Chapter 4

Impact of neurodegeneration and other pathology on clinical dysfunction in multiple sclerosis
Highlights

- Compared to healthy controls, reduced fractional anisotropy (FA) was found in 49% of the investigated white matter in cognitively preserved patients and in 76% of the investigated white matter in cognitively impaired patients.
- Additional white matter damage in the cognitively impaired patients was particularly found in areas that are highly relevant for cognition.
- Diffusion tensor imaging may be a powerful tool for monitoring cognitive impairment in MS.
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Cognitive impairment in MS: the impact of white matter integrity, gray matter volume and lesions


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Chris H Polman
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Frederik Barkhof
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**Abstract**

**Objective**
To investigate whether extent and severity of white matter (WM) damage, as measured with diffusion tensor imaging (DTI), can distinguish cognitively preserved (CP) from cognitively impaired (CI) multiple sclerosis (MS) patients.

**Methods**
Conventional MRI and DTI data were acquired from 55 MS patients (35 CP, 20 CI) and 30 healthy controls (HC). Voxelwise analyses were used to investigate fractional anisotropy (FA), mean diffusivity, radial and axial diffusivity of a WM skeleton. Regional gray matter volume was quantified and lesion probability maps were generated.

**Results**
Compared to HCs, decreased FA was found in 49% of the investigated WM skeleton in CP patients and in 76% of the investigated WM in CI patients. Several brain areas that showed reduced FA in both patient groups were significantly worse in CI patients, i.e., corpus callosum, superior and inferior longitudinal fasciculus, corticospinal tracts, forceps major, cingulum and fornices. In CI patients, WM integrity damage was additionally seen in cortical brain areas, thalamus, uncinate fasciculus, brain stem and cerebellum. These findings were independent of lesion location and regional gray matter volume, since no differences were found between the groups.

**Conclusion**
CI patients diverged from CP patients only on DTI metrics. WM integrity changes were found in areas that are highly relevant for cognition in the CI patients but not in the CP patients. These WM changes are therefore thought to be related to the cognitive deficits and suggest that DTI might be a powerful tool when monitoring cognitive impairment in MS.
**Introduction**

Cognitive impairment is frequently present in multiple sclerosis (MS) and affects up to 65% of all patients (1,2). Previous studies showed associations between conventional MRI measures (T1 and T2 lesion volumes and brain atrophy) and cognitive performance (3–5), and showed that brain atrophy was a better predictor of cognitive impairment than lesion volume (6–8).

Diffusion tensor imaging (DTI) (9), however, allows for quantitative measurements of the microstructural integrity of white matter (WM) tracts both in the normal-appearing WM and in areas with MS lesions. Reduced fractional anisotropy (FA) and increased mean diffusivity (MD) was previously shown for certain WM tracts (i.e., the corpus callosum, cingulum, posterior thalamic tract) and these abnormalities were related to impairment on several neuropsychological tests (10–14). Furthermore, FA of the whole brain normal-appearing WM was found to be a significant predictor of cognitive impairment (15).

To get more insight into the clinical representation of cognitive impairment in MS, we explored whether differences in WM integrity are present between *cognitively preserved* (CP) and *cognitively impaired* (CI) MS patients, using voxelwise analyses. The extent and severity of WM integrity damage were used as study parameters. Additionally, lesion probability maps were generated and gray matter (GM) voxel based morphometry (VBM) was performed to understand the relationship between DTI findings, WM lesions, regional GM volume and cognitive functioning.

**Methods**

**Standard protocol approvals, registrations and patient consents**

The institutional ethics review board approved the study protocol and all subjects gave written informed consent prior to participation.

**Participants**

All patients were diagnosed with clinically definite MS (16). Disease severity was measured on the day of scanning with a questionnaire based on the Expanded Disability Status Scale (EDSS) (17). Age- and sex-matched healthy controls (HC) were included in this study. The participants in the current study are partly overlapping with a previously reported functional
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MRI study. See Hulst et al. (18) for a more detailed description of the recruitment and eligibility procedure or neuropsychological test battery.

