ABSTRACT

BACKGROUND Whilst our knowledge of white matter (WM) pathology underlying cognitive impairment in relapsing remitting multiple sclerosis (MS) is increasing, equivalent understanding in those with secondary progressive (SP) MS lags behind.

OBJECTIVE To test the hypothesis that the extent and severity of WM tract damage differ between cognitively impaired (CI) and cognitively preserved (CP) SPMS patients.

METHODS Conventional MRI and diffusion MRI were acquired from 30 SPMS patients and 32 healthy controls (HC). Cognitive domains commonly affected in MS patients were assessed. Linear regression was used to predict cognition. Diffusion measures were compared between groups using tract based spatial statistics.

RESULTS Twelve patients were classified as CI, and processing speed was the most commonly affected domain. The final regression model including demographic variables and radial diffusivity explained the greatest variance of cognitive performance (R^2=0.48, p=0.002). SPMS patients showed widespread loss of WM integrity throughout the WM skeleton when compared with HC. When compared with CP patients, CI patients showed more extensive and severe damage of several WM tracts, including the fornix, superior longitudinal fasciculus and forceps major.

CONCLUSION Loss of WM integrity assessed using TBSS helps to explain cognitive decline in SPMS patients.

INTRODUCTION

Secondary progressive (SP) multiple sclerosis (MS) is associated with greater cognitive deficits than relapsing remitting MS (RRMS)(1). However, whilst our knowledge of WM pathology underlying cognitive impairment in RRMS is increasing, equivalent understanding in those with SPMS lags behind(2).

Diffusion tensor imaging (DTI) allows the assessment of WM integrity *in vivo*(3). Elements of cognitive decline in RRMS patients have been attributed to disconnection of regions involved in cognitive processing(4). Although, degeneration of chronically demyelinated axons is a prominent feature of progressive MS(5), other pathological processes could explain cognitive dysfunction in SPMS. For example, grey matter (GM) atrophy might play an important role since loss of GM is a well-known contributor to cognitive impairment and is more substantial in SPMS than RRMS(6). Recently, in SPMS patients more severe WM tract damage has been linked with cognitive impairment (7). However, given that pathology beyond WM integrity may influence cognitive outcomes, a more comprehensive approach is needed to examine the contribution of WM abnormalities to cognitive dysfunction in SPMS.

Therefore, we investigated the influence of WM integrity, GM atrophy, WM lesions and demographic factors on cognitive performance in SPMS patients. Furthermore, we tested the hypothesis that the extent and severity of WM abnormalities differ between cognitively preserved and cognitively impaired patients, after accounting for demographic factors.

MATERIAL AND METHODS

Participants and clinical assessments

SPMS patients(8) who were at least three months free from clinical relapses and attending the MS clinics at the National Hospital of Neurosurgery and Neurology were recruited. A group of healthy controls (HC), who did not suffer from any neurological or neuropsychiatric disorders and were not first-degree relatives of people with MS, also participated into this study. On the day of scanning, the MS participants underwent neurological assessment, including the Expanded Disability Status Scale (EDSS)(9) and the Multiple Sclerosis Functional Composite (MSFC) subtests(10).

The joint Medical Ethics Committee of the National Hospital for Neurology and Neurosurgery and the UCL Institute of Neurology, London approved the study. Written and informed consent was obtained from all participants.

Cognitive assessments

All subjects were invited to undergo cognitive assessment to examine five commonly affected cognitive functions in MS. Processing speed was assessed using the PASAT (3 seconds)(11) and Symbol Digit Modalities Test (SDMT)(12). Verbal memory was assessed using the immediate and 30-minute delayed Story Recall Test (SRT) from the adult memory and information processing battery (AMIPB)(13) and the Recognition Memory Test (RMT) for words(14). Visual memory was measured using the immediate and 30-minute delayed complex Figure Recall Test (FRT) from the AMIPB and RMT for faces(14). Executive function was assessed using the Stroop colour-word interference test(15) and Hayling Sentence Completion Test(16). Working memory was assessed with the digit-Span, a subtest of the Wechsler Adult Intelligence Scale-III(17). Premorbid IQ was assessed using the Wechsler Test of Adult Reading(18). Levels of anxiety and depression were measured using the Hospital Anxiety and Depression Scale (HADS)(19). For all cognitive domains, z-scores for each involved test were obtained with reference to the control participants and averaged. Patients were defined as cognitively impaired (CI) when their cognitive score was at least 2 standard deviations (SD) below that of controls on a minimum of 2 out of 5 tested cognitive domains. Additionally, an averaged cognition score was computed based on the five cognitive domains.

Image acquisition and analysis

MRI scanning was performed on a Philips Achieva 3T system (Philips Healthcare, Best, The Netherlands) using a 32-channel receive-only head-coil.

