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## Perfusion magnetic resonance imaging correlates of neuropsychological impairment in multiple sclerosis

Matilde Inglese<sup>1</sup>, Sumita Adhya<sup>1</sup>, Glyn Johnson<sup>1</sup>, James S Babb<sup>1</sup>, Laura Miles<sup>1</sup>, Hina Jaggi<sup>1</sup>, Joseph Herbert<sup>2</sup>, and Robert I Grossman<sup>1</sup>

<sup>1</sup>Department of Radiology, Hospital for Joint Disease, New York University School of Medicine, New York, New York, USA

<sup>2</sup>Department of Neurology, Hospital for Joint Disease, New York University School of Medicine, New York, New York, USA

### Abstract

Although cognitive impairment is common in multiple sclerosis (MS), its pathophysiology is still poorly understood. Abnormalities of cerebral blood flow (CBF) have long been acknowledged in MS and advances in perfusion magnetic resonance imaging (MRI) allow for their assessment *in vivo*. We investigated the relationship between regional perfusion changes and neuropsychological (NP) dysfunctions in patients with relapsing-remitting and primary-progressive MS. Absolute CBF, cerebral blood volume (CBV) and mean transit time were measured in 32 MS patients and 11 healthy controls using dynamic susceptibility contrast-enhanced T2\*-weighted MRI. A comprehensive NP test battery was administered to all patients. A mixed model analysis of covariance was performed for group comparisons in terms of perfusion measures in normal-appearing white matter (NAWM) and deep gray matter (GM). Pearson's correlations were used to describe the association of perfusion metrics with NP Z-scores. CBF and CBV values were significantly decreased in both NAWM and deep GM in MS patients compared with controls ( $P = 0.01$ ). In all patients, deep GM CBF was significantly associated with Rey Complex Figure Test (RCFT)-Copy ( $r = 0.5$ ;  $P = 0.001$ ) and deep GM CBV and NAWM CBV were significantly associated with Color-Word Interference Inhibition Switching test (D-KEFISIS) ( $r = 0.4$ ;  $P = 0.008$  and  $r = 0.4$ ;  $P = 0.02$ ). However, the only associations that remained significant after Bonferroni correction were between deep GM CBF and RCFT-Copy ( $P = 0.006$ ), and deep GM CBV and D-KEFISIS ( $P = 0.04$ ). Our results suggest a role for tissue perfusion impairment in NP dysfunction in MS. Large-scale studies are needed to characterize better this association.

### Keywords

multiple sclerosis; neuropsychological impairment; perfusion MR imaging; primary-progressive MS; relapsing-remitting MS

### Introduction

Cognitive impairment occurs in 40 to 65% of patients with multiple sclerosis (MS) and can have a considerable impact on occupational and social life (Amato *et al*, 2001; Rao *et al*,

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Correspondence: Dr M Inglese, Department of Radiology, New York University School of Medicine, 650 1st Avenue, 6th floor, New York, New York 10016, USA. E-mail: matilde.inglese@med.nyu.edu.

### Disclosure

The authors state no conflicts of interest.

1991b). The cognitive domains most commonly impaired are memory, attention, information processing, executive function, and visuospatial abilities (Rao *et al*, 1991a; Ron *et al*, 1991). The pathophysiology of neuropsychological (NP) deficits is, at least partially, unclear. The presence and extent of MS lesions affecting white matter (WM) tracts connecting cortical areas seem to be important factors, although microscopic changes in normal-appearing white matter (NAWM) and cortical and subcortical gray matter (GM) might also play a relevant role (Blinkenberg *et al*, 2000; Rovaris *et al*, 2000; Zivadinov *et al*, 2001a).

Magnetic resonance imaging (MRI) provides an opportunity to investigate the underlying pathological basis of cognitive dysfunction in MS (Zivadinov *et al*, 2001a). Although several MRI studies support the concept that MS cognitive dysfunction is related to the overall brain lesion burden, standard MRI markers such as T2-weighted (T2-W) lesion volume (LV) do not correlate closely with NP deficits (Foong *et al*, 1997; Rao *et al*, 1989; Rovaris *et al*, 1998). This is partly owing to the lack of pathological specificity of T2 visible lesions and to its insensitivity to microscopic changes in the normal-appearing brain tissue. Quantitative MRI techniques such as atrophy measures (Zivadinov *et al*, 2001b), magnetization transfer, diffusion tensor imaging, and proton MR spectroscopy have shown better correlation between MS pathology in normal-appearing brain tissue (Filippi, 2000) and cognitive performance (Deloire *et al*, 2005; Rovaris *et al*, 2002). Furthermore, more recent MRI studies have emphasized the role of subcortical GM structural and functional abnormalities as correlates of NP impairment in MS (Blinkenberg *et al*, 2000; Brass *et al*, 2006; Mainero *et al*, 2004; Paulesu *et al*, 1996).

