Chapter 3

The relationship between neurodegeneration and other pathology in multiple sclerosis
Highlights

- Normalized gray matter volume, normalized deep gray matter volume and cortical thickness are reduced in patients with long-standing MS compared to healthy controls.

- In patients with MS, whole brain gray matter and deep gray matter atrophy were particularly explained by white matter atrophy and lesion volume, while cortical atrophy was associated with normal-appearing white matter integrity loss.

- The relation between gray matter atrophy and white matter pathology is weaker in patients with progressive MS compared to patients with relapsing-remitting MS, suggesting a more independent neurodegenerative disease process in these patients.
Chapter 3.1

What explains gray matter atrophy in long-standing multiple sclerosis?

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Martijn D Steenwijk
Marita Daams
Petra JW Pouwels
Lisanne J Balk
Prejaas K Tewarie
Joep Killestein
Bernard MJ Uitdehaag
Jeroen JG Geurts
Frederik Barkhof
Hugo Vrenken
Chapter 3.1

Abstract

Purpose
To identify the focal and diffuse white matter (WM) pathology measures that are related to respectively whole brain, deep and cortical gray matter (GM) atrophy in long-standing multiple sclerosis (MS).

Materials and methods
The institutional review board approved the study; all subjects gave written informed consent. MRI was performed at 3T in 208 MS patients with long-standing disease (disease duration ≥ 10 years) and 60 healthy controls. Normalized gray and white matter volumes (NGMV and NWMV, respectively), normalized deep GM volumes (NDGMV), cortical thickness (CTh) and normalized lesion volumes (NLV) were quantified. Tissue integrity of normal-appearing WM (NAWM) and lesions was measured using diffusion tensor imaging. Multivariate associations between measures of GM atrophy and WM pathology were assessed in the patient group using multiple linear regression.

Results
NGMV, NDGMV and CTh were reduced in patients (all \( p < 0.001 \)). The final model for NGMV consisted of NWMV, NLV, age and sex (adjusted \( R^2 = 0.58; p < 0.001 \)). NWMV, NLV and sex were the explanatory variables for NDGMV (adjusted \( R^2 = 0.75; p < 0.001 \)). The model for CTh consisted of fractional anisotropy of NAWM, NLV, age and sex (adjusted \( R^2 = 0.32; p < 0.001 \)). The relationship between GM atrophy and WM pathology was weaker in primary and secondary-progressive compared to relapsing-remitting patients.

Conclusion
Whole brain and deep GM atrophy were particularly explained by WM atrophy and lesion volume, while cortical atrophy was associated with NAWM integrity loss. The weaker relationship between GM atrophy and WM pathology in progressive patients might indicate a more independent neurodegenerative disease process in these patients.
What explains gray matter atrophy in MS?

Introduction

Multiple sclerosis (MS) is a chronic inflammatory and neurodegenerative disease of the central nervous system. Although white matter (WM) lesions are still the most important MRI characteristic used in today’s MS diagnostics (1) and clinical trials (2–4), it has been recognized that gray matter (GM) atrophy is a crucial component of the disease (5). GM atrophy is present early in the disease (6,7), across different clinical subtypes (7–9), and is associated with physical disability and cognitive decline (6,10–18).

Although the exact mechanism underlying GM atrophy is unknown, several hypotheses have been postulated. They include primary damage of the GM, such as demyelination, neuronal loss and glial and synapse reduction, but also secondary damage due to axonal transection by lesions (19). Several studies used MRI to investigate the presumed relationship between GM atrophy and WM pathology. They mostly found an association between GM loss and lesion load (9,11,14,18,20–23), but were typically performed in patients with relatively short disease duration. Neurodegenerative aspects of the disease, however, may be more suitably addressed in cohorts of patients with long-standing disease (24). Measures of pathological and physical decline will be more pronounced in these patients, which might allow for a more reliable recognition of possible differences between clinical subtypes and disease mechanisms.

The purpose of this study was therefore to identify the focal and diffuse white matter (WM) pathology measures that are related to respectively whole brain, deep and cortical GM atrophy in long-standing MS.

