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# Lower Total Cerebral Arterial Flow Contributes to Cognitive Performance in Multiple Sclerosis Patients

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## Abstract

**Background:** The cognitive performance in multiple sclerosis (MS) patients declines with aging, longer disease duration, and possibly cardiovascular comorbidities.

**Objectives:** We investigated whether lower total cerebral arterial blood flow (CABF) measured at the level of the carotid and vertebral arteries, may contribute to worse cognitive performance in 132 MS patients and 47 healthy controls.

**Methods:** Total CABF was evaluated with extracranial Doppler, whereas structural T2-lesion volume (LV) and gray matter volume (GMV) were measured on 3T MRI. The cognitive performance was assessed by Symbol Digit Modalities Test (SDMT), Brief Visuospatial Memory Tests-Revised (BVMT-R), and California Verbal Learning Test Second Edition (CVLT-II). Analysis of covariance, partial correlation, and regression models were used to test the differences between study groups and cognition/CABF correlations. FDR-corrected (Benjamini-Hochberg) p-values (i.e. q-values) <0.05 were considered significant.

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Dejan Jakimovski, Karen Marr, Sirin Ghandi and Niels Bergsland have nothing to disclose.

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**Results:** Association between lower total CABF and the lower cognitive performance was observed only in MS patients (r=0.318, q<0.001 and r=0.244, q=0.012, for SDMT, and BVMTR, respectively). Lower GMV, higher T2-LV, and CABF were significantly associated with poorer performance on the processing speed measure of SDMT (adjusted $R^2$=0.295, t-statistics = 2.538, Standardized B=0.203 and q=0.020), but not with memory tests. Cognitively impaired MS patients had lower total CABF compared to cognitively preserved (884.5ml/min vs. 1020.2ml/min q=0.008).

**Conclusion:** Cognitively impaired MS patients presented with lower total CABF. Altered CABF may be a result of reduced metabolic rate and might contribute to abnormal cognitive aging in MS.

### Keywords

MS; cerebral arterial blood flow; MRI; cognition; Doppler

## Introduction

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS), which is characterized by the reoccurrence of focal neurological episodes associated with inflammation, axonal injury, and gliosis. [1] In the later stages of the disease, axonal dissection and Wallerian degeneration, the age-associated cerebral small vessel disease, and vascular comorbidities are suggested as important factors for development of MS neurodegeneration.[2, 3]

Recent treatment improvements and more favorable MS outcomes have contributed to an increased prevalence of older MS patients, an epidemiological phenomenon which has led to growing interest in the etiology of the long-term cognitive outcomes. [4] The cognitive impairment (CI) in MS patients can vary based on the examined population starting from 35% within the relapsing-remitting MS (RRMS) patients and increasing up to as many as 60% within the older, secondary-progressive MS (SPMS) patients. [4] The most commonly observed cognitive feature of CI MS patients is slowed processing of information, which can be measured by the Symbol Digit Modalities Test (SDMT). This test is a sensitive tool which the Multiple Sclerosis Outcome Assessments Consortium (MSOAC) and representatives from the Food and Drug Administration (FDA) and European Medicines Agency (EMA) recommended as a valid, reliable and cost-effective cognitive outcome measure for MS. [5] Moreover, multiple MRI studies have demonstrated that the cognitive impairment in MS is highly associated with lower cortical and deep gray-matter volume and higher number of cortical and white matter (WM) lesions. [6]

Recent studies also implicate reduced cortical cerebral blood volume in this vulnerable population of aged MS patients. [7] This reduction in both cerebral blood flow and cerebral blood volume was present even after correcting for patient's lesion burden and overall brain atrophy. [7] Abnormal perfusion findings are observed in both early and late stages of MS, with decreased perfusion in CI SPMS patients. [8]

The Canadian MS registry reveals an age-specific increase in the prevalence of cardiovascular comorbidities which include hypertension, hyperlipidemia, diabetes and heart

disease compared to the age-matched controls. [9] This increase of hypertension and hyperlipidemia in MS patients (10% within the age group of 20–44 years and up to 60% in the ≥60 years old MS patients), may additionally contribute to the higher atherosclerotic burden of the main arterial vessels and potentially cause an additional CI. [9] Along those lines, recent works show that MS patients have lower arterial neck vessel size when compared to age-, sex- and CVD-matched controls, suggesting that there is an overlap of pathophysiological pathways between the MS inflammation and atherosclerosis formation. [10] Moreover, larger arterial neck vessels are narrowed in patients compared to the controls over 5 years. [11] Furthermore, certain types of carotid artery morphological variates (especially K-type morphology) have been additionally associated with altered cognitive performance. [12] Lastly, over a period of 5 years, MS patients with presence of cardiovascular comorbidities developed higher whole brain volume loss and enlargement of lateral ventricles compared to those without. [13]

Against this background, studies investigating cardiovascular health and its effect on cognition in MS patients with long disease duration are currently missing and warranted. We hypothesized that lower total cerebral arterial blood flow (CABF), measured at the level of the main arterial neck vessels, contributes to worse cognitive performance in MS patients with longer disease duration.

