed with clinically definite MS according to the current McDonald diagnostic criteria (16). Based on the disease course patients were classified as being either relapsing-remitting (RR), secondary-progressive (SP) or primary-progressive (PP) (17). All patients were part of a prospective cohort in our MS center, having a disease duration of at
least 10 years since first symptom. Healthy controls were recruited using advertisements in the hospital and from non-related family and friends of the patients. Exclusion criteria were a history of neurological or psychiatric disease (for patients: other than MS) and patients were not allowed to participate if they received steroid treatment six weeks prior to participation. A part of the subjects in the current study has been described in recently published work (18–20). Compared to our previous study (19), one RRMS patient was excluded due to unavailability of DTI data.

Assessment of motor function

In patients, the degree of motor disability was assessed on the day of scanning using the Expanded Disability Status Scale (EDSS) (21). From the EDSS we derived two specific system scores: the pyramidal functional system (PFS) score and infratentorial functional system (IFS) score, the latter being derived by adding the scores for pyramidal, brainstem, cerebellar, sensory, bowel and bladder system (13). In addition, two subtests of the MS Functional Composite Measure (MSFC) (22) were used: the 25 foot Timed Walk Test (TWT) to assess lower limb function and the Nine-hole Peg Test (9-HPT) to assess upper limb function. The Multiple Sclerosis Walking Scale (MSWS; a self-report measure of walking ability) (23) was also assessed.

MR Imaging

MRI was performed on a 3T whole-body MR system (GE Signa HDxt, Milwaukee, WI, USA) using an eight-channel phased array head coil for brain imaging and a sixteen-channel combined head-neck-spine coil for cervical imaging. The brain protocol included a three-dimensional (3D) T1-weighted fast spoiled gradient recalled echo (FSPGR) sequence for volumetric measurements and a 3D fluid attenuated inversion recovery image (FLAIR) for

<table>
<thead>
<tr>
<th>Table e–1. Detailed information on scan parameters.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brain 3D FSPGR</strong></td>
</tr>
<tr>
<td>TR, ms</td>
</tr>
<tr>
<td>TE, ms</td>
</tr>
<tr>
<td>TI, ms</td>
</tr>
<tr>
<td>FA, °</td>
</tr>
<tr>
<td>Slice thickness, mm</td>
</tr>
<tr>
<td>In-plane resolution, mm²</td>
</tr>
</tbody>
</table>

Abbreviations: TR = repetition time; TE = echo time; TI = inversion time; FA = flip angle.
WM lesion detection. A 3D double inversion recovery (DIR) sequence was obtained for (juxta)cortical lesion detection. Furthermore, 2D echo-planar diffusion tensor images (DTI) were acquired, including 30 volumes with non-collinear diffusion gradients (b-value of 900 s/mm$^2$) and 5 volumes without diffusion weighting.

The cervical cord protocol included a 3D T1-weighted FSPGR sequence for cervical cord atrophy measurements and a 2D dual-echo T2-weighted spin-echo for cervical cord lesion detection. See Table e–1 for detailed scan parameters.

**Whole-brain MRI processing**

Normalized whole brain, GM and WM volumes were measured using the T1-weighted images and SIENAX (part of FSL 5.0.4, http://www.fmrib.ox.ac.uk/fsl). To minimize the impact of WM lesions on the atrophy measurements (24,25), the automated lesion-filling technique called LEAP (LEsion Automated Preprocessing) was applied (26). This algorithm fills the hypointense lesions in the T1-weighted image with intensities comparable to the normal-appearing white matter (NAWM) using the previously obtained lesion mask.

Cerebral WM lesions were automatically segmented on the FLAIR and T1-weighted images using the $k$NN-TTP algorithm (27) and were normalized for head size, resulting in normalized brain lesion volume (NBLV). For cortical and infratentorial lesion detection, the DIR images were reformatted to axial 3 mm thick slices. Cortical lesions (CLs) and infratentorial lesions were rated on the reformatted images in consensus by two operators (MD and MDS) according to recently published guidelines (28).