Materials and methods

Participants

The institutional ethics review board approved the study protocol and all subjects gave written informed consent prior to participation. The study was conducted between March 2011 and November 2012. All patients were diagnosed with clinically definite MS (1) and prospectively recruited from our MS center if they had a disease duration of at least 10 years since first symptom. Clinical subtype was confirmed on the day of scanning and disease severity was measured using the Expanded Disability Status Scale (EDSS) (25,26). To allow for comparison of the imaging measures in the MS patients with control values, healthy control subjects were recruited via advertisements in the hospital and via non-related family and friends. Patients were not allowed to participate if they could not undergo MRI investigation or received
steroid treatment in the six weeks prior to participation. Exclusion criteria were the presence or history of psychiatric or neurological disease (for patients: other than MS), incomplete data or insufficient image quality. Twenty four patients and five healthy controls were excluded on the basis of the exclusion criteria. The use of disease-modifying therapy was recorded for all patients.

**MR imaging**

MR imaging was performed by MDS and MD (each having three years of experience in MR acquisition) on a 3T whole body scanner (GE Signa HDxt, Milwaukee, WI, USA) using an eight-channel phased-array head coil. The protocol included a 3D T1-weighted fast spoiled gradient recalled echo (FSPGR) sequence (repetition time (TR) 7.8 ms, echo time (TE) 3 ms, inversion time (TI) 450 ms, flip angle 12º, sagittal 1.0 mm slices, 0.94 × 0.94 mm² in-plane resolution) for volumetric measurements and a 3D fluid attenuated inversion recovery sequence (FLAIR; TR 8000 ms, TE 125 ms, TI 2350 ms, sagittal 1.2 mm slices, 0.98 × 0.98 mm² in-plane resolution) for lesion detection. Furthermore, 2D echo-planar DTI images (TR 13000 ms, TE 86 ms, 2.4 mm slices, 2.0 × 2.0 mm² in-plane resolution) were acquired, including 30 volumes with noncollinear diffusion gradients (b-value 900 s/mm²) and 5 volumes without diffusion weighting.

**Image analysis**

Image analysis was performed by one author (MDS) with 5 years experience in medical image analysis. An overview of the image processing pipeline is displayed in Figure 1. WM lesions were segmented using kNN-TTP (27). In short, this method compares the voxels of a newly presented data set with a collection of manually labeled examples in a feature space. The features included are voxel-wise FLAIR and T1 signal intensity (see Figures 1A and 1B); normalized spatial coordinates; and tissue type priors describing the suspected tissue class to which the voxel would have belonged before the lesion developed. For every newly presented voxel, the algorithm locates the most similar examples in the training set and computes the probability of being a lesion (see Figure 1C). The resulting probability map is thresholded to obtain a binary lesion mask. Importantly, the training set for lesion segmentation was generated from images acquired identical as those in the present study. The resulting lesion volume was normalized for head size, thus creating normalized lesion volume (NLV).

Volumetrics were performed on the T1-weighted images. Lesion filling was applied to minimize the impact of hypointense lesions on atrophy measurements (see Figure 1D) (28). Normalized whole brain, GM and WM volumes (NBV, NGMV and NWMV respectively) were measured...
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With SIENAX (part of FSL 5.0.2, http://www.fmrib.ox.ac.uk/fsl) using optimized parameters for brain extraction (see Figure 1E) (29,30). Deep GM (DGM) volumes were measured using FSL-FIRST (31). This provided the volumes of the bilateral nucleus accumbens, amygdala, caudate, hippocampus, globus pallidus, putamen and thalamus, which were summed and normalized for head size to form a total normalized DGM volume (NDGMV). Cortical thickness was measured using FreeSurfer 5.1 (32,33). FreeSurfer uses the T1-weighted image to locate the WM and pial surface. The distance between both surfaces gives the cortical thickness at each location (see Figures 1F and 1G). All segmentations were checked and re-run if errors occurred. The mean thicknesses of both hemispheres were averaged to obtain the average cortical thickness (CTh) per subject.

