Regional cortical thinning in multiple sclerosis and its relation with cognitive impairment: A multicenter study

JM Tillema, HE Hulst, MA Rocca, H Vrenken, MD Steenwijk, D Damjanovic, C Enzinger, S Ropele, G Tedeschi, A Gallo, O Ciccarelli, A Rovira, X Montalban, N de Stefano, ML Stromillo, M Filippi, and F Barkhof on behalf of the MAGNIMS Study Group

Abstract

Objectives: The objectives of this paper are to compare in a multicenter setting patterns of regional cortical thickness in patients with relapsing–remitting multiple sclerosis (RRMS) and cognitive impairment (CI) and those cognitively preserved (CP), and explore the relationship between cortical thinning and cognitive performance.

Methods: T1-weighted isotropic brain scans were collected at 3T from seven European centers in 60 RRMS patients and 65 healthy controls (HCs). Patients underwent clinical and neuropsychological examinations. Cortical thickness (CTh) measures were calculated using FreeSurfer (failing in four) and both lobar and vertex-based general linear model (GLM) analyses were compared between study groups.

Results: Twenty (36%) MS patients were classified as CI. Mean global CTh was smaller in RRMS patients compared to HCs (left 2.43 vs. 2.53 mm, right 2.44 vs. 2.54 mm, \( p < 0.001 \)). Multivariate GLM regional analysis showed significantly more temporal thinning in CI compared to CP patients. Verbal memory scores correlated to regional cortical thinning in the insula whereas visual memory scores correlated to parietal thinning.

Conclusions: This multicenter study showed mild global cortical thinning in RRMS. The extent of thinning is less pronounced than previously reported. Only subtle regional differences between CI and CP patients were observed, some of which related to specific cognitive domains.

Keywords: Multiple sclerosis, MRI, cortical atrophy, cognitive impairment

Introduction

Cognitive impairment (CI) is present in up to 70% of all patients with multiple sclerosis (MS).\(^1\) Magnetic resonance imaging (MRI) studies have shown that atrophy measures are better predictors of CI than white matter (WM) lesion volume in MS.\(^2\)–\(^4\) Furthermore, other studies demonstrated relationships between CI and number of cortical lesions;\(^5\) diffusion tensor imaging (DTI) WM abnormalities;\(^6\) gray matter (GM) magnetization transfer ratio\(^7\) and deep GM volume loss.\(^3\)

Cortical lesions and cortical atrophy showed better correlations with CI compared to global atrophy and WM lesion measures. Cortical atrophy can be assessed on (MRI) with intensity-based segmentation methods or via surface-based methods. Regional areas of cortical thinning relate to clinical disability scores and WM lesion volume in MS.\(^8\) Bilateral thinning of the frontal and temporal cortex has been reported in MS using FreeSurfer\(^8\)–\(^10\) and other volumetric MRI analysis methods.\(^11,12\) Cortical thinning is already present in the earliest stages of MS,\(^13\) even in the absence of CI.\(^9\) Thinning of the parietal lobe occurs later in the disease and is prominent in patients with relapsing–remitting MS (RRMS) and CI, whereas these areas were preserved in patients with RRMS without CI.\(^9\)
To understand the relationship between specific regional thinning of the cortex and cognitive decline, studies investigating a well-defined, large cohort of patients with RRMS and overt CI are necessary. Focus on CI in MS is an important feature of current research as it affects quality of life, and better imaging correlates could facilitate future research focused on CI in MS. GM volume has already been used in clinical trials as a secondary outcome measure, and to investigate whether future studies could incorporate cortical thickness (CTh) measures, it is important to measure them prospectively in a multicenter setting.

To explore MRI correlates of cerebral cortical thinning in relation to CI in RRMS, we studied the relationship between regional cortical thinning and global CI as well as impairment in specific cognitive domains in a multicenter study in seven European centers of the Magnetic Resonance Imaging in MS (MAGNIMS) network.