Details on cervical cord lesion detection and cervical cord atrophy (MUCCA: mean upper cervical cord area) measurements in this cohort were published previously (19). In short, cervical cord lesions were counted manually on the cervical PD/T2-weighted images. MUCCA was quantified semi-automatically using Gaussian Mixture Modeling.

**DTI preprocessing and CST segmentation**

The diffusion images were corrected for head movement and eddy current distortions using FMRIB’s Diffusion Toolbox (FSL-FDT). Subsequently the diffusion tensor was fitted, from which the fractional anisotropy (FA), mean (MD), axial (AD) and radial diffusivity (RD) were calculated. The diffusion images were then used to segment the CSTs and derive tract specific pathology measures. As tractography in the presence of MS pathology might lead to unreliable results, this was done by means of a study specific atlas based on the controls. The pipeline was as follows:
Chapter 4.3

For each healthy control, subject-specific seed masks (i.e., the cerebral peduncles and motor cortex) were derived for CST tractography. The Johns Hopkins University (JHU)-atlas (29) and nonlinear registration (FSL-FLIRT) were used to obtain subject-specific masks of the cerebral peduncles. FreeSurfer 5.1 was used to obtain a subject-specific parcellation of the bilateral motor cortex (i.e., the union of the pre- and paracentral cortex) (30,31).

For each healthy control and hemisphere separately, probabilistic tractography (FSL-probtrackx2, 5000 streamlines per voxel) was run using the previously obtained masks as a seed region. Each probabilistic map was binarized using a 0.25% threshold of the total number of streamlines passing both the cerebellar peduncle and cortical seed mask. This threshold was determined visually and led to consistent segmentation results throughout the controls.

The binary maps were nonlinearly warped to JHU-space, and averaged to construct a single probabilistic map for each CST in standard space. The two probabilistic maps (left and right) with voxelwise values between zero and one formed the study-specific atlas of the CSTs.

Then the probabilistic atlas was propagated to the individual subject-space using nonlinear...
registration. For patients, lesion voxels were masked out of the registration cost function to enhance registration quality.

5. For each subject and tract separately, the weighted average NAWM FA, MD, AD and RD values of each tract were computed using the atlas probability values as a weighting factor. Weighting was performed to emphasize the integrity values in the center of the tract. In order to only include NAWM, the weighting factors of voxels belonging to GM, CSF or lesions were set to zero using the earlier derived segmentations.

6. In addition, weighted lesion volume inside each tract was computed as described above. This lesion volume was subsequently normalized for head size, resulting in normalized CST lesion volume (NLV\textsubscript{CST}). Note that NLV\textsubscript{CST} is a relative, instead of an absolute value, and cannot be compared with unweighted whole brain lesion volumes. See Figure 1 for an example of the segmented CST, lesions in the tract and the connected cortex.

**Cortical thickness of CST connected cortex**

FreeSurfer was also used to measure the cortical thickness in each subject. All cortical segmentations were checked and re-run if errors occurred. Analogous to NLV\textsubscript{CST}, weighted cortical thickness of the CST connected cortex was computed. To accomplish this, the previously obtained subject-specific CST probability map was sampled onto the WM surface derived by FreeSurfer and masked such that only the frontal and parietal cortex could contribute. Subsequently, the thickness of the cortex connected to the CST was computed by weighting with the sampled CST probabilities, resulting in CT\textsubscript{CST}.

**Cerebellar and brainstem volumes**

Volumes of the cerebellum and brainstem were calculated using FreeSurfer and normalized for head size, resulting in normalized cerebellar volume (NCbV) and normalized brainstem volume (NBstV).

**Statistical analysis**

Statistical analyses were performed in SPSS 20.0 (Chicago, IL, USA). Normality of the variables was assessed using Kolmogorov–Smirnov tests and visual inspection of histograms. When normally distributed, a multivariate general linear model (GLM) was used to assess group differences. When not normally distributed, the Mann–Whitney or the Kruskal–Wallis test was used. $P$-values < 0.05 were considered as statistically significant. When required, a Bonferroni-correction was applied to adjust for multiple testing. Partial correlations, corrected
### Table 1. Demographic, clinical and MRI measures.