Tissue integrity of the NAWM and lesions was measured using the DTI images, which were corrected for head movement and eddy current distortions using the FMRIB’s Diffusion Toolbox (also part of FSL). Subsequently the diffusion tensor was fitted to calculate fractional anisotropy (FA), mean (MD), axial (AD) and radial diffusivity (RD). To quantify NAWM integrity, the T1-weighted image of each subject was registered to the non-weighted diffusion

Figure 1. Overview of the image processing steps involved in a typical subject (female, 54 years, SPMS, EDSS = 3.5, disease duration 29 years). Using the T1-weighted (A) and FLAIR (B) image, a lesion segmentation (C) is obtained. Then the lesions in the original T1-weighted image are filled (D). Subsequently SIENAX is used to obtain gray matter, white matter and CSF segmentations (E) and FIRST is used to segment the deep gray matter structures (not shown). Then the FreeSurfer pipeline is used to obtain average cortical thickness (F, G). Finally, average DTI-metrics such as fractional anisotropy (H) are obtained in the normal-appearing white matter (I) and lesions (J).
image using boundary based registration. The resulting registration was used to transform the SIENAX WM and lesion mask into DTI space, which were combined to form an NAWM mask (see Figure 1I). The diffusion metrics in the NAWM were then averaged to obtain $\text{FA}_{\text{NAWM}}$, $\text{MD}_{\text{NAWM}}$, $\text{AD}_{\text{NAWM}}$, $\text{RD}_{\text{NAWM}}$. Average lesion integrity was quantified by averaging the diffusion metrics inside the lesion mask (see Figure 1J).

**Statistical analysis**

Statistical analyses were performed in SPSS 20.0 (Chicago, IL, USA). Kolmogorov–Smirnov tests and visual inspection of the histogram were used to assess normality of the variables. When variables were normally distributed, a multivariate GLM was used. Otherwise, the Mann–Whitney or Kruskal–Wallis test was used. If applicable, analyses were Bonferroni-corrected. \( P \)-values < 0.05 were considered statistically significant.

In the patients, univariate correlations of NWMV, NLV, $\text{FA}_{\text{NAWM}}$ and $\text{FA}_{\text{lesion}}$ with NGMV, NDGMV and CTh were assessed using partial correlations, with age and sex as covariates. Then multiple linear regression models were constructed with respectively NGMV, NDGMV and CTh as dependent variables. Disease duration and WM pathology measures showing univariate correlations with the respective dependent, were entered as candidate variables into a combined model using an automated forward stepwise selection technique. To limit the number of candidate variables, AD and RD were excluded as they are covered by FA and MD. The analyses were repeated for each clinical subtype to investigate potential differences.

The multivariate behavior of the main outcome measures in the patients was further explored using so-called parallel coordinate plots (PCPs) (34). Such a representation can be used to easily obtain a visual interpretation of multivariate trends in high dimensional data sets. To increase the visibility of patterns, a scattering points approach was adopted, and the data on the vertical axes (except for sex) were rank-ordered. The PCPs were constructed using Matlab R2011a (Natick, MA, USA).

**Results**

**Demographic and clinical characteristics**

A total of 208 (67% female) MS patients and 60 (62% female) healthy controls were included. In Table 1, the demographic and structural MRI data are summarized per group. The MS group was relatively old, had an average disease duration of 20 years and consisted of 130
What explains gray matter atrophy in MS?