Neuropsychological examination and cognitive impairment

In all subjects, the following cognitive functions were tested:

- Verbal memory and learning – assessed with the Verbal Learning and Memory Task (19), the Dutch equivalent of the Californian Verbal Learning Test (20).
- Information processing speed – measured by the Letter Digit Substitution Test, which is an adaptation of the Symbol Digit Substitution Test (21).
- Spatial memory – assessed with the Location Learning Test. This test consists of a stimulus card with a 5 × 5 grid in which 10 everyday objects are presented at different locations. Subsequently, a stimulus card with an empty grid is shown and the 10 objects are presented on small cards that have to be relocated. Delayed recall was assessed after approximately 20 minutes (22).
- Working memory – assessed with the Digit Span (forward and backward) subtest of the Wechsler Adult Intelligence Scale (23).
- Semantic memory (long-term verbal memory) – measured with a semantic word fluency test (24).

Patients were defined as CI when their neuropsychological test score was at least 2 standard deviations below that of the HCs on a minimum of two out of five tests, corresponding to a probability of 2.5% to fall into the normal population for each test. Otherwise, patients were categorized as CP.

Symptoms of depression, anxiety and fatigue are known nuisance factors when assessing cognition. The Hospital Anxiety and Depression Scale (HADS-A and HADS-D) was used to investigate the presence of these symptoms (25). Fatigue was assessed by the Checklist of Individual Strength (CIS–20) questionnaire (26).

Magnetic resonance imaging

Scanning was performed on a 1.5-T whole-body scanner (Siemens Sonata, Erlangen, Germany) using an 8-channel phased-array head coil. Diffusion-weighted echo-planar images (repetition time [TR] 8,500 ms, echo time [TE] 86 ms, 59 axial slices, 2.0 mm isotropic resolution) were acquired, including 60 volumes with noncollinear diffusion gradients (b-value of 700 s/mm$^2$) and 10 volumes without directional weighting.
Three-dimensional T1-weighted magnetization-prepared rapid acquisition gradient-echo (MPRAGE) images (TR 2,700 ms, TE 5 ms, inversion time [TI] 950 ms and 1.3 mm isotropic resolution) were obtained for volumetric measurements.

For WM lesion detection, axial turbo spin-echo proton density and T2-weighted images (TR 3,130 ms, TE 24/85 ms) were acquired, as well as spin-echo T1-weighted images (TR 485 ms, TE 12 ms), both with 3.0 mm thickness and 1.0 mm in-plane resolution.

Three-dimensional double inversion recovery (DIR) images were acquired to detect GM lesions (TR 6,500 ms, TE 355 ms, TI 350/2,350 ms and 1.2 mm isotropic resolution).

**DTI measures**

Diffusion tensor images were corrected for head movement and eddy current distortions using FMRIB’s Diffusion Toolbox. The diffusion tensor was fitted from which the FA, MD, axial (AD) and radial diffusivity (RD) were calculated. Voxelwise statistical analysis of these DTI metrics was carried out using tract based spatial statistics (TBSS) (27), part of the FSL toolbox (http://www.fmrib.ox.ac.uk/fsl). TBSS projects the FA data of all subjects onto a mean FA tract skeleton (this procedure was repeated for MD, AD and RD) before applying voxelwise cross-subject statistics for each DTI parameter using threshold-free cluster enhancement. The 3 different groups were contrasted to each other with age and sex added as covariates to the general linear model (GLM). Differences between groups were considered significant at \( p < 0.05 \) (corrected for multiple comparisons).

**Extent and severity of WM integrity damage**

The extent of WM integrity damage for the CP and CI MS patients was determined by calculating the number of abnormal voxels compared to HCs as a percentage of the total number of voxels within the WM skeleton. Additionally, a direct comparison was made between the CP and CI MS patients to investigate whether areas of WM damage were overlapping or nonoverlapping between these groups. The severity of WM integrity damage in the overlapping areas was explored statistically using a GLM.

**Brain volumes and regional GM volume**

Normalized whole brain/GM/WM was measured using the MPRAGE images and SIENAX (28). VBM (29) of the GM was performed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm). The default processing steps in the DARTEL toolbox were followed (30). Statistical analyses of the GM volume maps were performed in a GLM in which the 3 different groups were contrasted.
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**Table 1.** Demographic and clinical measures of healthy controls and cognitively preserved and cognitively impaired MS patients.