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>MS</th>
<th>RRMS</th>
<th>SPMS</th>
<th>PPMS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 54)</td>
<td>(n = 195)</td>
<td>(n = 124)</td>
<td>(n = 49)</td>
<td>(n = 22)</td>
</tr>
<tr>
<td>Age, y</td>
<td>50.89 ± 6.57</td>
<td>53.44 ± 9.60</td>
<td>50.47 ± 9.50</td>
<td>56.75 ± 6.43***</td>
<td>62.80 ± 7.73***</td>
</tr>
<tr>
<td>Gender, F/M</td>
<td>33/21</td>
<td>131/64</td>
<td>90/34</td>
<td>31/18</td>
<td>10/12</td>
</tr>
<tr>
<td>EDSS*</td>
<td></td>
<td>4.0 (1.0–8.0)</td>
<td>3.0 (1.0–7.5)</td>
<td>6.0 (2.5–8.0)</td>
<td>6.0 (2.5–8.0)</td>
</tr>
<tr>
<td>PFS*</td>
<td></td>
<td>2.0 (0–5.0)</td>
<td>2.0 (0–5.0)</td>
<td>3.0 (0–4.0)</td>
<td>3.0 (1.0–5.0)</td>
</tr>
<tr>
<td>IFS*</td>
<td></td>
<td>8.0 (0–17)</td>
<td>7.0 (0–14)</td>
<td>10 (5–16)</td>
<td>9 (5–17)</td>
</tr>
<tr>
<td>MSFC, sec</td>
<td></td>
<td>25.19 ± 54.48</td>
<td>6.58 ± 15.93</td>
<td>22.17 ± 10.93</td>
<td>59.96 ± 78.25</td>
</tr>
<tr>
<td>TWT score</td>
<td></td>
<td>25.19 ± 15.86</td>
<td>22.17 ± 10.93</td>
<td>29.67 ± 14.47</td>
<td>59.80 ± 78.22</td>
</tr>
<tr>
<td>MSWS</td>
<td></td>
<td>31.15 ± 15.57</td>
<td>24.87 ± 12.74</td>
<td>46.14 ± 9.69</td>
<td>45.87 ± 13.56</td>
</tr>
<tr>
<td>NBV, L</td>
<td>1.49 ± 0.07</td>
<td>1.42 ± 0.09***</td>
<td>1.43 ± 0.10***</td>
<td>1.39 ± 0.08***</td>
<td>1.40 ± 0.10***</td>
</tr>
<tr>
<td>NGMV, L</td>
<td>0.79 ± 0.05</td>
<td>0.76 ± 0.06***</td>
<td>0.77 ± 0.05*</td>
<td>0.73 ± 0.05***</td>
<td>0.73 ± 0.07***</td>
</tr>
<tr>
<td>NWMV, L</td>
<td>0.70 ± 0.04</td>
<td>0.66 ± 0.05***</td>
<td>0.66 ± 0.05***</td>
<td>0.66 ± 0.05***</td>
<td>0.67 ± 0.04**</td>
</tr>
<tr>
<td>RBLV, mL</td>
<td></td>
<td>17.14 ± 1.42</td>
<td>14.58 ± 1.05</td>
<td>23.64 ± 1.82</td>
<td>17.11 ± 1.29</td>
</tr>
<tr>
<td>NBLV, mL</td>
<td></td>
<td>22.65 ± 18.47</td>
<td>19.45 ± 1.39</td>
<td>31.27 ± 23.62</td>
<td>21.49 ± 15.25</td>
</tr>
<tr>
<td>Cortical lesions*</td>
<td></td>
<td>8 (0–129)</td>
<td>7 (0–14)</td>
<td>10 (5–16)</td>
<td>9 (5–17)</td>
</tr>
<tr>
<td>Infratentorial lesions*</td>
<td></td>
<td>1 (0–18)</td>
<td>1 (0–18)</td>
<td>2 (0–16)</td>
<td>2 (0–7)</td>
</tr>
<tr>
<td>NCBV, L</td>
<td>0.175 ± 0.016</td>
<td>0.165 ± 0.016***</td>
<td>0.167 ± 0.015**</td>
<td>0.161 ± 0.017***</td>
<td>0.162 ± 0.017**</td>
</tr>
<tr>
<td>NBstV, mL</td>
<td>29.60 ± 2.16</td>
<td>27.71 ± 3.10***</td>
<td>28.05 ± 2.63**</td>
<td>26.87 ± 3.24***</td>
<td>27.65 ± 3.48**</td>
</tr>
<tr>
<td>Weighted NLV CST mL</td>
<td>0.25 (0–3.33)</td>
<td>0.19 (0–2.12)</td>
<td>0.54 (0–3.33)</td>
<td>0.22 (0–1.82)</td>
<td></td>
</tr>
<tr>
<td>DTI CST</td>
<td></td>
<td>0.532 ± 0.021</td>
<td>0.529 ± 0.021</td>
<td>0.531 ± 0.020</td>
<td>0.525 ± 0.023</td>
</tr>
<tr>
<td>FA CST</td>
<td></td>
<td>0.760 ± 0.024</td>
<td>0.779 ± 0.029***</td>
<td>0.774 ± 0.028*</td>
<td>0.790 ± 0.031***</td>
</tr>
<tr>
<td>MD CST</td>
<td></td>
<td>1.260 ± 0.029</td>
<td>1.287 ± 0.040***</td>
<td>1.282 ± 0.038**</td>
<td>1.299 ± 0.043***</td>
</tr>
<tr>
<td>AD CST</td>
<td></td>
<td>0.510 ± 0.027</td>
<td>0.525 ± 0.030**</td>
<td>0.520 ± 0.029</td>
<td>0.535 ± 0.032***</td>
</tr>
<tr>
<td>RD CST</td>
<td></td>
<td>2.43 ± 0.15</td>
<td>2.29 ± 0.16***</td>
<td>2.32 ± 0.16***</td>
<td>2.22 ± 0.16***</td>
</tr>
<tr>
<td>CT CST mm</td>
<td></td>
<td>82.10 ± 7.81</td>
<td>72.48 ± 9.79***</td>
<td>74.36 ± 9.44***</td>
<td>70.46 ± 10.15***</td>
</tr>
<tr>
<td>MUCCA, mm²</td>
<td></td>
<td>26.10 ± 2.16</td>
<td>27.21 ± 3.10***</td>
<td>28.50 ± 2.63**</td>
<td>26.87 ± 3.24***</td>
</tr>
<tr>
<td>Cervical cord lesions*</td>
<td></td>
<td>6 (0–19)</td>
<td>5 (0–19)</td>
<td>7 (0–19)</td>
<td>6 (1–13)</td>
</tr>
</tbody>
</table>