Table 1. Demographic and MRI measures.$^a$

<table>
<thead>
<tr>
<th></th>
<th>HC (n = 60)</th>
<th>MS (n = 208)</th>
<th>RRMS (n = 130)</th>
<th>SPMS (n = 53)</th>
<th>PPMS (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>50.33 ± 7.08</td>
<td>53.70 ± 9.62**</td>
<td>50.68 ± 9.53</td>
<td>57.00 ± 6.76***</td>
<td>62.40 ± 7.66***</td>
</tr>
<tr>
<td>F/M</td>
<td>37/23</td>
<td>141/67</td>
<td>97/33</td>
<td>34/19</td>
<td>10/15</td>
</tr>
<tr>
<td>Disease duration, y</td>
<td>-</td>
<td>20.20 ± 7.08</td>
<td>19.03 ± 6.23</td>
<td>23.05 ± 8.50</td>
<td></td>
</tr>
<tr>
<td>EDSS$^b$</td>
<td>-</td>
<td>4.0 (3.0–6.0)</td>
<td>3.0 (2.5–4.0)</td>
<td>6.0 (4.0–7.0)</td>
<td></td>
</tr>
<tr>
<td>NBV, L</td>
<td>1.49 ± 0.07</td>
<td>1.41 ± 0.09***</td>
<td>1.43 ± 0.10***</td>
<td>1.39 ± 0.08***</td>
<td>1.41 ± 0.10***</td>
</tr>
<tr>
<td>NGMV, L</td>
<td>0.80 ± 0.05</td>
<td>0.75 ± 0.06***</td>
<td>0.76 ± 0.06*</td>
<td>0.73 ± 0.05***</td>
<td>0.74 ± 0.07***</td>
</tr>
<tr>
<td>NWMV, L</td>
<td>0.70 ± 0.03</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>NDGMV, mL</td>
<td>63.45 ± 4.71</td>
<td>57.27 ± 6.62***</td>
<td>58.00 ± 6.73***</td>
<td>55.21 ± 6.28***</td>
<td>57.60 ± 6.26***</td>
</tr>
<tr>
<td>CTh, mm</td>
<td>2.56 ± 0.09</td>
<td>2.47 ± 0.10***</td>
<td>2.48 ± 0.10***</td>
<td>2.43 ± 0.09***</td>
<td>2.45 ± 0.11***</td>
</tr>
<tr>
<td>LV, mL$^b$</td>
<td>-</td>
<td>11.24</td>
<td>9.70 (3.86–19.26)</td>
<td>13.93</td>
<td>12.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3.92–20.50)</td>
<td>(3.76–25.56)</td>
<td>(6.03–16.06)</td>
<td></td>
</tr>
<tr>
<td>NLV, mL$^b$</td>
<td>-</td>
<td>18.09</td>
<td>16.80</td>
<td>24.65</td>
<td>15.24</td>
</tr>
<tr>
<td>FA$_{\text{NAWM}}$</td>
<td>0.39 ± 0.02</td>
<td>0.37 ± 0.03***</td>
<td>0.37 ± 0.03***</td>
<td>0.36 ± 0.02***</td>
<td>0.37 ± 0.02**</td>
</tr>
<tr>
<td>MD$_{\text{NAWM}}$</td>
<td>0.83 ± 0.03</td>
<td>0.85 ± 0.03***</td>
<td>0.85 ± 0.03***</td>
<td>0.86 ± 0.03***</td>
<td>0.85 ± 0.03**</td>
</tr>
<tr>
<td>AD$_{\text{NAWM}}$</td>
<td>1.19 ± 0.03</td>
<td>1.20 ± 0.03</td>
<td>1.20 ± 0.03</td>
<td>1.20 ± 0.03</td>
<td>1.20 ± 0.03</td>
</tr>
<tr>
<td>RD$_{\text{NAWM}}$</td>
<td>0.65 ± 0.03</td>
<td>0.68 ± 0.04***</td>
<td>0.68 ± 0.04***</td>
<td>0.70 ± 0.04***</td>
<td>0.67 ± 0.04*</td>
</tr>
<tr>
<td>FA$_{\text{lesion}}$</td>
<td>-</td>
<td>0.28 ± 0.03</td>
<td>0.29 ± 0.03</td>
<td>0.28 ± 0.03</td>
<td>0.29 ± 0.03</td>
</tr>
<tr>
<td>MD$_{\text{lesion}}$</td>
<td>-</td>
<td>1.32 ± 0.09</td>
<td>1.30 ± 0.09</td>
<td>1.35 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>AD$_{\text{lesion}}$</td>
<td>-</td>
<td>1.71 ± 0.10</td>
<td>1.70 ± 0.11</td>
<td>1.75 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>RD$_{\text{lesion}}$</td>
<td>-</td>
<td>1.11 ± 0.09</td>
<td>1.10 ± 0.09</td>
<td>1.15 ± 0.07</td>
<td></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; NBV = normalized brain volume; NGMV = normalized gray matter volume; NWMV = normalized white matter volume; NDGMV = normalized deep gray matter volume; CTh = cortical thickness; LV = lesion volume; NLV = normalized lesion volume; FA = fractional anisotropy; MD = mean diffusivity; AD = axial diffusivity; RD = radial diffusivity. Note: MD, RD and AD were expressed as 10$^{-3}$ mm$^2$/sec.

$^a$Values listed are mean ± standard deviation for normally distributed variables, p-values were Bonferroni-corrected where applicable.

$^b$Variables were not normally distributed and therefore median (inter quartile range) is provided.

