Cognitive dysfunction is recognized as common sequelae of multiple sclerosis (MS) with the domains of episodic memory, working memory, speed of information processing, and attention most often affected. Cognitive impairment has a negative impact on quality of life, rehabilitation outcome, functional status and employment. While the associations between impaired cognition and disease-related variables such as disease duration and disability are equivocal, more robust correlations have been reported with magnetic resonance imaging (MRI) indices. In particular brain atrophy has emerged as a more powerful predictor of cognitive impairment than lesion volume. More recently, data extracted from normal appearing grey (NAGM) and white (NAWM) matter using diffusion tensor imaging (DTI) or magnetic transfer imaging have been shown to add to the variance derived from lesion and atrophy data.
correlate with cognitive impairment. However, these studies are few in number and it is unclear whether the indices obtained from normal appearing brain tissue (NABT) contribute anything beyond the lesion and atrophy data. For this reason, we undertook a DTI and cognitive study of patients with MS exploring the relative importance of lesions, atrophy, and NABT in the pathogenesis of cognitive impairment.

METHODS

(i) Sample

Sixty-two consecutive MS patients meeting modified McDonald criteria, and attending an out-patient MS clinic were enrolled. Patients were not paid to participate. Demographic data (age, education, marital status) and neurological data (expanded disability status scale [EDSS], duration of MS, disease course, and use of any disease-modifying drugs) were collected. Exclusion criteria were a history of drug/alcohol abuse, pre-MS psychiatric problems, traumatic brain injury with loss of consciousness, concurrent physical disease potentially affecting the central nervous system, and treatment with steroids in the three months prior to enrolment. Depression was measured using the Beck Depression Inventory-Fast Screen (BDI-FS) for medical patients, a scale that has been validated for use in MS.

(ii) Cognitive assessment

All subjects completed the Neuropsychological Screening Battery for Multiple Sclerosis (NSBMS), prior to undergoing MRI scanning on the same day. The NSBMS comprises the following tests: (a) the Selective Reminding Test as an index of verbal memory, (b) the 7/24 Spatial Recall Test as an index of non-verbal memory, (c) the Controlled Oral Word Association Test (COWAT) as a measure of lexical verbal fluency, and (d) the Paced Auditory Serial Addition Test (PASAT), as a marker of sustained attention and information processing speed.

In order to determine which subjects were cognitively impaired we followed the methodology specified by Rao in the NSBMS manual. Impairment was therefore defined as a score below the fifth percentile (based on established cut-offs from healthy individuals) on two or more of the four NSBMS measures. Our normative data were obtained from the published manual. Each MS patient’s cognitive result was based on these normative data taking into account gender, age, and years of education.

(iii) MR protocol

All images were acquired on a 1.5 T Signa scanner (GE Medical Systems, Milwaukee, WI). Scanning sequences included a T1-weighted acquisition (axial three-dimensional spoiled gradient echo sequence with a 5-ns echo time [TE], 35-ms repetition time [TR], 1 excitation, 35° flip angle, 22 x 16.5 cm field of view, 0.859 x 0.859 – mm in-plane resolution, and a 1.2 to 1.4 –mm slice thickness) and proton density (PD) and T2-weighted acquisitions (interleaved axial spin echo with TE of 30 and 80 ms, 3000 ms TR, 0.5 excitation, 22 x 22- cm field of view, 0.859 x 0.859 – mm in-plane resolution, and a 3-mm slice thickness).

An axial cardiac-gated DTI sequence was acquired with the following parameters: TR between 5000-8000 ms (depending on heart rate), TE 66 ms, 4 excitations, 22 slices, resolution 1.88 x 1.88 x 4 mm, along 11 gradient orientations, B = 1000, with two non-diffusion weighted baseline images (B=0). A fast spin-echo PD/T1 weighted sequence with TR/TE1/TE2 2500/20/85 msec to match the DTI slice prescription was acquired to facilitate registration with the T1-weighted image (see DTI analysis below).

(iv) Tissue segmentation and atrophy measurements

The T1-weighted image was segmented into grey matter, white matter, and cerebral spinal fluid (CSF) using an in-house automatic segmentation algorithm. Lesion volumes were subtracted from the white and grey matter tissue thereby leaving NAWM and NAGM. Atrophy of NAWM and NAGM were measured as the proportion of the total intracranial volume including CSF.