Abbreviations: HC = healthy controls; MS = multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary-progressive multiple sclerosis; PPMS = primary-progressive multiple sclerosis; EDSS = Expanded Disability Status Scale; PFS = Pyramidal Functional System; IFS = Infratentorial Functional System; MSFC = Multiple Sclerosis Functional Composite; TWT = Timed-Walk Test (part of MSFC); 9-HPT = 9-Hole Peg Test (part of MSFC); MSWS = Multiple Sclerosis Walking Scale; NBV = normalized brain volume; NGMV = normalized gray matter volume; NWMV = normalized white matter volume; RBLV = raw brain lesion volume; CST = Corticospinal Tract; FA = Fractional Anisotropy; MD = Mean Diffusivity; AD = Axial Diffusivity; RD = Radial Diffusivity; CT = Cortical Tract; MUCCA = Multivariate Cortical Surface Area; (continued)
Neuroimaging predictors for motor dysfunction in MS

Separate multiple stepwise linear regression analyses were performed to predict EDSS, TWT, 9-HPT and MSWS using respectively MUCCA, number of cervical cord, cortical and infratentorial lesions, CST MRI measures (NLV$\text{CST}$, MD$\text{CST}$ and CT$\text{CST}$), and cerebellar and brainstem volume as candidate predictors, and age and gender were entered as covariates. Only variables with significant correlations with motor disability were included in the regression models. 9-HPT, TWT and MSWS scores were not normally distributed and were therefore log-transformed prior to statistical analysis.