*p < 0.05, **p < 0.01 and ***p < 0.001 (compared with healthy controls)

$p < 0.05$, $^p < 0.01$ and $^||p < 0.001$ (compared with RRMS)

relapsing-remitting (RR), 53 secondary-progressive (SP) and 25 primary-progressive (PP) patients. Of the MS patients, 10 were using glatiramer, 40 beta-interferon and 9 natalizumab.
Table 2. Partial correlations between white matter pathology measures and gray matter atrophy measures in MS patients, corrected for age and sex.

<table>
<thead>
<tr>
<th></th>
<th>NGMV</th>
<th>NDGMV</th>
<th>CTh</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MS</td>
<td>RR</td>
<td>SP</td>
</tr>
<tr>
<td></td>
<td>(n = 208)</td>
<td>(n = 130)</td>
<td>(n = 53)</td>
</tr>
<tr>
<td>NWMV</td>
<td>0.67***</td>
<td>0.66***</td>
<td>0.60***</td>
</tr>
<tr>
<td>NLV</td>
<td>−0.52***</td>
<td>−0.57***</td>
<td>−0.36***</td>
</tr>
<tr>
<td>FA&lt;sub&gt;NAWM&lt;/sub&gt;</td>
<td>0.51***</td>
<td>0.52***</td>
<td>0.45***</td>
</tr>
<tr>
<td>MD&lt;sub&gt;NAWM&lt;/sub&gt;</td>
<td>−0.48***</td>
<td>−0.51***</td>
<td>−0.45***</td>
</tr>
<tr>
<td>AD&lt;sub&gt;NAWM&lt;/sub&gt;</td>
<td>−0.24***</td>
<td>−0.25**</td>
<td>ns</td>
</tr>
<tr>
<td>RD&lt;sub&gt;NAWM&lt;/sub&gt;</td>
<td>−0.53***</td>
<td>−0.55***</td>
<td>−0.48***</td>
</tr>
<tr>
<td>FA&lt;sub&gt;lesion&lt;/sub&gt;</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>MD&lt;sub&gt;lesion&lt;/sub&gt;</td>
<td>−0.19**</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>AD&lt;sub&gt;lesion&lt;/sub&gt;</td>
<td>−0.24***</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>RD&lt;sub&gt;lesion&lt;/sub&gt;</td>
<td>−0.16*</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

Abbreviations: NGMV = normalized gray matter volume; NDGMV = normalized deep gray matter volume; CTh = cortical thickness; MS = multiple sclerosis; RR = relapsing-remitting; SP = secondary-progressive; PP = primary-progressive; NWMV = normalized white matter volume; NLV = normalized lesion volume; FA = fractional anisotropy; MD = mean diffusivity; AD = axial diffusivity; RD = radial diffusivity; ns = not significant.

*p < 0.05, **p < 0.01 and ***p < 0.001
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Age was different between progressive patients and controls, but not between RRMS patients and controls. Sex distribution was equal in all groups ($p = 0.36$). PPMS and SPMS patients had higher EDSS scores than RRMS patients.

**MRI characteristics of MS patients**

The patients had a median lesion volume of 11.24 mL, consistent with moderate to advanced disease. Patients had lower NBV, NGMV, NWMV, NDGMV and CTh than controls (see Table 1). Furthermore, patients displayed decreased $\text{FA}_{\text{NAWM}}$ while $\text{MD}_{\text{NAWM}}$ and $\text{RD}_{\text{NAWM}}$ were increased. EDSS scores showed negative correlations with NGMV, NDGMV and CTh ($r = -0.303; p < 0.001$, $r = -0.272; p = 0.001$ and $r = -0.235; p < 0.001$, respectively).

All clinical subtypes showed reduced volume and thickness measures compared to controls, except NWMV in PPMS patients ($p = 0.17$). Independent of clinical phenotype, $\text{FA}_{\text{NAWM}}$ was reduced compared to controls, while $\text{MD}_{\text{NAWM}}$ and $\text{RD}_{\text{NAWM}}$ were increased. Compared to RRMS, SPMS patients showed lower NBV, NGMV, NDGMV and CTh, and larger NLV, but no differences could be detected in NWMV. Furthermore, differences were detected in $\text{FA}_{\text{NAWM}}$ and the diffusivity measures in lesions. No differences could be detected when comparing PPMS to RRMS, except for NGMV which was lower in PPMS patients.