**Methods**

**Participants**

This cross-sectional study was conducted in seven MAGNIMS (www.magnims.eu) centers, as previously described. Institutional ethics review boards in each participating center approved the study and all individuals provided written consent for participation. Inclusion criteria required all participants to be right-handed, aged between 20 and 65 years, have a diagnosis of RRMS, have no relapse or corticosteroids treatment within the month prior to scanning, and no clinically apparent motor impairment of the right upper limb. A group of healthy controls was also recruited at each site, gender matched, but not age matched (age was therefore included as a covariate in all statistical models).

Each center recruited patients with MS with known CI as well as cognitively preserved patients, and healthy controls. Clinical information on patients was collected, including the Expanded Disability Status Scale (EDSS) score at time of MRI, disease duration, use of disease-modifying treatments and years of education.

**Cognitive testing**

All patients underwent the Brief Repeatable Battery of neuropsychological tests (BRB-N) within 48 hours of obtaining the MRI by trained local personnel unaware of the MRI results, using validated translations of the neuropsychological testing. The BRB-N includes the Selective Reminding Test (SRT) assessing verbal memory, Spatial Recall Test (10/36 SRT) to assess visual memory, Paced Auditory Serial Addition Test (PASAT, 2” and 3”) and Symbol Digit Modalities Test (SDMT) to assess attention, and Controlled Oral Word Association Test (COWAT), and Word List Generation (WLG) to assess verbal fluency. Individual test scores were combined and z-scores were calculated for four cognitive domains (verbal memory, visual memory, attention and fluency), based on published data from healthy controls. In addition, the Wisconsin Card Sorting Test (WCST) was administered to evaluate executive functions. Patients with at least two abnormal tests (defined as a score more than 2 standard deviations (SDs) below the normative value) were considered cognitively impaired (CI).

**MRI data acquisition**

All participants at each individual site underwent MRI of the brain using a standardized protocol. MRI scans were obtained at 3.0 Tesla scanners at all sites (Amsterdam and Naples: GE Signa; Barcelona, Graz and London: Siemens Trio; Milan and Siena: Philips Intera). In all individuals, the following sequences of the brain were collected during a single session: a) dual-echo turbo-spin-echo (TSE): repetition time (TR) ranging from 4000 to 5380 ms, TE1 ranging from 10 to 23 ms, TE2 ranging from 90 to 102 ms, echo train length (ETL) ranging from 5 to 11, 44 contiguous, 3-mm thick axial slices, parallel to the anterior commissure-posterior commissure (AC-PC) plane, with a matrix size = 256 × 192 and a field of view (FOV) = 240 × 180 mm² (recFOV = 75%); and b) three-dimensional (3D) T1-weighted scan: TR = ranging from 5.5 to 8.3 ms (for GE/Philips scanners) and from 1900 to 2300 ms (for Siemens scanners); TE = ranging from 1.7 to 3.0 ms; flip angle ranging from 8 degrees to 12 degrees, 176 to 192 sagittal slices with thickness = 1 mm and in-plane resolution = 1 × 1 mm².

**Lesion measurements**

WM lesions maps were created centrally at the Neuroimaging Research Unit (Milan, Italy) by experienced observers. T2-hyperintense (T2-LL) and T1-hypointense lesion volumes (T1BH-LL) were measured on dual-echo TSE and 3D T1-weighted scans, respectively, using a local thresholding segmentation technique (Jim 5.0, Xinapse System, Leicester, UK).

**MRI data analysis: cortical thickness and surface-based cortical volume**

Surface-based cortical analysis was performed using FreeSurfer 5.1.0. Cortical thickness measures have...
demonstrated good test-retest reliability across scanner manufacturers and across field strengths. Briefly, this processing pipeline performs vertex-based cortical thickness calculations from pial and WM surfaces, obtained from the T1-weighted images, using previously in detail described and validated steps.

Visual inspection and standard manual corrections with subsequent continuation of the processing pipeline resulted in good results in the majority of the scans. In the MS group some adjustments of the WM segmentation maps were necessary when T1 hypo-intense lesions were not recognized in the initial segmentation step. Lesion color overlay maps on the initial segmentation was created. Lesions accurately classified as WM were colored green, lesions classified as non-WM, red. These maps were used to fill the WM-segmentation maps, accurately designating lesions as WM, which then fed back into the processing pipeline. To avoid artificial thinning of the CTh measures, we opted not to fill juxta-cortical and leukocortical lesions. Each scan was closely inspected visually for errors. The vast majority completed through the processing pipeline followed this filling method without difficulties and a typical example is provided in Figure 1.