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls</th>
<th>Cognitively preserved</th>
<th>Cognitively impaired</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td>44.5 (8.8)</td>
<td>46.7 (8.2)</td>
<td>50.2 (5.2)</td>
<td>0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>F/M</strong></td>
<td>19/11</td>
<td>28/7</td>
<td>11/9</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Educational level</strong></td>
<td>5.7 (0.9)</td>
<td>5.8 (0.7)</td>
<td>5.4 (1.1)</td>
<td>0.27</td>
</tr>
<tr>
<td><strong>RRMS/SPMS</strong></td>
<td>-</td>
<td>28/7</td>
<td>11/9</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>Disease duration, y</strong></td>
<td>-</td>
<td>11.6 (6.8)</td>
<td>11.9 (7.4)</td>
<td>0.86</td>
</tr>
<tr>
<td><strong>EDSS&lt;sup&gt;b&lt;/sup&gt;</strong></td>
<td>-</td>
<td>3.5 (2.0–7.5)</td>
<td>4.0 (2.0–7.0)</td>
<td>0.39</td>
</tr>
<tr>
<td><strong>HADS-A&lt;sup&gt;c&lt;/sup&gt;</strong></td>
<td>3.0 (2.0–6.0)</td>
<td>5.0 (4.0–8.0)</td>
<td>5.0 (4.0–8.0)</td>
<td>0.01&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>HADS-D&lt;sup&gt;c&lt;/sup&gt;</strong></td>
<td>1.0 (0–2.3)</td>
<td>4.0 (3.0–7.0)</td>
<td>4.0 (3.0–6.0)</td>
<td>&lt; 0.001&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>CIS–20&lt;sup&gt;c&lt;/sup&gt;</strong></td>
<td>25.0 (16.8–46.0)</td>
<td>72.5 (53.5–90.3)</td>
<td>82.0 (54.0–91.0)</td>
<td>&lt; 0.001&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Abbreviations: A = anxiety; CI = cognitively impaired; CIS–20 = Checklist Individual Strength, fatigue questionnaire; CP = cognitively preserved; D = depression; EDSS = Expanded Disability Status Scale; HADS = Hospital Anxiety and Depression Scale; HC = healthy control; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary-progressive multiple sclerosis.

<sup>a</sup>Data are mean (standard deviation) for normally distributed variables.

<sup>b</sup>Differences were found between HCs and CI patients.

<sup>c</sup>Variables were not normally distributed and therefore median (inter quartile range) are provided.

<sup>d</sup>Differences were found between each patient group and the HCs, but the CP and CI patients did not differ from each other.

to each other with age, sex and total brain volume added as covariates. We applied a statistical threshold of \( p < 0.05 \), family-wise error corrected to deal with multiple comparisons.

**Lesion measurements and lesion probability maps**

Cortical lesions were scored on axial reformats of the 3D DIR according to recently developed consensus guidelines (31). WM lesions were marked and manually outlined on the proton density/T2- and T1-weighted images using a local threshold technique to generate lesion probability maps (LPM) (32) in order to investigate the role of the location of WM lesions with regard to the DTI findings.

**Statistical analysis**

Statistical analyses of the demographic, clinical and volumetric variables were performed in SPSS 15.0 (Chicago, IL). When the variables were normally distributed, a multivariate GLM was used with age and sex included as covariates. When variables were not normally
distributed, the Mann–Whitney or the Kruskal–Wallis test was used ($p < 0.05$ was considered statistically significant).

**Results**

Fifty-five patients (39 females) and 30 age- and sex-matched healthy controls (19 females) participated in the study. Thirty-five MS patients were defined as CP, of which 22 patients displayed no impairment. Thirteen patients were impaired on one test. Spatial memory was impaired in 5 patients and the information processing speed in 5 others. Three patients showed impairment on working memory (Digit Span Backward).

Of the 20 CI MS patients, 9 patients were impaired on 2 tests, 6 patients on 3 tests, 4 patients on 4 tests, and 1 patient on all 5 tests. Spatial memory was most often impaired (16 patients), followed by information processing speed (14 patients), verbal memory and learning (11 patients) and working memory (Digit Span Backward; 10 patients). One patient was impaired on the Digit Span Forward. Six patients showed impaired semantic word fluency.