**Relation between GM atrophy and WM pathology**

Table 2 shows the partial correlations between measures of GM atrophy and WM pathology in the MS patients. In the total patient group, NGMV, NDGMV and CTh showed correlations with all WM measures except with $\text{FA}_{\text{lesion}}$. Within the clinical subtypes, GM atrophy measures displayed similar correlations with NWMV and NLV, although no correlation was detected between CTh and any of the WM measures in SPMS.

$\text{FA}_{\text{NAWM}}$ showed significant positive correlations with all GM measures in RRMS patients, with NGMV and NDGMV in SPMS patients, but no correlations in PPMS patients. In the patients with RRMS and SPMS, diffusivities within the lesions were correlated with NDGMV, whereas in the PPMS patients, diffusivities within the lesions were correlated with cortical thickness.

**Multiple linear regression of GM atrophy measures in MS patients**

Since $\text{FA}_{\text{lesion}}$ did not show a significant correlation with any of the GM atrophy measures, it was not used as a candidate variable for multiple linear regression. As displayed in Table 3, the final model for NGMV consisted of NWMV, NLV, age and sex, and explained 58% of the variance. NWMV, NLV and sex were the final explanatory variables for NDGMV. This model
Table 3. Multiple linear regression of NGMV, NDGMV and CTh in MS patients and clinical subtypes, age and sex were entered as covariates.\(^a\)

<table>
<thead>
<tr>
<th>MS (n = 208)</th>
<th>NGMV (F(4, 203) = 73.59, p &lt; 0.001,) adjusted (R^2 = 0.58)</th>
<th>NDGMV (F(4, 203) = 155.99, p &lt; 0.001,) adjusted (R^2 = 0.75)</th>
<th>CTh (F(4, 204) = 33.68, p &lt; 0.001,) adjusted (R^2 = 0.32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NWMV (0.499^{***})</td>
<td>NWMV (0.598^{***})</td>
<td>FA(_{NWM}) (-0.286^{***})</td>
<td></td>
</tr>
<tr>
<td>NLV (-0.226^{***})</td>
<td>NLV (-0.407^{***})</td>
<td>NLV (-0.216^{**})</td>
<td></td>
</tr>
<tr>
<td>Age (-0.376^{***})</td>
<td>Age (-0.045)</td>
<td>Age (-0.277^{***})</td>
<td></td>
</tr>
<tr>
<td>Sex(^b) (0.235^{***})</td>
<td>Sex(^b) (0.165^{***})</td>
<td>Sex(^b) (0.164^{**})</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RRMS (n = 130)</th>
<th>NGMV (F(5, 124) = 37.86, p &lt; 0.001,) adjusted (R^2 = 0.59)</th>
<th>NDGMV (F(5, 124) = 93.85, p &lt; 0.001,) adjusted (R^2 = 0.78)</th>
<th>CTh (F(4, 204) = 22.77, p &lt; 0.001,) adjusted (R^2 = 0.40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NWMV (0.426^{***})</td>
<td>NWMV (0.54^{***})</td>
<td>NLV (-0.357^{***})</td>
<td></td>
</tr>
<tr>
<td>NLV (-0.231^{**})</td>
<td>NLV (-0.407^{***})</td>
<td>FA(_{NWM}) (0.247^{*})</td>
<td></td>
</tr>
<tr>
<td>MD(_{NWM}) (-0.153^{*})</td>
<td>MD(_{NWM}) (-0.119^{*})</td>
<td>Age (-0.331^{***})</td>
<td></td>
</tr>
<tr>
<td>Age (-0.358^{***})</td>
<td>Age (-0.062)</td>
<td>Sex(^b) (0.118)</td>
<td></td>
</tr>
<tr>
<td>Sex(^b) (0.208^{**})</td>
<td>Sex(^b) (0.179^{***})</td>
<td></td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>SPMS (n = 53)</th>
<th>NGMV (F(3, 49) = 14.79, p &lt; 0.001,) adjusted (R^2 = 0.44)</th>
<th>NDGMV (F(4, 48) = 37.52, p &lt; 0.001,) adjusted (R^2 = 0.74)</th>
<th>CTh (F(2, 52) = 2.538, p = 0.89,) adjusted (R^2 = 0.06)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NWMV (0.563^{***})</td>
<td>NWMV (0.654^{***})</td>
<td>Age (-0.160)</td>
<td></td>
</tr>
<tr>
<td>Age (-0.347^{**})</td>
<td>NLV (-0.385^{***})</td>
<td>Sex(^b) (0.272^{*})</td>
<td></td>
</tr>
<tr>
<td>Sex(^b) (0.345^{**})</td>
<td>Age (0.075)</td>
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<tr>
<th>PPMS (n = 25)</th>
<th>NGMV (F(3, 21) = 21.25, p &lt; 0.001,) adjusted (R^2 = 0.72)</th>
<th>NDGMV (F(3, 21) = 14.65, p &lt; 0.001,) adjusted (R^2 = 0.63)</th>
<th>CTh (F(3, 21) = 9.46, p &lt; 0.001,) adjusted (R^2 = 0.51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NWMV (0.817^{***})</td>
<td>NWMV (0.723^{***})</td>
<td>MD(_{lesion}) (-0.623^{***})</td>
<td></td>
</tr>
<tr>
<td>Age (-0.374^{**})</td>
<td>Age (-0.127)</td>
<td>Age (-0.358^{*})</td>
<td></td>
</tr>
<tr>
<td>Sex(^b) (0.267^{*})</td>
<td>Sex(^b) (0.377^{**})</td>
<td>Sex(^b) (0.311)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary-progressive multiple sclerosis; PPMS = primary-progressive multiple sclerosis; NGMV = normalized gray matter volume; NDGMV = normalized deep gray matter volume; CTh = cortical thickness; NWMV = normalized white matter volume; NLV = normalized lesion volume; FA = fractional anisotropy; MD = mean diffusivity.