Results

Demographic and clinical characteristics

195 (67% female) patients with MS and 54 (61% female) healthy controls were included (subjects greatly overlapping with previous work (19)). Between groups, gender was well balanced and age was comparable (patients being slightly older). See Table 1 for demographic, clinical and MRI data. The group consisted of 124 RRMS patients, 49 SPMS patients and 22 PPMS patients and had an average disease duration of 19.98 (± 6.99) years and had a median EDSS score of 4 (range 1.0–8.0).

Global brain and cervical cord pathology

Whole brain and cervical cord findings were described in more detail in previous studies (18,19). In short, brain volumes of patients were lower compared to controls ($p < 0.001$). NBLV was higher in SPMS patients compared to RRMS patients, but not in PPMS patients.
compared to RRMS patients. A smaller MUCCA was found in patients, and differed between MS subtypes. 95.1% of the patients presented with cervical cord lesions, with a median of 6 lesions per patient (range 0–19) (19). SPMS patients had more cortical and infratentorial lesions compared to RRMS patients (see Table 1). Cerebellar and brainstem volumes were lower in patients compared to controls. Compared to RRMS patients, SPMS patients showed lower brainstem, but not cerebellar, volumes.

**Corticospinal tract pathology**

CST lesions were present in all patients (see Table 1 for weighted lesion volumes). A larger lesion volume was present in the CSTs of SPMS patients compared to RRMS patients. MD, AD and RD were higher in the CSTs of patients compared to controls, most pronounced in SPMS patients. FA of the CST showed no differences. CT\(_{CST}\) was thinner in patients compared to controls. Interestingly, the weighted lesion volume of the CST was larger in SPMS patients compared to RRMS patients. Over time, CST volumes and lesion volumes decreased in both patient groups, with more pronounced changes in SPMS patients. The CST lesions were associated with motor dysfunction, as shown in Table 2. The table includes partial correlations between MRI measures and motor dysfunction, corrected for age and gender. The abbreviations used are as follows:

- **EDSS**: Expanded Disability Status Scale
- **PFS**: Pyramidal Functional System
- **IFS**: Infratentorial Functional System
- **TWT**: Timed-Walk Test
- **9-HPT**: 9-Hole Peg Test
- **MSWS**: Multiple Sclerosis Walking Scale
- **NBV**: normalized brain volume
- **NBLV**: normalized brain lesion volume
- **NCbV**: cerebellar volume
- **NBstV**: normalized brainstem volume
- **FA\(_{CST}\)**: fractional anisotropy in the corticospinal tract
- **MD\(_{CST}\)**: mean diffusivity in the corticospinal tract
- **AD\(_{CST}\)**: axial diffusivity in the corticospinal tract
- **RD\(_{CST}\)**: radial diffusivity in the corticospinal tract
- **CT\(_{CST}\)**: cortical thickness of cortical area connected to corticospinal tract
- **MUCCA**: Mean Upper Cervical Cord Area