During inflammation, vascular dysfunction and local production of toxic metabolites may lead to defective microcirculation and subsequent ischemia, contributing to clinical manifestations (Wakefield *et al*, 1994). The assessment of cerebral hemodynamics has been made possible through MRI by using dynamic susceptibility contrast (DSC)-enhanced T2\*-weighted MRI (Ostergaard *et al*, 1996). This technique utilizes signal changes that accompany a tracer passing through the cerebral vasculature, which enable a quantitative estimation of cerebral blood flow (CBF). Dynamic susceptibility contrast-enhanced perfusion MRI has been applied to the study of MS and has demonstrated perfusion impairment in both lesional (Haselhorst *et al*, 2000) and structurally intact WM (Law *et al*, 2004).

Previous hemodynamic and metabolic studies using positron emission tomography (PET) and single-photon emission computed tomography (Brooks *et al*, 1984; Paulesu *et al*, 1996) have demonstrated a generalized coupled reduction in both cerebral oxygen utilization and blood flow in MS patients. The reduction was found in both white and cortical/subcortical GM and correlated with cognitive impairment, rather than with locomotor disability. Unlike PET and single-photon emission computed tomography, perfusion MRI offers higher signal-to-noise ratio (SNR) and contrast-to-noise ratio, higher spatial resolution, absence of ionizing radiation, and shorter acquisition time (Sorensen and Reimer, 2000).

We hypothesized that decreased perfusion in NAWM and subcortical GM, as assessed by DSC MRI, would reflect neuronal/axonal dysfunction and therefore, would be related to cognitive deficits in MS patients. To this end, we investigated the relationship between regional perfusion impairment and a comprehensive battery of NP tests.

## Materials and methods

### Subjects

Thirty-two MS patients meeting the revised International Panel Criteria (McDonald *et al*, 2001) were prospectively enrolled in the study. Eighteen (12 female and 6 male) patients had relapsing-remitting MS (RR-MS) and 14 (7 female and 7 male) had primary-progressive MS

(PP-MS). Exclusion criteria were (a) a current or past medical or psychiatric disorder other than MS, (b) current or past substance abuse, and (c) MS relapse or corticosteroid use in the previous 6 weeks. Disability was assessed by a single experienced neurologist blind to the MRI findings using the Expanded Disability Status Scale (EDSS) score within 1 week of MRI. The patients with RR-MS had a mean age of 48 years (range: 31 to 71), mean education of 18 years (range: 16 to 21), mean disease duration of 7.6 years (range: 1 to 34) and median EDSS score of 1.0 (range: 0 to 6.5). The patients with PP-MS had a mean age of 55 years (range: 29 to 75), mean education of 17 years (range: 12 to 21), mean disease duration of 5 years (range: 1 to 19) and median EDSS score of 4 (range: 3 to 7). Only RR-MS patients were under immunomodulatory treatment. Six patients were on interferon- $\beta$ 1a (Avonex, Biogen, Cambridge, MA, USA) for an average of 2 years (range: 1 to 3); three patients were on interferon- $\beta$ 1a (Rebif, Serono, Rockland, MA, USA) for an average of 1.6 years (range: 1 to 2), and nine patients were on glatiramer acetate (Copaxone, Teva, Petah Tiqvah, Israel) for an average of 3.1 years (range: 1 to 5). Patients were permitted to remain on psychoactive drugs, such as antidepressants and anxiolytics, as part of their routine MS medical care. For MRI comparison, 11 age- and gender-matched healthy controls (4 male and 7 female) were recruited. Their mean age was 51 years (range: 29 to 65). Different aspects of a subgroup of these patients and controls have been reported as part of a separate publication (Adhya *et al*, 2006). Approval for this study was obtained from the Institutional Board of Research Associates of New York University Medical Center and informed consent was obtained from all subjects.

### Neuropsychological Testing

Cognitive functioning was assessed using a battery of NP tests examining cognitive domains, which have been found to be vulnerable to impairment in individuals with MS (Lezak, 2004). Neuropsychological tests were administered to all MS patients within 24 h of MRI. The following cognitive domains were assessed: (1) visuospatial memory (Rey Complex Figure Test (RCFT)); (2) verbal fluency (The D-KEFS Verbal Fluency Test (VFT)); (3) verbal learning and memory (California Verbal Learning Test-II); (4) attention and concentration (digits backward portion of the Digit Span subtest, Wechsler Adult Intelligence Scale-III (DB)); (5) processing speed (Symbol Digit Modalities Test, 3-sec trial of the Paced Auditory Serial Addition Test (PASAT-3 secs)); (6) working memory (DB, Symbol Digit Modalities Test, and PASAT-3 secs); (7) executive functioning (D-KEFS Color-Word Interference Test: Inhibition (D-KEFSI) and Inhibition Switching (D-KEFSIS)). The RCFT assesses visuospatial ability as well as short- and long-term visuospatial memory. The RCFT is composed of three parts, each of which elicits a separate score: (1) copy of a complex geometric figure (RCFT-Copy), (2) immediate recall of the figure (RCFT-Immediate), and (3) delayed recall of the figure (RCFT-Delayed). Depressive symptoms were assessed with the Depression subscale of the Brief Symptom Inventory (BSI-D) (Derogatis and Melisaratos, 1983). The Depression subscale of the BSI assesses clinical indications of depression such as dysphoric mood and affect as well as reduced motivation and a loss of interest. Higher scores indicate greater depressive symptomatology.