\(^a\) Values listed are the standardized betas of the final model for respectively the total patient group, RRMS, SPMS and PPMS. First, in order, the retained explanatory variables after stepwise multiple linear regression are displayed. Second, the covariates are listed in random order.

\(^b\) Male: 0; female: 1.

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

accounted for 75% of the variance. Finally, the model for CTh consisted of \(\text{FA}_{NWM}\), NLV, age.
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and sex, and accounted for 32% of the variance. Figure 2 displays the multivariate behavior of the MS patients in PCPs. In these plots, each subject is represented by a curve passing several vertical lines indicating the different variables that were measured. For example, in Figure 2E, the curve for each subject is colored by the corresponding value for NGMV. As can be seen, patients with a high NGMV (orange-red lines) who typically have high NDGMV and high CTh as well, are mostly younger females with high NWMV, small NLV and high $F_{\text{NAWM}}$, which are the main explanatory variables for GM atrophy. In addition, they have a relatively short disease duration and low EDSS. Vice-versa, patients with a low NGMV (blue lines) typically have low NDGMV and low CTh, are more often older males, and have low NWMV and high NLV.

The multiple linear regression analyses were repeated in the clinical subgroups. As described in the materials and methods, to limit the number of variables, only WM measures showing a correlation with the respective GM atrophy measure for the clinical subgroup concerned, were used as a candidate variables.

In RRMS patients, $\text{MD}_{\text{NAWM}}$ turned out to be an additional explanatory variable for NGMV and NDGMV compared to the models for the total patient group, but with low significance ($p = 0.035$ and $p = 0.024$, respectively). The total amount of explained variance for each RRMS model was approximately the same compared to the total patient group models (RRMS vs total; NGMV: 59 vs 58%; NDGMV: 78 vs 75%; CTh: 40 vs 32%).

In SPMS patients, NLV was no longer part of the model for NGMV. The model for NGMV explained 44% of the variance, compared to 58% in the total patient group. The model for NDGMV in SPMS patients contained the same variables as the analogous model for NDGMV in the total patient group, and explained 74% of the variance. As none of the WM measures showed a correlation with CTh in the SPMS patients, only disease duration was a candidate variable in the model for CTh. Disease duration was not selected however, and the model for CTh was not significant ($p = 0.089$).

Despite the small group size, the regression analyses were repeated for the PPMS patients (see Table 3). Compared to the total patient group, NLV disappeared in all models. The models for PPMS patients explained more variance in NGMV (72% compared with 58%) and CTh (51% compared with 32%) than the total group, while the model for NDGMV explained a lower amount of the variance (63% compared with 75%).
Figure 2. Parallel coordinate plots illustrating the multivariate behavior of all MS patients (A-J) and in the three clinical subtypes (K-M). Each curve corresponds to a single patient and shows the values observed for that patient on each of the outcome measures. To increase the visibility of patterns, data on the vertical axes (except for sex) were rank-
Discussion

This study demonstrates that whole brain, deep and cortical GM atrophy have different relations to WM pathology in patients with long-standing MS. More specifically, whole brain and deep GM atrophy were particularly explained by WM atrophy and lesion volume, while cortical atrophy was associated with NAWM integrity loss. The relation between GM atrophy and WM pathology was found to be weaker in progressive compared to relapsing-remitting patients. This has important implications, as it suggests a different underlying disease process in advanced MS.