**Table 2. Partial correlations between MRI measures and motor dysfunction, corrected for age and gender.**

<table>
<thead>
<tr>
<th></th>
<th>EDSS</th>
<th>PFS</th>
<th>IFS</th>
<th>TWT</th>
<th>9-HPT</th>
<th>MSWS</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBV</td>
<td>–0.264***</td>
<td>–0.198**</td>
<td>–0.258***</td>
<td>–0.257***</td>
<td>–0.252**</td>
<td>ns</td>
</tr>
<tr>
<td>NBLV</td>
<td>0.307***</td>
<td>0.198**</td>
<td>0.307***</td>
<td>0.186*</td>
<td>0.310***</td>
<td>0.271***</td>
</tr>
<tr>
<td>Cortical lesions</td>
<td>0.200**</td>
<td>0.187*</td>
<td>0.260***</td>
<td>0.168*</td>
<td>0.289***</td>
<td>0.170*</td>
</tr>
<tr>
<td>Infratentorial lesions</td>
<td>0.428***</td>
<td>0.384***</td>
<td>0.399***</td>
<td>0.335***</td>
<td>0.385***</td>
<td>0.395***</td>
</tr>
<tr>
<td>NCbV</td>
<td>–0.242**</td>
<td>ns</td>
<td>–0.168*</td>
<td>–0.238**</td>
<td>–0.178*</td>
<td>ns</td>
</tr>
<tr>
<td>NBstV</td>
<td>–0.293***</td>
<td>–0.202**</td>
<td>–0.278***</td>
<td>–0.188*</td>
<td>–0.219**</td>
<td>ns</td>
</tr>
<tr>
<td>Weighted LV(_{CST})</td>
<td>0.338***</td>
<td>0.193**</td>
<td>0.291***</td>
<td>0.244**</td>
<td>0.278***</td>
<td>0.208**</td>
</tr>
<tr>
<td>FA(_{CST})</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>–0.185*</td>
<td>ns</td>
</tr>
<tr>
<td>MD(_{CST})</td>
<td>0.225**</td>
<td>0.187**</td>
<td>0.251***</td>
<td>0.238**</td>
<td>0.218**</td>
<td>0.177*</td>
</tr>
<tr>
<td>AD(_{CST})</td>
<td>0.231**</td>
<td>0.181*</td>
<td>0.205**</td>
<td>0.208**</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>RD(_{CST})</td>
<td>0.178*</td>
<td>0.158*</td>
<td>0.230**</td>
<td>0.211**</td>
<td>0.222**</td>
<td>0.169*</td>
</tr>
<tr>
<td>CT(_{CST})</td>
<td>–0.255**</td>
<td>–0.160*</td>
<td>–0.236**</td>
<td>ns</td>
<td>–0.310***</td>
<td>–0.247**</td>
</tr>
<tr>
<td>MUCCA</td>
<td>–0.296***</td>
<td>–0.402***</td>
<td>–0.381***</td>
<td>–0.204**</td>
<td>–0.201**</td>
<td>–0.292***</td>
</tr>
<tr>
<td>Cervical cord lesions</td>
<td>0.364***</td>
<td>0.368***</td>
<td>0.376***</td>
<td>0.190*</td>
<td>0.163*</td>
<td>0.375***</td>
</tr>
</tbody>
</table>

Abbreviations: EDSS = Expanded Disability Status Scale; PFS = Pyramidal Functional System; IFS = Infratentorial Functional System; TWT = Timed-Walk Test; 9-HPT = 9-Hole Peg Test; MSWS = Multiple Sclerosis Walking Scale; NBV = normalized brain volume; NBLV = normalized brain lesion volume; NCbV = cerebellar volume; NBstV = normalized brainstem volume; weighted LV\(_{CST}\) = weighted corticospinal tract lesion volume; FA\(_{CST}\) = fractional anisotropy in the corticospinal tract; MD\(_{CST}\) = mean diffusivity in the corticospinal tract; AD\(_{CST}\) = axial diffusivity in the corticospinal tract; RD\(_{CST}\) = radial diffusivity in the corticospinal tract; CT\(_{CST}\) = cortical thickness of cortical area connected to corticospinal tract; MUCCA = Mean Upper Cervical Cord Area.

*p < 0.05, **p < 0.01 and ***p < 0.001
to controls. Compared to RRMS patients, more cortical thinning was found in SPMS patients, but not in PPMS patients (see Table 1).