Raw NP scores were normalized using published norms and then converted to Z-scores based on the normal distribution. In cases where no published norms were available (DB and PASAT-3 secs), test scores were normalized against 38 normal controls comparable with the patients on variables such as age and years of schooling. In such cases, raw scores were converted directly to Z-scores based on the normal distribution using control means and standard deviations (s.d.). Subjects were classified as impaired if they failed two or more NP tests. The cut-off score for failing a NP test was a Z-score of  $-1.6$  s.d. (5th percentile) or below.

## Magnetic Resonance Imaging Acquisition

Magnetic resonance imaging was performed using a 3.0-T scanner (Trio, Siemens Medical Systems, Erlangen, Germany) with an eight-channel phased-array head coil. The following sequences were collected in all subjects during a single MR session: (a) dual-echo turbo spin-echo (repetition time (TR) = 5,500 ms, echo time (TE) = 12/99 ms, 48 contiguous, 3-mm-thick axial slices with a  $256 \times 205$  matrix, and a  $220 \times 190$ mm field of view (FOV); parallel imaging acceleration factor of 2; (b) gradient-echo echo-planar imaging (TR = 1,000 ms, TE = 32 ms; 10 contiguous, 3-mm-thick axial slices with a  $128 \times 128$  matrix;  $220\text{mm} \times 220\text{mm}$  FOV; flip angle,  $30^\circ$ ; signal bandwidth, 1,396 Hz/pixel; in-plane voxel size,  $1.7\text{mm} \times 1.7$  mm). Dynamic susceptibility contrast enhanced MR images were acquired during the first pass of a standard-dose bolus (0.1 mmol/kg) of gadopentetate dimeglumine (Magnevist; Berlex Laboratories, Wayne, NJ, USA). Contrast was injected at a rate of 5 mL/sec, followed by a 20-mL bolus of saline also at a rate of 5 mL/sec. A total of 60 images were acquired at 1-sec intervals, with the injection occurring at the fifth image, so that the bolus would typically arrive at the 15th to 20th image. (c) Post-Gd T1-weighted spin-echo (TR = 471 ms, TE = 12 ms, 50 contiguous, 3-mm-thick, axial slices with a  $256 \times 205$  matrix, and a  $220\text{mm} \times 220\text{mm}$  FOV).

## Image Processing and Evaluation

Absolute cerebral blood volume (CBV), CBF, and mean transit time (MTT) were calculated using the method of Rempp *et al* (1994). Mean transit time, CBV, and CBF were calculated by placing regions of interest in the following brain regions: frontal and periventricular NAWM, the splenium of the corpus callosum, thalamus, putamen, and the head of the caudate nucleus bilaterally. Regions of interest were fixed in size (radius 1 image pixel, 1.7 mm) and were placed to avoid arterial and venous structures, and MS lesions as described elsewhere (Adhya *et al*, 2006). Perfusion measurements were obtained by two authors blind to patients' identity. To exclude interobserver and minimize intraobserver variability as to the location of regions of interest placement between patients, each patient data set was reviewed by both authors at the same time (Wetzel *et al*, 2002).

Classification of T2-W and T1-W LV was performed in each patient by a single observer, unaware of subject identity, employing a segmentation technique based on user-supervised local thresholding (Jim 3.0, Xinapse System, Leicester, UK). Hypointense WM T1-W lesions were defined as those lesions with signal intensity between that of the GM and the cerebrospinal fluid on T1-W scans (van Waesberghe *et al*, 1998). In both T2-W and T1-W images, the value of total brain LV was calculated by multiplying lesion area with slice thickness.