**Subject descriptives**

In Table 1, the demographic data of the subjects are summarized per group. Patients and controls did not differ with regard to sex or educational level. Age was different between CI patients and HCs ($p = 0.04$), but not between CP patients and HCs ($p = 0.74$) or CI patients ($p = 0.36$). The MS-specific characteristics mean disease duration, disease type and median EDSS score were not different between CP and CI patients. Both patient groups differed from the HCs regarding fatigue, anxiety and depression measures. CP patients did not differ from the CI patients on any of these measures.

**Conventional MRI measures**

The results for the MRI measures are displayed in Table 2. There was an effect of group for normalized brain volume (NBV; $F = 13.8$, $p < 0.001$), normalized GM volume (NGMV; $F = 9.7$, $p < 0.001$) and normalized WM volume (NWMV; $F = 10.4$, $p < 0.001$). Post hoc Bonferroni-corrected analyses revealed reductions in NBV for both CP and CI patients compared to HCs ($p = 0.011$ and $p < 0.001$, respectively) and for the CI patients compared to the CP patients ($p = 0.02$). NWMV was lower in CP and CI patients compared to HCs ($p = 0.008$ and $p < 0.001$), but did not differ between the two patient groups ($p = 0.217$). Reductions in NGMV were seen in the CI patients compared to the HCs ($p < 0.001$) and in
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Table 2. Structural MRI measures of healthy controls, cognitively preserved and cognitively impaired MS patients.a

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls</th>
<th>Cognitively preserved</th>
<th>Cognitively impaired</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBV, L</td>
<td>1.47 (0.06)</td>
<td>1.41 (0.07)</td>
<td>1.36 (0.09)</td>
<td>&lt; 0.001b</td>
</tr>
<tr>
<td>NGMV, L</td>
<td>0.77 (0.04)</td>
<td>0.75 (0.04)</td>
<td>0.71 (0.06)</td>
<td>&lt; 0.001c</td>
</tr>
<tr>
<td>NWMV, L</td>
<td>0.69 (0.04)</td>
<td>0.66 (0.04)</td>
<td>0.64 (0.05)</td>
<td>&lt; 0.001d</td>
</tr>
<tr>
<td>T2 lesion volume, mL</td>
<td>-</td>
<td>4.35 (2.66–6.94)</td>
<td>7.06 (3.74–11.94)</td>
<td>0.08</td>
</tr>
<tr>
<td>T1 lesion volume, mL</td>
<td>-</td>
<td>1.84 (0.80–3.32)</td>
<td>2.89 (0.08–6.79)</td>
<td>0.07</td>
</tr>
<tr>
<td>Number of cortical lesionsa</td>
<td>-</td>
<td>5 (3.0–8.5)</td>
<td>7 (2.0–13.0)</td>
<td>0.69</td>
</tr>
</tbody>
</table>

Abbreviations: CI = cognitively impaired; CP = cognitively preserved; HC = healthy control; NBV = normalized brain volume; NGMV = normalized gray matter volume; NWMV = normalized white matter volume.

a Data are mean (standard deviation).
b Differences between all groups.
c Differences between CI patients and HCs, and between CI and CP patients.
d Differences between both patient groups and HCs; no differences between the CP and CI patient group.
a Because of non-normal distribution, median and inter quartile range are provided.

CI patients compared to CP patients (p = 0.03). No differences in NGMV were found between CP patients and HCs (p = 0.12). Patient groups did not differ regarding T1 hypointense lesion volume (p = 0.08), T2 hyperintense lesion volume (p = 0.07) or number of cortical lesions (p = 0.81).

Diffusion tensor imaging

CP MS patients vs HCs

Comparing individual voxels of the WM skeleton between CP patients and HCs showed lower FA values for CP patients in 49% of the investigated WM (see Figures 1A and 2). Reduced FA was seen across the brain, including the most important WM bundles, such as the corpus callosum, optic radiations, superior and inferior longitudinal fasciculus, inferior frontal occipital fasciculus, corticospinal tracts, forceps minor and major, fornices and cingulum.