Relation between cervical cord, brain and CST metrics and motor dysfunction in MS patients

Cervical cord pathology and conventional brain metrics versus motor dysfunction

Table 2 displays all partial correlations, corrected for age and gender. Higher EDSS, PFS, IFS and TWT scores were associated with smaller MUCCA, higher amount of cervical cord lesions, higher NBLV, lower NBV, and more infratentorial and cortical lesions. Higher scores on the 9-HPT were associated with the same MRI measures, with exception of cervical cord lesions, and higher MSWS scores were associated with the same MRI measures with the exception of NBV. A lower cerebellar volume was associated with higher EDSS, TWT and 9-HPT scores, and a lower brainstem volume was associated with higher 9-HPT, TWT, EDSS, PFS and IFS scores.

CST measures versus motor dysfunction

Higher EDSS, PFS and IFS scores were associated with a higher lesion volume, MD, AD and RD in the CST, and lower thickness of the connected cortex (but not FA) (see Table 2). Higher TWT scores were associated with a higher lesion volume, MD, AD and RD (but not FA and thickness of the connected cortex) in the CST. Higher 9-HPT scores were associated with a higher lesion volume, FA, MD and RD in the CST, and lower thickness of the connected cortex (but not AD). Higher scores on the self-reported MSWS were associated with higher lesion volume in the CST, higher MD, RD and a lower thickness of the connected cortex (but not FA and AD).

Predicting motor function in MS patients from CST, infratentorial and cervical cord measures

Gender and age were used as covariates in all regression models (see Table 3). EDSS was associated with infratentorial and cervical cord lesions, lesion volume in the CST and MUCCA (explaining 40.3% of the variance). TWT was associated with infratentorial lesions and cerebellar volume (explaining 15.0% of the variance). 9-HPT was associated with infratentorial lesions and the thickness of the cortex connected to the CST (explaining 24.5% of the variance). MSWS was associated with infratentorial and cervical cord lesions, thickness of the cortex connected to the CST and MUCCA (explaining 35.4% of the variance).
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**Table 3.** Stepwise linear regression of EDSS, TWT, 9-HPT and MSWS in MS patients.\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>EDSS</th>
<th>TWT</th>
<th>9-HPT</th>
<th>MSWS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>F</strong></td>
<td>21.275, (p &lt; 0.001), adjusted (R^2 = 0.403)</td>
<td>8.953, (p &lt; 0.001), adjusted (R^2 = 0.150)</td>
<td>14.921, (p &lt; 0.001), adjusted (R^2 = 0.245)</td>
<td>15.687, (p &lt; 0.001), adjusted (R^2 = 0.354)</td>
</tr>
<tr>
<td>Age</td>
<td>0.407***</td>
<td>0.151*</td>
<td>0.195**</td>
<td>0.317***</td>
</tr>
<tr>
<td>Gender</td>
<td>ns</td>
<td>ns</td>
<td>Gender</td>
<td>ns</td>
</tr>
<tr>
<td>Infratentorial lesions</td>
<td>0.254***</td>
<td>0.294***</td>
<td>0.317***</td>
<td>0.219**</td>
</tr>
<tr>
<td>Cervical cord lesions</td>
<td>0.191**</td>
<td>NCbV</td>
<td>CT(_{\text{CST}})</td>
<td>0.250***</td>
</tr>
<tr>
<td>Weighted NLV(_{\text{CST}})</td>
<td>0.184**</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MUCCA</td>
<td>-0.131*</td>
<td>-</td>
<td>-</td>
<td>-MUCCA</td>
</tr>
</tbody>
</table>

Abbreviations: EDSS = Expanded Disability Status Scale; TWT = Timed-Walk Test (part of MSFC); 9-HPT = 9-Hole Peg Test (part of MSFC); MSWS = Multiple Sclerosis Walking Scale; ns = not significant; NCbV = normalized cerebellar volume; weighted NLV\(_{\text{CST}}\) = weighted normalized corticospinal tract lesion volume; CT\(_{\text{CST}}\) = cortical thickness of cortical area connected to corticospinal tract; MUCCA = Mean Upper Cervical Cord Area.