## Statistical Analysis

For each subject, CBF, CBV, and MTT values were averaged over sides and over the three regions of WM and the three regions of GM to generate one measure from the NAWM and one measure from the deep GM. An analysis of covariance was performed to compare subject groups. In each case, the perfusion measure within NAWM and deep GM constituted the dependent variable, patient age and gender the covariates and subject group the classification factor. Using data only from MS patients, least-squares regression was used to evaluate the association of NAWM and deep GM perfusion metrics and T2-W and T1-W LVs with the Z-scores from each NP test, and the association of each perfusion measure with EDSS, T2-W, and T1-W LVs, adjusting for age, gender, and disease duration. It is noted that the NP Z-scores were each found to be reasonably normally distributed within each patient group as were the T2-W and T1-W LVs after subjected to a natural log transformation. Thus, the dependent variables for the regression analyses consisted of the NP test Z-scores, the natural log of the LVs, and the ranks of the EDSS scores (since EDSS is ordinal rather than numeric). A separate analysis was conducted for each subject group and each dependent variable. In each case, the candidate predictor variables included the NAWM and deep GM CBF, CBV, and MTT (for

all analyses), and the T2-W and T1-W LVs (when the dependent variable was a NP Z-score). The candidate covariates included age, gender, BSI-D and disease duration. Using results from the regression analysis, Pearson correlations were used to quantify the association of each perfusion measure with the Z-scores from each NP test, after adjusting for age, gender, BSI-D, and disease duration.

All reported *P*-values are two-sided type 3 significance level (i.e., *P*-values to assess the effect of one factor adjusted for the effects of all other factors in the same model) with the *P*-values for the correlations between a specific imaging measure and each of the NP test scores subjected to a Bonferroni correction. Results were declared statistically significant when the relevant *P* < 0.05. All statistical computations were performed using SAS for Windows version 9.0 (SAS Institute, Cary, NC, USA, 2002).

## Results

### Comparison of Neuropsychological Z-Scores between Patients Groups

The mean  $\pm$  s.d. of each NP test Z-score for RR-MS and PP-MS patients is given in Table 1. After adjusting for age, gender, education, disease duration, and BSI-D, PP-MS patients showed a significant decrease in Z-scores with respect to the following NP tests: RCFT-Copy ( $P < 0.0001$ ), California Verbal Learning Test-II ( $P = 0.002$ ), and DB ( $P = 0.003$ ). The statistical significance was retained after Bonferroni correction ( $P < 0.03$ ). RR-MS patients (6/18) were under treatment with interferon- $\beta$ 1a (30  $\mu$ g administered intramuscularly once weekly (Avonex)), which has shown a favorable effect on measures of information processing and learning memory (Fischer *et al*, 2000). However, no significant difference ( $P > 0.1$ ) in terms of NP test scores was found between RR-MS on treatment with interferon- $\beta$ 1a and RR-MS patients on treatment with other disease-modifying therapies.

### Comparison of Perfusion Metrics between Subject Groups

After adjusting for age and gender, significant differences were found between the PP-MS patients and the healthy controls with respect to the CBF ( $P \leq 0.001$ ) and CBV ( $P \leq 0.001$ ) in both WM and GM regions. Also, significant differences were found between the RR-MS patients and the healthy controls with respect to the CBF ( $P \leq 0.009$ ) in both WM and GM regions. Although CBV values were lower in RR-MS compared with controls, the difference reached statistical significance in the WM ( $P \leq 0.001$ ) but not in the GM ( $P = 0.3$ ). After adjusting for age, gender, and disease duration, significant differences were found between the patients with PP-MS and RR-MS with respect to CBF ( $P \leq 0.001$ ) and CBV ( $P \leq 0.005$ ) in both WM and GM. There were no significant differences among the three groups of subjects in terms of MTT ( $P > 0.2$  for all comparisons). Results pertaining to group comparisons with respect to average CBF and CBV and MTT are given in Table 2.

### Correlations between Perfusion Metrics and Neuropsychological Tests

In RR-MS patients, after adjusting for BSI-D, deep GM CBV ( $r = 0.4$ ;  $P = 0.07$ ) as well as NAWM CBV ( $r = 0.4$ ;  $P = 0.06$ ) showed a trend toward a significant association with D-KEFISIS.

In PP-MS patients, after adjusting for BSI-D, deep GM CBF was significantly associated with RCFT-Copy ( $r = 0.8$ ;  $P = 0.0003$ ) and deep GM CBV with D-KEFISIS ( $r = 0.6$ ;  $P = 0.03$ ). NAWM CBF was significantly associated with VFT ( $r = 0.5$ ;  $P = 0.05$ ) and Symbol Digit Modalities Test ( $r = 0.5$ ;  $P = 0.04$ ) and NAWM CBV with VFT ( $r = 0.5$ ;  $P = 0.04$ ). In the group of patients as a whole, after adjusting for age, gender, disease duration, and BSI-D, deep GM CBF was significantly associated with RCFT-Copy ( $r = 0.5$ ;  $P = 0.001$ ), deep GM CBV with D-KEFISIS ( $r = 0.4$ ;  $P = 0.008$ ), and NAWM CBV with D-KEFISIS ( $r = 0.4$ ;  $P = 0.02$ ). Statistical