Diffusivity measures were increased in CP patients compared to HCs. MD was increased in 63% of the investigated WM. All brain areas with reduced FA also displayed an increase in MD. Additionally, increased MD was found in some juxtacortical areas without changes in FA. RD was increased in 62% of the investigated WM. The areas with increased RD overlapped with areas that showed increased MD. AD was increased in 46% of the investigated WM, partly overlapping with the MD and RD findings, although no changes in AD were found in
Figure 1. Fractional anisotropy differences between cognitively preserved and impaired patients compared to healthy controls: (A) In green the white matter (WM) skeleton is shown; all areas in blue display reduced fractional anisotropy (FA) in CP MS patients ($p \leq 0.05$); 49% of the investigated WM was affected. (B) The areas with reduced FA in CP MS (continued)
patients are shown in blue ($p \leq 0.05$); the lesion probability map is shown in red, reflecting lesion locations where $\geq 10\%$ of the patients had a T2 lesion. Cognitively impaired (CI) patients compared to healthy controls: (C) In green the WM skeleton is shown; all areas in blue display reduced FA in CI MS patients ($p \leq 0.05$); 76% of the investigated WM was affected. (D) The areas with reduced FA in CI MS patients are shown in blue ($p \leq 0.05$); the lesion probability map is shown in red, reflecting lesion locations where $\geq 10\%$ of the patients had a T2 lesion.

**Figure 2.** Extent of white matter integrity damage. The bars indicate the percentage of affected voxels (in the investigated white matter [WM]) of patients compared to healthy controls (HC) (blue and red bars) and indicate the extent of WM integrity damage. The green bars indicate differences between cognitively impaired (CI) and cognitively preserved (CP) MS patients. This is shown for all the different diffusion tensor imaging metrics (fractional anisotropy [FA], mean diffusivity [MD], radial diffusivity [RD] and axial diffusivity [AD]).

The body of the corpus callosum, corticospinal tracts, forceps minor, parts of the cingulum and superior longitudinal fasciculus (see Figure 2; blue bars).

**CI MS patients vs HCs**
In CI patients, decreased FA values and increased diffusivity measures were found in comparison to HCs. In the CI patients, FA values were lower in 76% of the investigated WM (see Figures 1C and 2). Again, reduced FA was seen in the most important WM bundles, as
mentioned above. Additionally, reduced FA was found in juxtacortical areas following the u-fibres, the uncinate fasciculus, as well as thalamus, brain stem and cerebellum.

MD was increased in 64% of the investigated WM. All areas with increased MD showed reduced FA. In the anterior thalamic radiation, cerebellum and brain stem reduced FA was present without changes in MD. In these areas an increase in RD was found (74% of the investigated WM showed increased RD). AD was increased in 34% of the investigated WM, which included the corticospinal tracts, the inferior and superior longitudinal fasciculus, thalamus, cingulum and fornices (see Figure 2; red bars).

**Direct comparison of CP vs CI MS patients**

*Severity* - CI patients had worse measures of FA, MD and RD compared to CP patients (see Figure 2; green bars). Most profound were the lower FA values in CI patients (50% of the total investigated WM) compared to CP patients. Reductions in FA (and increases in MD and RD) in overlapping brain regions (areas that showed changes in both the CP and CI patient group compared to HCs) were more severe in the CI patients. These overlapping areas included the corpus callosum, the superior and inferior longitudinal fasciculus, the corticospinal tracts, the forceps minor, the entire cingulum and fornices (see Figure 3).

*Extent* - Besides more severe damage in overlapping areas, more extensive differences in WM integrity were seen in the thalamus, the uncinate fasciculus, juxtacortical areas, brain stem and cerebellum; areas that were only affected in the CI patients.
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Table e–1. Brain areas with significant regional GM atrophy in cognitively preserved and cognitively impaired MS patients compared to healthy controls.*