Our results confirm findings from previous studies in which MS patients displayed extensive GM atrophy (9,11,14,18,20–23). In line with earlier reports, GM atrophy was most pronounced in SPMS patients and correlated with EDSS (9,11,35). WM pathology was not restricted to focal lesions, but included widespread damage to the NAWM as previously indicated by several smaller studies (36–38). NAWM damage in terms of lower FA and higher MD was present in all clinical subtypes and driven by increased RD. Despite the generally advanced stage of the disease, we did not observe increases in AD_{NAWM}. This might be explained by the fact that we used average AD_{NAWM} whereas other studies showing increased AD in MS patients particularly investigated the centers of the tracts (37,39,40). Diffusion tensor imaging measures inside the lesions revealed increased diffusivity in SPMS patients compared to RRMS, but no differences in FA. Combined histopathological and MRI studies showed that increased RD serves as a surrogate for demyelination, while increased AD, although still topic of debate, has been linked to axonal injury (41,42). This suggests that the NAWM tissue damage found in the present study particularly consists of subtle demyelination, while both demyelination and axonal damage were present in lesions.

The degree of GM atrophy showed strong correlations with measures of WM pathology. In line with the literature, lesion volume was a recurring explanatory variable for GM atrophy (11,43). Whole brain and deep GM atrophy were further explained by WM atrophy, while cortical atrophy was further explained by NAWM tissue damage. In general, age was negatively associated with whole brain GM volume and cortical thickness, but not significant...
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in the models for deep GM atrophy. Corroborating previous findings, male sex was found to be predictor for GM atrophy (17). In the regression analyses of the RRMS patients, MD of NAWM turned out to be an additional, borderline significant, explanatory variable for NGMV and NDGMV. This is in line with a previous study, which showed that MD is a significant explanatory variable for thalamic atrophy in patients with clinically isolated syndrome (22). Interestingly, compared to the models for RRMS patients, the models for the progressive patients showed a weaker relationship between measures of GM atrophy and WM pathology. For instance, lesion volume disappeared as an explanatory variable for NGMV and CTh in the SPMS patients, although NLV in this subgroup was higher than in RRMS patients. In the PPMS patients, only WM atrophy was found to be an explanatory variable for whole brain and deep gray matter volume. Cortical thickness in these patients showed an association with AD inside lesions. This could be explained by retrograde neurodegeneration due to axonal injury, but the value of this finding remains to be elucidated, as the number of PPMS patients was small and we did not control for lesion load, size or the heterogeneity of damage inside individual lesions.

Some limitations apply to this work. The MS patients were on average older than controls. To prevent an unwanted influence of age on the study outcomes, we added age (and sex) as covariates in both the correlation and regression analyses. Second, the SPMS and PPMS groups were relatively small for the large number of explanatory variables in the regression analyses. Although univariate analyses were first performed to reduce the number of variables, especially the results concerning PPMS should be interpreted with great care. Third, fifty-nine (28%) of the MS patients in this study received disease modifying therapy (DMT) which could have influenced the results. We did not control for this, because a thorough investigation of the potential influence of DMT would require differentiation of the DMT actually used, making the groups in this comparison too small. Future studies are necessary to investigate the effect of DMT on the relation between GM atrophy and WM pathology. Finally, especially in the models for cortical thickness, it cannot be ruled out that cortical lesions could have had an impact on atrophy. Further studies are needed to overcome this limitation.

In conclusion, WM atrophy and lesion volume were found to be the most important explanatory variables for whole brain and deep GM atrophy, while cortical atrophy was mainly associated with NAWM integrity loss. Analyzing the individual clinical subgroups revealed a weaker relationship between GM atrophy and WM pathology measures in the progressive clinical subtypes. This might indicate that in progressive patients, at least in patients with long-standing MS, the neurodegenerative process is less dependent on WM pathology.
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References


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