trends were also observed between GM CBF and California Verbal Learning Test-II ( $P = 0.06$ ), DB ( $P = 0.07$ ), and VFT ( $P = 0.09$ ). However, the only associations that remained significant after Bonferroni correction were between deep GM CBF and RCFT-Copy ( $P = 0.002$ ) in PP-MS patients and between deep GM CBF and RCFT-Copy ( $P = 0.006$ ), and deep GM CBV and D-KEFSSIS ( $P = 0.04$ ) in all patients (cf., Figure 1). No association was found between deep GM or NAWM MTT and NP scores in either group of patients ( $P > 0.2$ ).

### Correlations between T2-Weighted, T1-Weighted Lesion Volumes, and Neuropsychological Tests

The mean T2-W LV was 4.6mL (range: 0.12 to 27.8) in RR-MS patients and 7.0mL (range: 0.39 to 32.9) in PP-MS patients. The mean T1-W LV was 0.91mL (range: 0.04 to 3.7) in RR-MS and 2.9mL (0.04 to 21.5) in PP-MS. After adjusting for BSI-D, T2-W LV was significantly associated with VFT ( $r = -0.6$ ;  $P = 0.02$ ) and with PASAT-3 secs ( $r = -0.8$ ;  $P = 0.002$ ) in PP-MS patients, whereas T1-W LV was significantly associated with PASAT-3 secs ( $r = -0.8$ ;  $P = 0.001$ ) in PP-MS patients and with D-KEFSSIS ( $r = -0.5$ ;  $P = 0.04$ ) in RR-MS patients. However, after Bonferroni correction, only the correlations between PASAT-3 secs and T2-W LV ( $P = 0.05$ ) and T1-W LV ( $P = 0.02$ ) in PP-MS patients were retained. In the patient group as a whole, after adjustment for age, gender, disease duration, and BSI-D, T2-W LV was significantly associated with VFT ( $r = -0.4$ ;  $P = 0.02$ ) and with PASAT-3 secs ( $r = -0.5$ ;  $P = 0.006$ ) and T1-W LV with VFT ( $r = -0.4$ ;  $P = 0.03$ ) and PASAT-3 secs ( $r = -0.6$ ;  $P = 0.0004$ ). However, after Bonferroni correction, only the correlation between T1-W LV and PASAT-3 secs was retained ( $P = 0.007$ ).

### Correlations between Perfusion Metrics, T2-Weighted, and T1-Weighted Lesion Volumes and Expanded Disability Status Scale

Neither the NAWM nor the deep GM perfusion metrics were significantly associated with T2-W or T1-W LVs in either patient group ( $P > 0.09$ ) or in the two groups together ( $P > 0.2$ ). Neither the NAWM ( $P > 0.2$ ) nor the deep GM ( $P > 0.4$ ) perfusion metrics were significantly associated with EDSS in either patient group or in the two groups together ( $r = -0.4$ ;  $P > 0.05$ ). T2-weighted LV ( $r = 0.6$ ;  $P = 0.01$ ) and T1-W LV ( $r = 0.5$ ;  $P = 0.02$ ) were significantly associated with EDSS in the RR-MS patients but not in the PP-MS patients ( $P > 0.4$ ). T2-weighted LV and EDSS ( $r = 0.4$ ;  $P = 0.02$ ) were also significantly associated when the patients were considered as a whole group. Neither perfusion metrics nor T2-W and T1-W LV were associated with disease duration either in the two patient groups separately or in the patient group as a whole ( $P > 0.1$ ).

## Discussion

This is the first study investigating the relationship between brain perfusion impairment, as measured by MRI, and cognitive dysfunctions in patients affected by MS. Almost 70% (10 out of 14) of PP-MS and 44% (8 out of 18) of RR-MS exhibited impairment on two or more NP tests. This is in line with the estimates of prevalence of cognitive deficits in MS that range from 40 to 65%, with higher frequency in the chronic progressive clinical subtype (Heaton *et al*, 1985; Rao *et al*, 1991a).

An ischemic component has long been acknowledged in histological studies of MS (Wakefield *et al*, 1994). Indeed, several mechanisms may lead to hypoxia-like tissue injury in MS. Cytotoxic T cells may recognize their antigen on endothelial cells and activate a clotting cascade, which, in turn, leads to thrombosis. Likewise, specific antibodies may recognize their antigen at the vessel wall and induce vascular damage by complement activation. Furthermore, inflammatory edema may impair microcirculation through focal tissue swelling, whereas exudation of inflammatory cells and intravascular fibrin deposition may induce acute and

chronic venous obliterations (Lassmann, 2003). In addition, obliterative vasculitis might result in chronic ischemia through the modulation of vascular tone and CBF. The mild diffuse ischemia resulting from all these processes might trigger a pathological cascade leading to mitochondrial dysfunction, nitric oxide production, and release of calcium and glutamate. Together these factors contribute to the onset and progression of neuroaxonal injury, which is considered the pathological substrate of irreversible clinical and neurocognitive deficits.