<table>
<thead>
<tr>
<th>Anatomical region</th>
<th>Cluster size</th>
<th>Coordinates</th>
<th>T</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP &lt; HC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left thalamus</td>
<td>239</td>
<td>–14, –34, 3</td>
<td>8.24</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Right thalamus</td>
<td>329</td>
<td>15, –31, 4</td>
<td>7.69</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>124</td>
<td>3, 4, –0</td>
<td>6.89</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Heschl's gyrus</td>
<td>27</td>
<td>54, –7, 4</td>
<td>7.13</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Planum Polare</td>
<td>82</td>
<td>44, 2, –17</td>
<td>6.88</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Left Putamen</td>
<td>143</td>
<td>–33, –6, –0</td>
<td>6.46</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>–22, 11, –14</td>
<td>5.84</td>
<td>0.006</td>
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<tr>
<td>Insula</td>
<td>85</td>
<td>36, –3, –3</td>
<td>6.29</td>
<td>0.001</td>
</tr>
<tr>
<td>Precentral gyrus</td>
<td>34</td>
<td>6, –15, 55</td>
<td>6.24</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>–3, –31, 61</td>
<td>6.04</td>
<td>0.003</td>
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<tr>
<td>Right amygdala</td>
<td>87</td>
<td>26, 3, –14</td>
<td>6.22</td>
<td>0.001</td>
</tr>
<tr>
<td>CI &lt; HC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left putamen</td>
<td>545</td>
<td>–28, –10, –6</td>
<td>8.40</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>119</td>
<td>–22, 12, –12</td>
<td>5.93</td>
<td>0.004</td>
</tr>
<tr>
<td>Left thalamus</td>
<td>487</td>
<td>–15, –33, –0</td>
<td>8.00</td>
<td>&lt; 0.001</td>
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<tr>
<td>Right thalamus</td>
<td>652</td>
<td>14, –21, 10</td>
<td>7.89</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Left caudate</td>
<td>192</td>
<td>–10, 20, –8</td>
<td>7.42</td>
<td>&lt; 0.001</td>
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<tr>
<td>Right caudate</td>
<td>246</td>
<td>12, 22, –0</td>
<td>6.92</td>
<td>&lt; 0.001</td>
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<tr>
<td>Insula</td>
<td>872</td>
<td>36, –4, –3</td>
<td>7.39</td>
<td>&lt; 0.001</td>
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<tr>
<td></td>
<td>30</td>
<td>–42, –6, 3</td>
<td>5.79</td>
<td>0.007</td>
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<tr>
<td>Cingulate gyrus</td>
<td>148</td>
<td>–2, –30, 28</td>
<td>6.72</td>
<td>&lt; 0.001</td>
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<tr>
<td></td>
<td>36</td>
<td>–2, 2, 33</td>
<td>6.02</td>
<td>0.003</td>
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<td></td>
<td>20</td>
<td>–9, 11, 37</td>
<td>5.87</td>
<td>0.006</td>
</tr>
<tr>
<td>Heschl's gyrus</td>
<td>27</td>
<td>54, –7, 4</td>
<td>6.67</td>
<td>&lt; 0.001</td>
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<tr>
<td>Subcallosal cortex</td>
<td>118</td>
<td>2, 8, –6</td>
<td>6.63</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Precentral gyrus</td>
<td>102</td>
<td>–8, –21, 46</td>
<td>6.61</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>81</td>
<td>6, –16, 55</td>
<td>6.37</td>
<td>0.001</td>
</tr>
<tr>
<td>Inferior frontal gyrus</td>
<td>43</td>
<td>51, 10, 13</td>
<td>6.06</td>
<td>0.003</td>
</tr>
<tr>
<td>Left amygdala</td>
<td>42</td>
<td>–33, 0, –21</td>
<td>5.90</td>
<td>0.005</td>
</tr>
</tbody>
</table>

*Regions of GM that showed reduced volume in CP and CI MS patients compared to HCs at a threshold of $p < 0.05$, family wise error corrected, with age, sex and total GM volume as covariates. Only clusters with > 20 voxels are shown.

Spatial correspondence DTI findings to conventional MRI measures

Lesion probability maps - The LPMs showed WM lesions in MS-specific areas such as the periventricular areas (see Figures 1, B and D) that also displayed DTI abnormalities. A large part of the DTI abnormalities did not coincide with the LPM, and no apparent differences in
LPMs could be detected between the CP and CI patients. Post hoc analysis confirmed this: no differences in median lesion fraction of the skeleton (lesion volume inside the WM skeleton/total volume of the skeleton) could be detected between CP (2.0%) and CI patients (2.9%).

Cortical lesions - To investigate the clinical relevance of the pronounced differences of DTI metrics inside the thalamus of CI patients, Spearman correlations were calculated between FA and MD of the skeletonized WM bundles in the thalamus, and the number of cortical lesions (CLs) as measured on DIR. Higher MD was correlated with a higher number of CLs ($r = 0.524, p = 0.045$). No correlations were detected between FA and number of CLs.