T1-weighted scans and normalised brain volumes

For brain volume measurements, all subjects underwent a three-dimensional inversion-prepared fast spoiled gradient recall (3D FSPGR) T1-weighted sequence (TR 13.3 ms, TE 4.2 ms, inversion time 450 ms, 124 contiguous axial slices, slice thickness of 1.5 mm, FOV 300×225 mm, matrix size 256×160 (reconstructed to 256×256 for a final in plane resolution of 1.17 mm)). NGMV was computed from segmented T1-weighted images using SIENAX software.

T2-weighted scans and lesion probability mask

For WM lesion detection, turbo spin-echo dual-echo proton density- and T2-weighted images were obtained (FOV 240x180 mm^2, repetition time 3500 ms, echo time 19/85 and 50 axial slices, 1x1 x3 mm^3). Lesion marking was carried out by an experienced rater (VS) using JIM version 5. To assess whether diffusion differences were co-localized with focal lesions, a lesion probability map (LPM) was created(20).

Diffusion MRI and TBSS

All subjects underwent a whole-brain, cardiac gated, spin-echo diffusion-weighted sequence (TR = 24000 ms; TE = 68 ms; 72 axial slices with an isotropic 2 mm resolution) with 61 volumes with non-collinear diffusion gradients (b-value of 1200 s mm^{-2}) and 7 volumes without diffusion weighting. After correction of motion and eddy current distortions using FMRIB's Linear Image Registration Tool, a diffusion tensor model was fitted on a voxel-by-voxel basis using DTIFIT from the FMRIB's Diffusion Toolbox (FSL, FMRIB Image Analysis Group, Oxford, UK). From the tensor fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD) and mean diffusivity (MD) images were derived. The default tract-based spatial statistics (TBSS) pipeline (version 5.2) was used to perform voxel-based diffusion tensor MRI analyses across subjects(21).

Group comparisons of diffusion measures were conducted using a permutation algorithm ("randomise" from FSL) with 10,000 permutations using threshold-free cluster enhancement (TFCE) ($p<0.05$ family wise error (FWE) corrected). In particular, we performed the following group comparisons, each consisting of two contrasts: (I) cognitively preserved (CP) versus HC, (II) CI versus HC and (III) CI versus CP. All group comparisons for diffusion measures were conducted while treating age, gender and premorbid IQ as covariates.

In addition to the whole brain analysis, we created regions of interest (ROIs) based on the John Hopkins University Atlas(22) which is commonly used as a ROI template for WM tracts(7). Additionally, ROI masks were created of the corpus callosum and fornix. To estimate the degree of damage in the patient groups, the following two analyses were performed: 1) the "extent" of damage was determined by calculating the percentage of significantly abnormal ($p<0.05$) voxels for the different diffusion metrics; and 2) the "severity" of damage was calculated per patient, by converting the diffusion measures to Z-scores, based on the mean and standard deviations of WM values in HC(23).

Statistical analysis

Statistical analyses were performed using SPSS software, version 21 (Chicago, Illinois, USA). Variables were checked for normality using Kolmogorov-Smirnov testing and histogram inspection. T2 lesion volumes were log-transformed. Chi-square tests were used to compare group differences for categorical variables.

Predicting averaged cognition employed linear regression analyses. Age, sex and premorbid IQ were first entered in block 1. To examine the additional explained variance of the MRI measures, NGMV, WM integrity and WM lesion load were entered one by one in block 2. Due to strong multicollinearity of the four diffusion measures, they could not be included as separate predictors in linear regression model. Therefore, the metric which showed the strongest correlation with cognition, was included as a measure of WM integrity.

For group differences regarding diffusion measures, a one-way between group analysis of covariance (ANCOVA) including age, gender and premorbid IQ as covariates was conducted, using post-hoc Bonferroni comparisons to assess specific group differences. A more stringent alpha level was considered as significant ($p<0.01$) to correct for multiple comparisons.

RESULTS

Demographic characteristics

30 patients with SPMS and 32 HC underwent MRI, and all patients and 23 HCs underwent cognitive assessment (**Table I**). Twelve patients were classified as cognitively impaired (CI), of which four patients showed impairments in two cognitive domains, five in three cognitive domains, two in four domains and one in five domains.

[Insert table I here]

Cognitive profile SPMS

For all SPMS patients together, processing speed was most commonly impaired (15 patients, 50%), followed by executive function (10 patients, 33%). For CI patients, the group means of executive functioning and processing speed were respectively 4.1 and 2.9 SD below the mean of the HC (**Table II**).