In our study, decreased brain perfusion correlated with cognitive deficits in PP-MS patients and in the group of patients as a whole. This is in line with the findings of previous studies using PET and single-photon emission computed tomography (Blinkenberg *et al*, 2000; Brooks *et al*, 1984; Lycke *et al*, 1993; Paulesu *et al*, 1996; Pozzilli *et al*, 1991; Roelcke *et al*, 1997), which have demonstrated a generalized coupled reduction in both cerebral oxygen utilization and blood flow in patients with established MS. Hypoperfusion was reported in both WM and GM and correlated with cognitive impairment rather than with locomotor disability, thus reiterating that the EDSS is heavily weighted toward locomotor disability and that the cerebral functional system is of limited sensitivity. Most PET and single-photon emission computed tomography studies, however, investigated only patients affected by RR-MS and, with very few exceptions (Paulesu *et al*, 1996; Pozzilli *et al*, 1991), cognition was assessed using the mini mental status scale or the cerebral functional system of the EDSS. Unlike PET and single-photon emission computed tomography, DSC perfusion MRI has several advantages: higher signal- to-noise ratio and contrast-to-noise ratio, higher spatial resolution, absence of ionizing radiation that makes the method more amenable to serial studies and shorter acquisition time (Sorensen and Reimer, 2000). In addition, at 3.0 T, the intrinsic signal-to-noise ratio of DSC perfusion is higher and sensitivity to T2\* changes due to contrast agent is increased. This can be traded off for a shorter TE and hence a larger number of slices with improved brain coverage within a fixed TR (Manka *et al*, 2005).

A second finding of our study, and perhaps more interesting, is the association between the decrease of CBF in the deep GM and the NP performance of MS patients. Previous imaging studies have suggested a role for basal ganglia and thalami in the NP dysfunction affecting patients with MS (Blinkenberg *et al*, 2000; Mainero *et al*, 2004; Paulesu *et al*, 1996). Using PET, Paulesu *et al* (1996) showed a significant reduction of cerebral glucose metabolism in the left thalamus and in both hippocampi of MS patients with memory deficits compared with unimpaired MS patients. In a functional MRI study, RR-MS patients with no or only mild NP deficits showed significantly greater brain activation compared with controls in the prefrontal, temporal cortex, basal ganglia bilaterally, and in the left thalamus during the administration of a memory test (Mainero *et al*, 2004). Recent advances in neuroscience have shown that cognitive functions are controlled by widely distributed neural networks, and not simply by certain restricted cortical areas. In particular, basal ganglia and thalami have revealed a level of organization and functional specificity paralleling that of the cerebral cortex itself. Accordingly, deep gray nuclei should no longer be viewed as structures having a role independent of the cerebral cortex and thalamus, with which they have a highly specific afferent and efferent connection. Specifically, the ‘dorsolateral prefrontal’ circuit, although not fully characterized, is believed to participate in processes subserving spatial memory (Alexander *et al*, 1986; Goldman *et al*, 1971). It is worth noting that, among the NP tests, the RCFT-Copy, a measure of visuospatial skills, showed the best correlation with the deep GM hypoperfusion. Admittedly, our results have to be interpreted with caution, as one PP-MS patient was identified as an outlier with respect to the RCFT-Copy test Z-score. Although the association between the RCFT-Copy Z-score and the GM CBF decrease was moderate and still significant after the removal of the outlier (Figure 1) from the analysis, the statistical significance was not retained after correction for multiple comparisons. Nevertheless, it is of interest that the GM CBF decrease was greater in patients with more severe impairment on the the RCFT-Copy test. Disappointingly, decreased perfusion in the NAWM and deep GM was associated only with

some NP test scores. This might have several explanations. First, MS-related cognitive dysfunction is inherently heterogeneous, affecting different cognitive domains to different degrees in different patients, with progression rates varying across patients and across cognitive domains. Second, although MS is a diffuse and widespread disease, microstructural and functional impairment may show regional degrees of severity in different patients at different stages of the disease. Finally, a statistical issue should also be considered: although several NP test scores did not reach statistical significance when correlated with decreased perfusion in both NAWM and deep GM, some of them showed a statistical trend.