(v) Lesion analysis

Magnetic resonance images were processed on a Sun computer workstation (Palo Alto, CA) using the Analyze AW 6.0 software package (Biomedical Imaging Resonance, Mayo Clinic, Rochester, MN). Hypointense lesions were identified on the T1/PD-weighted images, and hyperintense lesions were defined on the T2/PD-weighted datasets that had been co-registered to the T1-weighted images. Lesions < 3 mm in diameter in 3-dimensional space were excluded from the analysis. Hyperintense lesions were defined using a semi-automated local thresholding technique in Analyze, and borders were adjusted manually as needed. Hypointense lesions were identified on the segmented T1-weighted images. All voxels that segmented as CSF on the T1-weighted image and were within a traced hyperintense lesion were relabeled as black holes. The measurement technique showed high intrarater (intraclass correlation coefficient = 0.98) and interrater (intraclass correlation coefficient = 0.99) reliability on a sample of 20 brains that were not part of the study sample. Total T1 hypointense and T2/PD hyperintense lesion volumes were obtained.

(vi) DTI analysis

The DTI data were corrected for motion and eddy-current distortions using the nonlinear registration tool provided in the FMRIB software library (“eddy_correct”, http://www.fmrib.ox.ac.uk/fsl/). Fractional anisotropy (FA) and mean diffusivity (MD) maps were created from the corrected data using DTI studio (version 2.4.01). To generate whole-brain FA and MD data, the tissue segmentations were moved into DTI space. This was accomplished by nonlinear registration of the T1-weighted images with their first baseline DTI volume. For this dataset, the optimal pipeline consisted of a multistage affine registration of T1 to DTI and T2 to DTI using the spin-echo and fast spin-echo images as intermediate steps (AIR v. 5.25, Automated Image Registration, http://bishopw.loni.ucla.edu/AIR5/). Once the images were roughly aligned, the T1 and DTI images were fully registered using nonlinear registration (align_warp, 3-7th order, AIR 5.25). Finally, rotation matrices were combined to generate the nonlinear rotation required to move the T1-weighted image and subsequent segmentation image into DTI space, which were resliced using nearest neighbor interpolation. The tissue
Segmentation masks were combined with the FA and MD maps to obtain average values for NAWM and NAGM (see Figure).

**Statistical Analysis**

In the primary analysis, comparisons between subjects with and without cognitive impairment were undertaken for all demographic, neurological and imaging data. The latter included total hypointense and hyperintense lesion volume, NAWM and NAGM atrophy, FA of NAWM, and MD of NAGM. Statistical significance was set at \( p < 0.05 \).

In the secondary analysis, predictors of cognitive impairment were sought using a logistic regression analysis. Variables entered into the analyses were those that differentiated the two groups in the primary analysis. Prior to entering the putative predictor variables into the regression analysis, correlations between them were explored. In the cases where two variables were significantly correlated \( (r > 0.8) \), the variable with the more robust \( t \)-test was entered into the equation.

The regression analysis was modeled as follows: First, to control for the disease-related and demographic mismatch, EDSS, education and disease course were entered into the analysis. Thereafter we adopted a block entry design with each imaging variable entered sequentially according to their level of statistical significance derived from the primary analysis. At each step in the analysis an omnibus test of model coefficients was derived with a chi-square indicating whether the change in variance was significant or not.

**Ethics**

This study was approved by the Research Ethics Board at Sunnybrook Health Sciences Centre and St. Michael’s Hospital, both affiliated with the University of Toronto. Written, informed consent was obtained from all subjects.

*Figure: Stepwise process to derive FA and MD from NABT (a) FA image in DTI space, (b) FA normal appearing white matter (NAWM) image, (c) MD image in DTI space, (d) MD normal appearing grey matter (NAGM) image.*
RESULTS

(i) Sample characteristics

The mean age of the sample was 40.7 (sd = 9.6) years. Fifty-one (82.3%) subjects were female, 13 (21%) were single, 42 (68%) married, and 7 (11%) divorced. The mean duration since diagnosis was 94.6 (sd = 70.1) months. Forty-nine (79%) subjects had relapsing-remitting MS, 11 (18%) had secondary progressive MS, and 2 (3%) had primary progressive MS. Thirty-four (55%) subjects were using disease modifying treatment. The median EDSS was 2.5. All subjects had, as a minimum education, completed high school.