**Lobar analysis**

The cerebral cortex was parcellated into six lobar structures (cingulate, frontal, insula, occipital, parietal and temporal), with extraction of average hemispheric lobar CTh and surface-based cortical volumetric measures. Surface-based volumes were normalized by multiplying the native cortical GM volumes with the scaling factor for registration to the Montreal Neurological Institute (MNI) template. Given the correlation between age and CTh (Figure 2), the correlation between age and performance on cognitive testing, and significant group differences, an additional analysis was performed on age- and sex-matched CP and CI individuals.

**Statistical analysis**

Statistical analysis of demographic, clinical data and regional thickness and surface-based volumes was performed using SPSS 21.0 (Chicago, IL). For normally distributed variables, a multivariate general linear model (GLM) was used for group comparisons, using center, age and sex as covariates. A $p$ value < 0.05 was considered statistically significant.

Group comparisons and correlations between clinical and thickness measures were also performed using vertex-based GLM in the FreeSurfer software, correcting for center, age and sex. Gaussian smoothing kernel was with full width at half maximum (FWHM) of 10 mm; cluster-wise correction was performed using Monte Carlo Z simulation, thresholding at $p < 0.001$, 10,000 iterations and cluster-level threshold was set at $p < 0.05$. 

![Figure 1](image_url) Panel (a) is an example of typical outline in MS patient showing that most WM lesions cause no significant problems with surface measures (e.g. large left frontal lesion). Panel (b) shows where (larger) juxta-cortical lesions can introduce segmentation and outline errors. In the left-hand panel the uncorrected error is shown, in the middle panel the segmentation maps identify the areas where in the pipeline the WM lesion is erroneously accounted for as GM, and the right-hand panel shows the correction after filling of these areas (in the segmentation maps only, not in native T1 images). MS: multiple sclerosis; WM: white matter; GM: gray matter.
Regional mean measurements of CTh and volumes were correlated to clinical and cognitive testing scores (z-scores for verbal memory, visual memory, attention and fluency) using partial correlations, correcting for center, age and sex.

Results
Sixty patients with RRMS were enrolled in this study, of whom four patients were excluded because of significant juxtacortical lesion burden, leading to irreparable extensive errors in surface outlining (two CI, two CP). Sixty-five healthy controls underwent the same imaging protocol. Table 1 summarizes demographic and clinical characteristics. Control participants were younger than patients (35.9 vs. 39.2 years, \( p = 0.048 \)) but had similar sex distribution (\( p = 0.65 \)). Twenty (36%) patients were defined as CI (seven individuals failed two tests and 13 failed three or more), and 36 (64%) as CP (seven individuals failed one test). In CI patients, the most commonly affected domain was attention (mean z-score = −1.4), followed by visual memory (z = −1.1) and verbal memory (z = −0.85), and these z-scores were all significantly lower than the CP group (\( p < 0.001 \)). Fluency was not significantly different between CI...
and CP patients \((p = 0.10)\). Between CP and CI MS patients, differences in age \((p = 0.012)\), EDSS \((p = 0.03)\), lesion volume \((p < 0.01)\) and disease duration \((p = 0.03)\) (Table 1) were found, none in sex and years of education.

### Global CTh

The average cortical thickness (CTh) was similar for left and right hemispheres (LH/RH) in controls and MS subgroups (Figure 3). Average CTh significantly differed between controls (LH 2.54 mm (SD 0.11); RH 2.54 mm (SD 0.11)) and MS patients (LH 2.44 mm (SD 0.13); RH 2.45 mm (SD 0.13), \(p < 0.001\)). The global hemispheric thickness did not differ between CI and CP patients (LH \(p = 0.60\), RH \(p = 0.71\)).

### Lobar analysis

Regional CTh measurements were compared between subgroups and controls using multivariate GLM analysis (Table 2) correcting for age, sex, center and disease duration. This shows that there are regional differences on a lobar level between the patients and controls, more widespread in patients with CI. In CP patients, this is seen in the frontal, temporal, parietal and cingulate lobe, where it was seen in all lobes in patients with CI, albeit subtly, comparable to the global analysis. The only region in this analysis that had a statistically significant difference was within the temporal lobe \((p < 0.05)\).