T2-weighted and T1-W LVs showed a moderate correlation with PASAT-3 secs. The presence of widespread lesions leads to the disruption of WM pathways, which connect distant brain regions involved in the complex processes required to perform PASAT correctly. In most of the previous studies, correlations between T2-W LV and NP performance have been weak or moderate (Foong *et al*, 1997; Rao *et al*, 1989; Rovaris *et al*, 2002; Swirsky-Sacchetti *et al*, 1992). This is most likely owing to the poor pathological specificity of the abnormalities detected on T2-W scans and to the inability of conventional MRI to assess the extent of subtle changes in the normal-appearing brain tissue. Accordingly, the use of quantitative MRI techniques (Deloire *et al*, 2005; Rovaris *et al*, 2002) to assess MS pathology in normal-appearing brain tissue has shown more encouraging results in terms of correlation with cognitive dysfunction (Filippi, 2000). Interestingly, in our cohort of MS patients, there was no correlation between regional hypoperfusion and T2-W and T1-W LVs suggesting that both focal WM lesions and the diffuse hypoperfusion of NAWM and NAGM might be independently associated with NP deficits.

An alternative explanation to our findings is that tissue damage such as axonal loss and demyelination may lead to lower oxygen use and lower blood flow demand with subsequent CBF decrease. Although our study is preliminary, the lack of correlation between measures of tissue damage (T2 and T1 LVs) and perfusion metrics and between disease duration and perfusion measures suggest that the decrease of CBF is not the mere consequence of lower blood flow demand due to more tissue damage.

Although our study provides the first attempt to correlate MRI measures of brain perfusion and cognitive performance in patients with MS, it has several caveats. First of all, owing to the relatively small patient sample, our findings have to be regarded as preliminary and their interpretation has to be cautious. It is now important to confirm these findings in a larger patient group, examined serially from the early stage of the disease. Second, although the correlations we found between hypoperfusion and the severity of cognitive dysfunction is encouraging, there is still much NP variance to be explained. The assessment of cortical GM and lesional perfusion that may have accounted for the variance in cognitive impairment would have been beneficial. Studies involving technical implementations, particularly regarding improved focal lesions as well as cortical GM segmentation on the perfusion maps, are ongoing at our center. Third, the effect of immunomodulating treatment (only RR-MS patients were under treatment) on vascular permeability and vascular inflammation is not known. Therefore, perfusion studies in MS patients before and after immunomodulating therapy are needed to assess for a possible effect of treatment on perfusion parameters.

In conclusion, in our preliminary study, MRI perfusion metrics are associated with NP dysfunction. This assumes increasing relevance as cognitive impairment is very common in MS and has a negative impact on the quality of life. Not only does DSC MRI have the potential to investigate the adverse effect of neuroaxonal injury on brain metabolism in MS, but also the association with cognitive deficits suggests that this technique may complement conventional MRI and provide a potentially useful non-invasive evaluation of these symptoms.

## Acknowledgements

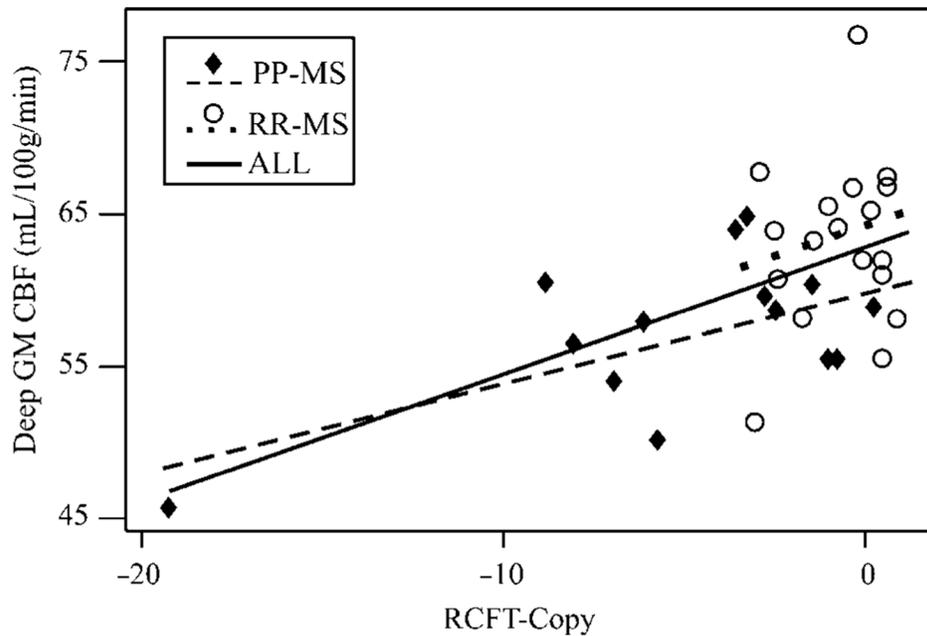
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**Figure 1.** Scatter plot of the correlation between the mean deep gray matter (GM) CBF expressed in mL/100 g per min versus the mean Rey Complex Figure Test (RCFT) Z-score in patients with RR-MS ( $\circ$ ) and in patients with PP-MS ( $\blacklozenge$ ). Lower scores of (RCFT) Z-score indicate greater visuospatial memory impairment. Note that the least-squares regression line indicates a significant linear relationship in PP-MS (dashed line;  $r = 0.8$ , adjusted  $P = 0.002$ ) and in the whole group of patient (solid line;  $r = 0.5$ , adjusted  $P = 0.006$ ) but not in RR-MS patients (dotted line;  $r = 0.1$ ,  $P > 0.5$ ). It is noted that the partial correlation of deep GM CBF and RCFT-Copy in the PP-MS patients and in the patient group as a whole remained significant without Bonferroni correction ( $r = 0.67$ , adjusted  $P = 0.0125$ ;  $r = 0.47$ , adjusted  $P = 0.007$ , respectively) after removal of the patient with the outlying value of  $-19.2$  for RCFT-Copy.