[Insert table II here]

MRI characteristics

CP and CI patients had both lower NGMV than HC (for each $p<0.05$), but no difference in NGMV was observed between patient groups ($p=0.122$) (**Table III**). Mean skeleton FA, RD and MD values were more abnormal in CI patients than in CP patients, who showed lower WM integrity when compared with HC (all tests had $p<0.05$). CP and CI patients did not differ with respect to WM lesion load ($p=0.09$) and mean skeleton AD ($p=0.07$) (**Table III**). Lesion load was not correlated with diffusion measures.

[Insert table III here]

Predictors of cognition

Mean skeleton RD was selected as a measure of WM integrity, because it showed correlation with cognition ($r=-0.359$; $p<0.05$). The regression model with age, sex and premorbid IQ explained 42% of the variance of cognition in SPMS patients ($F=6.32$; $p=0.002$). This indicated that worse cognitive performance was associated with lower premorbid IQ ($\beta=0.672$; $p<0.001$), female sex ($\beta=-0.274$; $p=0.09$) and higher age ($\beta=-0.213$; $p=0.18$). A regression model only containing premorbid IQ explained 30% of the total variance of cognition ($F=11.93$, $p=0.002$). Entering MRI measures to the

regression model containing only demographic factors, led to a small increase in explained variance of 2% by including NGMV (R^2=0.44; F=4.95; p=0.004), 4% by including lesion load (R^2=0.46; F=5.42; p=0.003) and 6% by including mean skeleton RD (R^2=0.48; F=5.78; p=0.002). The test statistics refer to the final regression model. A model containing demographic factors and MRI measures together explained 51% of the variance (F=3.97; p=0.007).

TBSS findings: extent of WM damage

The extent of whole-skeleton WM damage is summarised in **figure I**. Relative to CP patients, lower FA values were observed for 50% of the investigated WM, and higher RD, MD and AD values were seen for respectively 47%, 22% and 10% of the investigated WM in CI patients.

[Insert figure 1 here]

TBSS findings: extent of tract-specific WM damage

CI and CP patients compared with HC

When looking at the voxel-based differences in diffusion measures between groups, a widespread reduction in FA was observed throughout the skeleton in both patient groups when compared with HC. CP patients showed the greatest extent of FA reduction in the fornix, corpus callosum, right cingulate gyrus, forceps major and forceps minor when compared with HC (**Figure 2A**). Although largely overlapping results were observed for the other diffusion measures, the right inferior fronto-occipital fasciculus was amongst the most extensively affected tracts when looking at RD, MD and AD. Additionally, the bilateral uncinate fasciculi showed an extensive increase in RD and MD relative to HC, whereas extensively increased AD was observed in the corticospinal tracts (**Figure 2B-D**).

Similar WM tracts were affected in CI patients; however this patient group showed an additional loss of WM integrity of several tracts. When looking at the most extensively damaged WM tracts, the right inferior longitudinal fasciculus and right inferior fronto-occipital belonged to WM tracts with the highest percentage of decreased FA per tract volume (**Figure 2E**). Additionally, the right superior longitudinal fasciculus was amongst the most extensively affected tracts in CI patients relative to HC when looking at MD and AD (**Figure 2F-G**).

These diffusion abnormalities overlapped with the distribution of WM lesions, but changes were also detected outside MS WM lesions (**Figure 2**).

[Insert figure 2 here]

CI patients compared with CP patients

CI patients showed the most extensive FA reductions in the fornix, corpus callosum, bilateral uncinate fasciculi, left superior longitudinal fasciculus (temporal and frontal part) and left inferior fronto-occipital fasciculus compared with CP patients (with changes in more than 57% of tract voxels) (**Figure 3A**). Similar regions showed extensive differences for the other diffusion measures. The left corticospinal tract, however, demonstrated the greatest increases in RD and AD, in CI relative to CP patients (**Figure 3B-D**).

[Insert figure 3 here]

TBSS findings: severity of tract-specific WM damage

CI patients showed, relative to CP patients, significant decreases in FA and increases in RD within the fornix, forceps major and left superior longitudinal fasciculus ($p<0.01$). Additionally, FA of the bilateral anterior thalamic radiation and inferior fronto-occipial fasciculi also differed between CP and CI patients ($p<0.01$) (**Supplementary tables I-IV**).

DISCUSSION

We found that demographic variables had the largest contribution in explaining cognition in SPMS. Whole-brain MRI measures generally explained little additional variance, although the final models remained significant. Of the whole brain MRI measures, when combined with the demographic variables, WM integrity (i.e. RD) appeared to be the strongest predictor, altogether accounting for 48% of the variance in cognition in SPMS. CI patients demonstrated more extensive and severe microstructural changes in WM tracts when compared with CP patients.