(ii) Between-group demographic, neurological, and psychiatric comparisons

Eleven subjects (18%) met the criteria for cognitive impairment. Comparisons between subjects with and without cognitive impairment are shown in Table 1. Subjects with cognitive impairment had lower education (p=.001), were more likely to have secondary progressive disease (p=.05) and a higher EDSS score (p=.001). There was no difference between the two groups with respect to depression recorded by the BDI-FS, nor in their use of disease-modifying drugs (see Table 1). Group comparisons for the individual cognitive tests are shown in Table 2.

(iii) Between-group comparisons on MRI variables

Differences between groups on lesion, atrophy, and DTI measures are shown in Table 3. Patients with cognitive impairment had larger hypointense and hyperintense lesion volumes, greater NAWM atrophy, lower NAWM FA and higher NAGM MD. There was no difference in NAGM atrophy.

(iv) Predictors of cognitive impairment

A logistic regression analysis was used to investigate predictors of cognitive impairment. The disease and demographic variables that differentiated the two groups were first entered into the model. Education, EDSS, and disease course were able to explain 46.4% of the variance ($R^2=.464$; percentage correctly classified 85.5, $\chi^2=20.50$, $p<.001$). Lesion and atrophy data were then entered into the model sequentially according to their level of statistical significance derived from the primary analysis. Hypointense lesion volume raised the variance percentage to 54.8% ($R^2=.548$, percentage correctly classified 91.9). An omnibus test of model coefficients revealed that this change was statistically significant ($\chi^2=4.57$, $p=.033$). Adding hyperintense lesion volume, followed by NAWM atrophy did not significantly change the result ($R^2=.548$, percentage correctly classified 91.9, $\chi^2=0$, $p=.996$, and $R^2=.550$).

Table 1: Demographic, disease-related, and psychiatric variables

<table>
<thead>
<tr>
<th></th>
<th>Not Cognitively Impaired (n=51)</th>
<th>Cognitively Impaired (n=11)</th>
<th>t-test / $\chi^2$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>40.8(9.9)</td>
<td>40.6(8.6)</td>
<td>t=0.09</td>
<td>0.93</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>40/ 11</td>
<td>11/0</td>
<td>$\chi^2=2.9$</td>
<td>0.19*</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-school</td>
<td>6</td>
<td>7</td>
<td>$\chi^2=14.7$</td>
<td>*<em>0.001</em></td>
</tr>
<tr>
<td>College or higher</td>
<td>45</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marital Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>11</td>
<td>2</td>
<td>$\chi^2=2.0$</td>
<td>0.60*</td>
</tr>
<tr>
<td>Married/ Common-Law</td>
<td>33</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>7</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of MS symptoms (months)</td>
<td>121.3(89.7)</td>
<td>164.6(114.0)</td>
<td>t=-1.4</td>
<td>0.172</td>
</tr>
<tr>
<td>Time Since Diagnosis (months)</td>
<td>89.0(72.6)</td>
<td>120.8(52.2)</td>
<td>t=-1.4</td>
<td>0.174</td>
</tr>
<tr>
<td>Disease Course</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapsing-Remitting</td>
<td>43</td>
<td>6</td>
<td>$\chi^2=7.2$</td>
<td><em>0.05</em></td>
</tr>
<tr>
<td>Secondary Progressive</td>
<td>6</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary Progressive</td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any Disease Modifying Drugs (Y/N)</td>
<td>28/23</td>
<td>6/5</td>
<td>$\chi^2=0.0$</td>
<td>0.98</td>
</tr>
<tr>
<td>EDSS</td>
<td>2.6 (1.8)</td>
<td>4.8 (1.8)</td>
<td>t=-3.6</td>
<td>**0.001</td>
</tr>
<tr>
<td>Beck Depression Inventory- Fast Screen</td>
<td>7.2 (4.5)</td>
<td>9.6 (3.4)</td>
<td>t=-1.6</td>
<td>0.108</td>
</tr>
</tbody>
</table>

* = p<.05; ** = p<.01; * - using Fisher’s exact test
percentage correctly classified 90.3, $\chi^2 = .110$, $p = .740$, respectively). When DTI data were added to the model FA of NAWM raised the variance from 55% to 65% ($R^2 = .650$, percentage correctly classified 93.5), a statistically significant change ($\chi^2 = 5.96$, $p = .015$). Thereafter, adding MD of NAGM did not raise the variance percentage ($R^2 = .651$, percentage correctly classified 93.5, $\chi^2 = .031$, $p = .861$).