\(^a\)Values are standardized betas of the final model.

\(*p < 0.05, **p < 0.01\) and ***\(p < 0.001\)

**Discussion**

In this study, we aimed to investigate the relationship between several types of pathology (i.e., WM lesions, cortical lesions, atrophy, normal-appearing WM damage) in different parts of the motor system and clinical measures assessing motor dysfunction in long-standing MS. The rationale for this approach was that most previous studies have limited their focus on single MRI measures, such as either brain (lesion) volumes or DTI metrics in patients with a relatively short disease duration (6). In the context of motor deficits, the CST is of particular interest as damage may lead to coordination problems, spasticity, muscle weakness and increased tendon reflexes (32). Its dysfunction can be measured by clinical assessment and the course of the tract can be reconstructed relatively easily using DTI.

Motor dysfunction, as measured by EDSS, TWT, 9-HPT and MSWS, was associated with both conventional MRI measures and DTI measures within the CST and thickness of the connected cortex. In accordance with earlier studies (33,34), we did not find any significant differences in FA within the CST between patients and controls. This may be explained by the fact that FA describes the degree to which the diffusion within a voxel is oblong (FA
close to 1) or spherical (FA close to 0). As patients displayed proportionally large axial and radial diffusion changes, no differences in FA were found between groups. However, group differences in MD within the CST were present, and were associated with motor symptoms supporting the results from an earlier study (8).

EDSS showed a strong correlation with infratentorial lesions, a moderate correlation with NBLV, CST and cervical cord lesions and a weak correlation with MD, AD and RD within the CST, thickness of the cortex connected to the CST and NBV. This is in line with earlier studies, describing modest correlations between EDSS scores and conventional MRI markers such as NBV and NBLV (35), and a strong correlation with infratentorial lesions (36).

PFS and IFS scores showed moderate to strong correlations with infratentorial measures (MUCCA, number of cervical cord lesions and infratentorial lesions). Some of the metrics showed stronger associations with these scales than with total EDSS score, however the amount of variance that could be explained for these scales was comparable to the model of EDSS (data not shown).

Also in line with a recent study (36), infratentorial lesions showed a moderate correlation with TWT scores, whereas the correlation between other MRI markers and TWT scores was weak. In accordance with previous studies, we found an association between motor dysfunction and CST lesions, and MD, but not FA, within the CST (8,12).

In all stepwise linear regression models, infratentorial lesions occur, indicating the important role of this MRI marker in motor dysfunction. Moreover, cerebellar volume seems to be mainly involved in walking disability, whereas thickness of the cortex connected to the CST plays a role in upper limb function. The most important predictors for clinical dysfunction were infratentorial and cervical cord lesions, MUCCA, CST lesions and thickness of the cortex connected to the CST whereas diffuse CST damage showed to have a modest influence. In a clinical setting, measures such as MUCCA, cervical and infratentorial lesions may be implemented relatively easily.

While aiming to include the major structures (both cervical and brain) of the central nervous system that are known to be involved in motor functioning, our study was not exhaustive. We measured diffusivity in one specific white matter tract in an attempt to improve the relation to relevant clinical scores of motor disability. Future studies could consider the role of several other brain regions (i.e., corpus callosum, basal ganglia, inter-hemispheric connections between motor cortices and the supplementary motor area). Although the effect of (juxta) cortical lesions on cortical atrophy is generally thought to be limited (37), it can not be ruled out that lesions in the cortex connected to the CST may have had an influence on clinical measures. As opposed to the current cross-sectional study, longitudinal studies (35) may
reveal stronger associations and may explain more variance. Lastly, a possible limitation is that we considered the variables as being statistically independent, while it would be plausible to assume that the included neuroimaging markers do not occur completely independent from one another.

In conclusion, our results show that motor dysfunction has a complex substrate and cannot be described by a single neuroimaging marker, but is the consequence of cerebellar and spinal cord pathology, as well as CST damage. However, of these metrics, diffuse CST damage showed to have a modest influence on motor dysfunction.
References

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