Cognitive dysfunction of SPMS patients

Consistent with previously published studies using similar criteria(1), 40% of our SPMS patients was defined as cognitively impaired, with a predominant involvement of processing speed and executive function. Together with age, gender and premorbid IQ, WM integrity significantly explained 48% of the variance of cognitive performance in SPMS patients. Although, WM lesion volume and NGMV together with age, gender and premorbid IQ explained more variance than a model containing only these demographic factors, WM integrity (i.e. RD) explained the most additional variance. Although RD was more severely and extensively abnormal in CI than CP patients, mean skeleton RD added little to the regression model to predict cognitive impairment. A possible explanation could be that different measures of cognition were used as dependent variable for the regression model (e.g. averaged cognition) and TBSS analysis (e.g. CP and CI). A recent study reported that WM skeleton FA significantly predicted cognition in SPMS patients(7). However, the diffusion measures FA and RD are highly correlated, and reduced FA is probably driven by an increased RD. Corresponding to a number of studies(24), higher premorbid IQ seems to influence the expression of cognitive deficits in SPMS. This could reflect the role of education and early life intellectual enrichment as major protective factor against cognitive impairment.

WM tracts critical for cognitive function were more affected in CI patients

TBSS enabled us to examine changes in WM integrity of the major WM tracts across the brain, independent of demographic factors. In doing so, we demonstrate that cognitive dysfunction in patients with SPMS is related to loss of WM integrity within a number of WM tracts, including the fornix, corpus callosum, bilateral uncinate fasciculi, left superior longitudinal fasciculus and left inferior fronto-occipital fasciculus. Many of these tracts have previously been linked to cognition in healthy participants, such as the uncinate fasciculus that connects parts of the limbic system with the orbitofrontal cortex and is involved in executive function(25) and the fornix, the major efferent pathway from the hippocampus, is known to be crucial for the formation of memories(26).

Additionally, in CI patients more WM abnormalities were also observed in the cortical spinal tract which is not primarily involved in cognition. However, a number of studies have shown that cognitive impairment is linked to wider motor and sensory disturbances in MS (for example 27).

In addition, by visually examing figure 1 and 2 it seems that relative to HC, CI patients showed predominantly more extensive loss of WM integrity in tracts linking cortical structures one to the other than CP patients. This difference may play a significant and important role in the establishment of cognitive impairment and has previously been documented in a mixed sample of MS patients(28). However, CP patients also showed loss of WM integrity in regions known to be involved in cognitive processes, such as the fornix and uncinate fasciculi. Greater capacity for functional reorganization or the protective value of higher premorbid IQ are likely to play a role to maintain cognitive function(28,29).

The structural correlates of cognitive dysfunction in SPMS patients

Our observed changes in diffusion measures between CP and CI patients were unlikely to be primarily due to WM lesions, given no correlation was observed between lesion load and diffusion measures. This finding corresponds to previous DTI studies in which the involvement of NAWM in RRMS has been shown (28,30). Whether damage to axons traversing lesions or a more diffuse injury process underlies NAWM changes is uncertain. Nevertheless, recent evidence indicates that WM integrity is often reduced in NAWM near lesions(31) suggesting, that the role of lesions in cognitive dysfunction may be underestimated by considering only abnormalities in regions that are close to lesions, rather than broader, network-level effects of lesions. Diffusion changes were especially severe in FA and RD opposed to AD, thought to be indicative more of demyelination than axonal loss(32). However, this should be interpreted with caution, because the principal diffusivities are calculated *in vivo* in regions of low coherence of WM tracts(33). Therefore, it is likely that a combination of pathological changes is associated with the changes in diffusion measures.

Limitations and conclusions

A few limitations apply to this work. Larger patient groups are desirable in future studies when contrasting CI vs. CP SPMS patients. Using the currently observed differences in diffusion metrics between CI and CP patients, and the observed standard deviations, we calculated that a sample size of N=17 per group would be able to detect significant differences in diffusion metrics, with 80% power and an alpha level set at 0.05. Additionally, it is desirable to aim for even numbers of subjects when contrasting two groups. For the TBSS analysis it is not possible to test or correct for differences in variance between groups. Although we considered a more stringent alpha level as significant (*p<0.01*) for the group comparisons regarding diffusion measures conducted in SPSS, a formal

correction for multiple comparisons was not made. However, we reported 13 significant results associated with a *p*-value less than 0.01, whilst one would expect on average 1 out of 100 false positive results, so it is unlikely that a type I error accounts for all these significant results. Since we used the SIENAX tool to estimate the volume of brain tissues, it may have underestimated changes in deep GM (34). Given these regions are linked to cognitive processes(35), it is possible that better estimation of changes in these regions would have affected our results.

In conclusion, as with RRMS(4,30), damage to WM tracts also appears to be a potential mechanism for cognitive impairment in SPMS patients. The present study shows that loss of WM integrity assessed using TBSS helps to explain cognitive decline, and emphasizes the relevance of studying patterns of WM disruption throughout the brain in relation to cognitive deficits.

DISCLOSURES

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