**DISCUSSION**

This study confirms the previously reported association between cognitive impairment on the one hand and lesion volume (hypointense and hyperintense) and atrophy of NAWM on the other. Of note, however, is that fractional anisotropy derived from NAWM added significantly to the hypointense lesion data in explaining the presence of cognitive impairment.

Before discussing the significance of these findings some comment on our sample is required. This is necessary because the percentage of cognitively impaired patients in our study was low relative to much but not all, published data. We recruited our subjects from a consecutive sample of community based patients attending their yearly clinic appointment. In a landmark publication, Rao et al. reported that approximately 40% of community based patients had cognitive dysfunction, a figure subsequently replicated. However, a recent study from Italy of 550 MS patients found only 20% of subjects were cognitively impaired, albeit based on an unusual cut-off point of 1 SD below normative data. While direct comparisons are often problematic, what is notable about this study is that the subjects all had relapsing-remitting MS and mild disability, a profile that overlaps with our sample composition. However, a subsequent re-analysis of the Italian data using a different, more conventional threshold for determining cognitive impairment raised the percentage of patients deemed impaired. We do not have this degree of flexibility given that we have closely

**Table 2: Cognitive test performance of patients with (n=11) and without (n=51) cognitive impairment based on the four cognitive parameters specified by Rao** for the Neuropsychological Screening Battery for MS (NSBMS)

<table>
<thead>
<tr>
<th></th>
<th>Not Cognitively Impaired (n=51)</th>
<th>Cognitively Impaired (n=11)</th>
<th>t-test/ Mann-Whitney U-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Selective Reminding Test- Consistent Long-Term Retrieval</strong></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>7/24 Spatial Recall Test- Total Recall (Trials 1-5)</td>
<td>32.3</td>
<td>2.9</td>
<td>25.5</td>
<td>7.6</td>
</tr>
<tr>
<td>PASAT – 2 seconds Total Correct</td>
<td>34.1</td>
<td>8.6</td>
<td>15.8</td>
<td>8.2</td>
</tr>
<tr>
<td>COWAT Total Correct Responses</td>
<td>40.2</td>
<td>10.0</td>
<td>21.0</td>
<td>10.4</td>
</tr>
</tbody>
</table>

**Table 3: MRI differences between patients with (n=11) and without (n=51) cognitive impairment**

<table>
<thead>
<tr>
<th></th>
<th>Not Cognitively Impaired (n=51)</th>
<th>Cognitively Impaired (n=11)</th>
<th>t-test/ Mann-Whitney U-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hypointense lesion volume (cm³)</strong></td>
<td>Mean</td>
<td>SD</td>
<td>95% CI</td>
<td>Mean</td>
</tr>
<tr>
<td><strong>Hyperintense lesion volume (cm³)</strong></td>
<td>7.97</td>
<td>8.98</td>
<td>5.45 - 10.5</td>
<td>17.4</td>
</tr>
<tr>
<td><strong>Normal appearing white matter (NAWM) atrophy</strong></td>
<td>.333</td>
<td>.039</td>
<td>.322 - .344</td>
<td>.295</td>
</tr>
<tr>
<td><strong>Normal appearing grey matter (NAGM) atrophy</strong></td>
<td>.478</td>
<td>.022</td>
<td>.471 - .484</td>
<td>.470</td>
</tr>
<tr>
<td>Fractional Anisotropy (FA) of NAWM</td>
<td>.265</td>
<td>.014</td>
<td>.261 - .268</td>
<td>.251</td>
</tr>
<tr>
<td>Mean Diffusivity (MD) of NAGM (μm² ms⁻¹)</td>
<td>2.91</td>
<td>.18</td>
<td>2.86 - 2.96</td>
<td>3.06</td>
</tr>
</tbody>
</table>

** = p<.05; ** = p<.01; a - represented as proportion of total intracranial volume
followed the guidelines for determining global cognitive impairment as specified by Rao in the NSBMS manual. As such, our figure of 18% is less amenable to change. This leads us to conclude that our sample had relatively fewer cognitively impaired patients than anticipated. In part this may reflect the fact that our sample was highly educated with 79% having a college education or higher. Furthermore, it is unlikely that our lower prevalence is due to the "Will Rogers Phenomenon" reported in other areas of MS research as recent studies using newer diagnostic criteria still arrive at figures for cognitive impairment in the region of 40%.