The additional subanalysis between the age-matched CP and CI group showed comparable findings, with significant changes between CP and CI participants in only the temporal lobe \((p = 0.050)\), with a trend toward significance in the cingulate \((p = 0.055)\), where in addition to age/gender distribution, disease duration and lesion volume were not significantly different.

### Vertex-wise CTh

Compared to controls, MS patients showed widely distributed cortical thinning, with predominant clusters bilaterally in the middle temporal, lateral occipital and inferior parietal gyri as well as the cuneus (Figure 4). Direct comparison between CP and CI patients showed no statistical differences in vertex-based CTh measures. In patients, no significant correlations were seen between CTh and global z-scores of the BRB-N, EDSS, and disease duration.

### Correlation between performance on cognitive subtests and CTh

The correlations between the z-scores for each of the tested cognitive domains and regional CTh in the total MS group showed weak but significant correlations, with greater thinning in the insula (driven by right hemispheric changes) associated with worse verbal memory \((p = 0.035, r = 0.29)\). Also, we observed greater thinning in the left parietal cortex correlated with a poorer performance on visual memory test \((p = 0.044, r = 0.28)\). All these findings were obtained with partial correlations, correcting for age, sex and center.

### Correlation between EDSS and conventional MRI measures and CTh

In the MS patients, partial correlations with correction for age, sex and center showed significant correlations between lobar CTh and EDSS (parietal: \(-0.34\),...
Log-T2-LV was correlated to insula, occipital, parietal and temporal thinning (−0.30 to −0.40 range, \( p < 0.05 \)); log-T1BH-LV was correlated to insula, parietal and temporal thinning (−0.34 to −0.39, \( p < 0.05 \)).

**Discussion**

In this multicenter study we found global cortical thinning in MS patients compared to healthy controls. Only subtle regional differences in the extent of temporal lobe cortical thinning were detected between CP and CI patients, while no striking differences in global CTh were observed between these groups. In addition, correlating CTh with specific domains of the applied cognitive battery showed associations between insular thinning and verbal memory and parietal thinning and performance on visual memory-specific tests in the MS group as a whole.

The magnitude of differences in CTh between the MS subgroups was smaller than previously reported in single-center studies. Similar to recent studies on CTh in MS, cortical thinning in patients compared to healthy controls was less pronounced than previously reported. The average global thickness differences between controls and MS patients (both CI and CP) were on the order of 0.1 mm in our multicenter study.

Our findings show presence of mild global cortical thinning in MS, without cognitive status profoundly affecting these results. Only subtle but significant temporal lobe thinning was found in patients with MS and cognitive dysfunction. The subtlety of these findings is reflected in the fact that no significant differences were found using vertex-based surface comparisons between the two subgroups (CP and CI). Admittedly, the relatively small sample size, multicenter nature and the possible regional heterogeneity of the cortical thinning could contribute. Worse performance in specific cognitive domains was correlated to regional CTh measures. Attention and visual memory scores were most commonly affected in the CI group, and verbal memory scores correlated to regional cortical thinning in the insula, whereas visual memory scores correlated to parietal thinning. These significant correlations between regional thinning and meaningfully associated neuropsychological metrics strengthen the notion that such variables could be useful in future studies. Unraveling the interplay between specific cognitive domains in MS and the combination of MRI metrics, e.g. regional WM lesions, WM integrity and cortical GM volume loss, is very important in understanding the pathogenesis of cognitive dysfunction in MS. Finding this correlation in such a
heterogeneous disease and study group provides evidence this measure could be a helpful additional tool in future studies.