**Table 1**Mean  $\pm$  s.d. of NP tests Z-scores of RR-MS and PP-MS patients

Neuropsychological tests	RR-MS patients Z-score	PP-MS patients Z-score	P-value
RCFT-Copy	-2.3 $\pm$ 4.4	-5.1 $\pm$ 5.1	< <b>0.0001</b>
RCFT-Immediate	-1.3 $\pm$ 1.0	-2.0 $\pm$ 1.1	0.5
RCFT-Delayed	-1.6 $\pm$ 1.0	-2.1 $\pm$ 1.2	0.3
VFT	0.7 $\pm$ 1.5	0.3 $\pm$ 1.8	0.5
CVLT	0.7 $\pm$ 1.0	-0.6 $\pm$ 0.9	<b>0.002</b>
DB	-0.06 $\pm$ 1.2	-1.0 $\pm$ 0.9	<b>0.003</b>
SDMT	-0.2 $\pm$ 0.9	-1.2 $\pm$ 1.5	0.1
PASAT-3 secs	-0.5 $\pm$ 1.5	-1.1 $\pm$ 1.4	0.6
D-KEFSI	0.2 $\pm$ 0.9	0.06 $\pm$ 1.1	0.2
D-KEFSIS	0.1 $\pm$ 1.0	-0.1 $\pm$ 1.4	0.2

BSI-D, Depression subscale of the Brief Symptom Inventory CVLT, California Verbal Learning Test-II; DB, Digits Backward portion of WAIS-III Digit Span subtest; D-KEFSI, D-KEFS Color-Word Interference Test: Inhibition; DKEFSIS, D-KEFS Color-Word Interference Test: Inhibition Switching; PASAT-3 secs, 3 sec trial of the Paced Auditory Serial Addition Test; RCFT-Copy, Rey Complex Figure Test-Copy; RCFT-Delayed, Rey Complex Figure Test-Delayed Recall; RCFT-Immediate, Rey Complex Figure Test-Immediate Recall; s.d., standard deviation; SDMT, Symbol Digit Modalities Test; VFT, D-KEFS Verbal Fluency Test.

Each NP score is adjusted for age, gender, education, disease duration, and BSI-D and corrected by Bonferroni test. Statistically significant comparisons are indicated in bold.

**Table 2**

Mean  $\pm$  s.d. of CBF, CBV, and MTT values averaged over the normal-appearing white matter and over the deep gray matter nuclei of healthy controls, RR-MS, and PP-MS patients

	Healthy controls		RR-MS patients		PP-MS patients	
	Deep GM	WM	Deep GM	NAWM	Deep GM	NAWM
CBF (ml/100 g/min)	68.2 $\pm$ 7.1	42.4 $\pm$ 5.9	62.9 $\pm$ 7.8	32.7 $\pm$ 4.9	57.2 $\pm$ 8.4	27.3 $\pm$ 4.5
CBV (ml/100 g)	4.8 $\pm$ 0.6	3.1 $\pm$ 0.7	4.5 $\pm$ 0.7	2.4 $\pm$ 0.5	3.9 $\pm$ 1.0	1.9 $\pm$ 0.6
MTT (seconds)	4.3 $\pm$ 0.6	4.4 $\pm$ 0.7	4.3 $\pm$ 0.7	4.3 $\pm$ 0.6	4.0 $\pm$ 0.9	4.2 $\pm$ 1.0

CBF, cerebral brain flow; CBV, cerebral brain volume; GM, gray matter; MTT, mean transit time; NAWM, normal-appearing white matter; PP-MS, primary-progressive multiple sclerosis; RR-MS, relapsing-remitting multiple sclerosis; s.d., standard deviation; WM, white matter.

Values are given as means  $\pm$  s.d. and adjusted for age, gender, and disease duration.