The link between cognitive dysfunction and imaging indices derived from NABT can be traced to the early MRI literature, in particular measurement of \( T_1 \) relaxation times. This observation has been supported by data obtained from positron emission tomography and MR spectroscopy. More recently, the emphasis has shifted to MRI derived indices of NABT, namely DTI and magnetic transfer imaging, with promising results although not all studies were able to demonstrate significant DTI differences between cognitively impaired and intact subjects. Here our results replicate the results of a diffusion weighted imaging (DWI) study, a technique considered less sensitive than DTI in detecting higher-order components of water diffusion in tissue. The sensitivity of DTI as a marker of cerebral integrity is further underscored by a study involving patients with benign MS, some of whom were cognitively impaired. When this subgroup of impaired subjects was compared to patients with secondary progressive MS, no differences were found with respect to \( T_1 \) lesion volume, atrophy, and DTI measurements of normal appearing white and grey matter.

Our most notable finding was that DTI derived data boosted the variance when it came to explaining cognitive problems. Two DTI indices were obtained, fractional anisotropy and mean diffusivity. Fractional anisotropy provides a measure of the longitudinal diffusion of water along fibres and is commonly referred to as a marker of the structural integrity of white matter tracts, whereas MD gives a measure of water diffusion that is less influenced by the inherent directionality in the underlying tissue microstructure. Both may be affected independently of lesions as demonstrated by Dineen et al who found that areas of FA damage within white matter tracts did not overlap well with lesion distribution probability maps. Our results provide further support for this by illustrating that FA of NAWM in particular added significantly and independently to the hypointense lesion volume in accounting for the variance in cognitive dysfunction. This finding also extends results from the earlier DWI study which revealed that entropy, a measure of the degree of disorder of diffusion within brain tissue, added to the grey matter fraction in accounting for unique variance in performance on a single cognitive measure, the Symbol Digit Modalities Test. These authors, however, did not assess the independent contribution of DWI to overall cognitive impairment.

We did not find an association between atrophy of NAGM and cognitive abnormalities, a result at odds with recent imaging data. This was counterbalanced by our result showing that MD of NAGM differentiated between cognitively impaired and intact groups, possibly by providing an indicator of cortical demyelination or retrograde degeneration of grey matter neurons secondary to axonal damage. While this cerebral marker did not emerge as an independent predictor of cognition we do not discount it based on our data given that the small sample size of the cognitively impaired group may have compromised this result.

Our study is not without limitations, the first being the absence of scan-rescan data attesting to potential variability in data acquisition. Second, the small number of cognitively impaired patients introduces a note of caution when it comes to interpreting our results and could explain why age did not differ between our patients with RRMS and SPMS. This suggests that our cognitively impaired group could be atypical, a factor that may also underlie our grey matter atrophy findings. Finally, our cognitive battery was limited and did not contain a sensitive cognitive measure such as the Symbol Digit Modalities Test which could have led to a more robust correlate with NAGM emerging. Of note, however, is that the battery we used, namely the NSBMS, has good proven validity in MS populations with a 71% sensitivity and a 94% specificity. Furthermore the manual provides normative data according to relevant demographics (age, education, gender) which makes it a practical cognitive tool for use with MS patients.

CONCLUSIONS

In summary, our findings illustrate that lesions and atrophy tell only part of the story when it comes to explaining cognitive dysfunction. Data extracted from NABT reveals that more pervasive and subtle markers of a disordered anatomy contribute independently to cognitive dysfunction. From this we can conclude that even in a sample with mild to moderate physical disability it is important to look beyond the more conventional markers of brain pathology for clues as to the origins of cognitive compromise.

ACKNOWLEDGEMENTS

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