Total GM atrophy and regional analysis using other analysis modalities have previously been reported to show regional variances in GM volume in MS, most pronounced in the frontal, temporal and parietal lobes from the earlier stages of the disease.11,30–33 Studies on regional cortical volume loss (i.e. cortical thinning) and CI in MS are scarce. However, the few studies that have been performed showed regional variation between CI and CP patients with RRMS. A recent voxel-based morphometry (VBM) study found left superior temporal gyrus, left insula and right middle occipital gyrus atrophy in CI compared to CP RRMS patients.34 Another study did not replicate these findings and although volumetric differences between CI and CP were found, no significant regional difference between these groups was seen.5 Both studies included a relatively small sample of patients from single centers. CI in MS patients has also been related to cortical lesions,5 DTI abnormalities,6 GM magnetization transfer ratio7 and deep GM atrophy.3 This underscores that multifactorial interplay between lesions, normal-appearing (NA) WM integrity, and deep and likely regional cortical GM atrophy, is important in the development of CI in MS. Likely, the heterogeneity in topographic distribution of inflammatory and degenerative aspects of MS-related pathology limit the use of one single analysis modality to explain all variance in cognitive dysfunction in MS. Our multicenter study supports that subtle variations may be present between CI and CP MS patient groups; however, not strongly differentiating between them. It also is reflected in the fact that overall patients with CI had larger lesion volumes than CP patients. In the multivariate GLM analysis, the difference in lesion volume did not retain significance, likely reflecting multicenter and age effects. Also, the influence of WM lesions was not assessed topographically. Lesion volume did correlate mostly to temporal, insular and parietal thinning. A recent study addressed the correlation between regional cortical thinning and WM lesions.29 Such correlations between pathological locations and micro-structural properties of the involved WM tracts could be useful in studying the development of cognitive dysfunction.

Imaging correlations of CI in RRMS thus far are lacking a specific substrate and have equal dissociative properties as the paradox between classical WM lesion detection and the extent of physical disability. Combining imaging modalities in future longitudinal investigations with uniform imaging protocols will further elucidate the role of specific structural involvement in their development of difficulties in specific cognitive domains.

There are some technical limitations to the current study. Prior studies revealed a correlation between the number of cortical5 and juxta-cortical lesions35 and CI. We purposefully did not aggressively fill the WM lesions with normal WM intensities to avoid artificial underestimation of CTh. This may have resulted in an overestimation of CTh in cases where CI could have been correlated to number of juxta-cortical lesions. Our filling methods gave cortical segmentations that were judged to be correct on visual inspection in the majority of the cases; we had to exclude only four patients because of the quality of the surface measurements being affected by a large number of juxta-cortical lesions. Furthermore, intra-cortical lesions were not assessed with dedicated pulse-sequences in this study. Studies exploring the correlation between other types of cortical lesions and atrophy are needed, to understand both the methodological impact of lesions on atrophy measures and the causality between these important disease features. Limitations of this study also include the older age, slightly higher EDSS scores and disease duration of the CI patients compared to CP patients and controls. Age affects CTh measures and is correlated to performance on the BRB-N.19 This limitation was addressed by performing a subanalysis with younger CP patients in a subgroup analysis where these demographic and clinical features were not significantly different, with comparable regional CTh patterns. Another limitation is that controls did not undergo in-depth cognitive testing, which might have enhanced the analysis of disease-specific correlations between regional thinning and performance within specific cognitive domains. Lastly, the study was performed with slightly different acquisition protocols in each center. This was corrected for by using an equal number of control MRIs from each center performed on the same scanner and using each center as a covariate for analysis. Future uniform scanning parameters could address such limitations to some degree, where on the other hand the current study reflects the variability in daily practice between different centers that would be the typical setting for everyday practice and within clinical trials.

The use of regional CTh measures could become a useful additional tool in such future multicenter studies. The current study shows that multicenter CTh studies are feasible and that subtle differences between MS and healthy controls are detectable. CTh measures did not strongly predict cognitive status, but could be a useful tool incorporated into future larger
studies investigating relationships between specific cognitive domains and anatomical regional involvement, possibly in combination with measures specifically addressing anatomical and WM integrity with functional measures.

Conflict of interest
None declared.

Funding
The MS Centre Amsterdam is supported by the Dutch MS Research Foundation (grant number 09-358d). Dr Hulst is supported by the Dutch MS Research Foundation (grant number 08-648). Dr Damjanovic was supported by a MAGNIMS-ECTRIMS fellowship. The NMR Unit is supported by the MS Society of Great Britain and Northern Ireland and the National Institute for Health Research (NIHR) University College London Hospital (UCLH) Biomedical Research Centre (BRC).

References