Regional GM volume - In both patient groups, a reduction in regional GM volume was seen in several areas of the brain (e.g., thalamus, putamen, insula) compared to HCs (see Table e–1 and Figure e–1). In CP patients, reduced GM volume was most prominent in the thalamus (bilaterally). CI patients showed most pronounced damage in the left putamen, thalamus bilaterally and the insula bilaterally. The amount of reduced GM volume was more extensive.
(larger cluster sizes) compared to CP patients. In a direct comparison, the patient groups did not differ on regional GM volume.

**Discussion**

Using a stringent definition of cognitive impairment, we were able to distinguish CP from CI MS patients based on microstructural WM integrity differences as measured with DTI. More extensive and more severe changes in DTI metrics were seen in CI patients compared to CP patients.

While the overlapping affected areas (more severely damaged in CI patients) may be relatively clinically unspecific, the areas that were additionally affected in the CI patients are more relevant for cognition. The uncinate fasciculus connects the anterior part of the temporal lobe with the orbital and frontal cortex (33) and is thought to be involved in emotion, decision-making and episodic memory. In patients with Alzheimer disease, reduced FA was found in this particular WM tract (34). This is interesting in light of our findings, especially since 16 of our 20 CI patients were impaired on memory function.

Previous studies showed positive correlations between MS lesions in the juxtacortical areas and cognitive impairment (35). The more pronounced DTI changes found in these areas in our CI patients (more stringently defined than is commonly done in the field, i.e., test scores of 2 standard deviations instead of 1.5 standard deviation below the HCs) reflect more subtle pathological processes compared to lesions, and thus likely have an independent effect on cognitive performance as well.

Of great interest is the prominent effect of WM integrity differences within the thalamus. Thalamic atrophy was previously shown to play a major role in cognitive impairment (36). Thalamic GM reductions were also found in our study, but did not differ between CP and CI patients, while decreased thalamic FA was found in the CI patients only. Other studies reported increased FA values within the thalamus of MS patients (37,38). These contradictory results can possibly be explained by the fact that the current study used TBSS, which only maps the WM bundles within the thalamus, instead of measuring the FA in this GM structure as a whole.

The most evident differences between CP and CI patients were seen in the FA measures, which are mostly driven by increased RD. Animal models suggest that an increase in RD is associated with demyelination, while the increase of AD reflects axonal injury (39). Our data
suggest that measures of subtle demyelination more clearly differentiate between CP and CI patients than measures of axonal injury.

Some limitations apply to this work. The CI patients were older compared to HCs. Most importantly, they were not older than the CP patients \( (p = 0.36) \). To prevent an unwanted influence of age on the main study outcome, we added age (and sex) as covariates in both the TBSS and VBM analyses.

The two patient groups did not differ in regional GM volume. Recently, regional GM atrophy differences were reported between CP and CI patients from different MS subtypes \( (40) \). Even though we did not detect differences between the two patient groups, visual inspection of the regions that were found for CP and CI patients compared to the HCs are in line with these previous results. Additionally, total GM volume (as measured with SienaX) was different between CP and CI patients. The absence of differences in regional GM volume in the direct comparison between CP and CI patients may be due to our relatively small sample size.

A trend towards a higher WM lesion load in CI patients was observed which might have been of influence on the outcome since TBSS measures both in lesions and in normal-appearing WM. However, the distribution of WM lesions on the LPMs were approximately the same in both patient groups, suggesting that subtle WM changes are at least partly independent of focal WM damage.

In this study, several structural imaging techniques were used to differentiate CP from CI MS patients. However, only DTI measures, which allow for measurement of subtle WM integrity changes, diverged between CP and CI MS patients. In CI patients, more extensive WM integrity changes were seen in the uncinate fasciculus, juxtacortical areas and thalamus. These structures all play an important role in cognition. A next step will be to investigate the sensitivity and specificity of DTI to detect changes over time and the exact relation with cognitive deterioration. DTI is easy to obtain and a robust and reliable postprocessing pipeline is available, although standardized acquisition protocols are necessary. Ultimately, DTI might become a useful outcome measure to monitor or predict the cognitive effects of MS.

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Chapter 4.1

References


Chapter 4.1