## Materials and Methods

### Study population

The participants were part of a larger, prospective, cardiovascular, environmental, and genetic study in MS (CEG-MS) that enrolled patients from 2014 until 2017. [14] The inclusion criteria for this study consisted of: 1) age between 18 and 75 years old; 2) having MRI, neuropsychological examination, and Doppler scans completed within 30 days of the clinical examination; 3) being a healthy control (HC) without known history of neurological disorder, and 4) MS diagnosis as defined by the 2010-revised McDonald criteria. [15] On the other hand, the exclusion criteria were: 1) known history of morphological vascular abnormalities (Klippel-Trenaunay-Weber, ParkesWeber, Servelle-Martorell or Budd-Chiari syndromes), 3) history of major depressive disorder, mood disorders or other confirmed psychiatric diseases, and 3) pregnant and nursing mothers. Both the primary-progressive MS (PPMS) and SPMS patients were grouped into a single progressive MS (PMS) group. The demographic, clinical data and information regarding cardiovascular comorbidities were collected using a previously published structured interview-based questionnaire and cross-reference with electronic medical records. [16] Additionally, the MS patients were examined by experienced neurologist and the Expanded Disability Status Scale score was used to determine the level of physical disability. The study was approved by the local Institutional Review Board (IRB) and all study participants signed written consent form.

### Neuropsychological examination

Both the MS and HC groups underwent neuropsychological examination by trained examiners under supervision of a board-certified neuropsychologist. We applied the Brief International Cognitive Assessment for MS (BICAMS), comprising the SDMT, [17] and the

learning trials from the Brief Visuospatial Memory Tests-Revised (BVMT-R)[18] and the California Verbal Learning Test Second Edition (CVLT-II). [19] In all three tests, higher scores indicate better cognitive performance.

The post-hoc comparison between the CI and cognitively preserved (CP) MS patients was derived using the >−1.5 z-score classification (equivalent to 1.5 standard deviations) when compared to the performance of the HCs. In order to reduce the effect of age, site and method of examination, the z-scores were calculated based on the performance of the matched-HCs from this study cohort. MS patients with at least one >−1.5 z-score test were classified as CI-MS patients.

### Doppler ultrasound

Arterial neck vessel examination was performed using an echo-color Doppler (ECD Esoate – Biosound My Lab 25 Gold, Genoa, Italy) equipped with a 7.5–10 MHz transducer (ECD Easote – Biosound, Genoa, Italy). The examination was performed by a blinded technologist who used a standardized scanning protocol. Briefly, warm Aquasonic 100 water-soluble, hypoallergenic transmission gel was placed on the neck area with the head placed in a neutral, forward position. The left and right common carotid artery (CCA) blood flow was obtained in supine position approximately 1.5 cm before the bifurcation and at the proximal neck level of the CCA. Similarly, the bilateral vertebral artery (VA) blood flow was obtained at the level of C5-C6 level. Anterior to posterior wall diameter (AP), at 180° alignment was obtained for the computed generated CCA and VA cross-sectional area. For deriving the time-averaged velocity (TAV) a manual waveform tracing over four second period was used. The ECD frequency, 55°- 60° angle of incident, and spectral gate size were adjusted according to standard vascular protocol. Each individual flow from the bilateral CCA and VA was added together and hereafter referred as total CABF.

### Magnetic resonance imaging acquisition and analysis

The MRI scans were obtained using a 3T GE Signa Excite HD 12 Twin Speed 8-channel scanner (General Electric, Milwaukee, WI, USA) with an 8-channel head and neck (HDNV) coil. The primary MRI sequences used for the purposes of this study included an axial 3D-spoiled-gradient recalled (SPGR) T1-weighted image (WI) and 2D fluid attenuated inversion recovery (FLAIR). The slice thickness of the sequences was 3mm and 1mm for the 2D FLAIR and 3D T1-WI, respectively. Detailed description of the MRI acquisition protocol has been reported previously. [20] The T2-lesion volume (LV) was obtained by two trained operators using a semi-automated edge detection and contouring/thresholding technique previously described [Java Image Manipulation (JIM), Xinapse Systems, Essex, UK]. [21] The gray matter volume (GMV) analysis was obtained utilizing the SIENAX software (version 2.6, FMIRB, Oxford, UK) after inpainting to mitigate the impact of hypointense lesions. [22] SIENAX estimates total and regional brain tissue volumes which are normalized for the skull size.

### Statistical analysis

All statistical analysis was performed using SPSS 24.0 (IBM, Armonk, NY, USA). The differences between the demographic, neuropsychological, Doppler, and MRI variables

between the MS patients and HCs, and between CI and CP MS patients were calculated using $\chi^2$- test, Student's *t*-test, Mann-Whitney U-test and analysis of covariance (ANCOVA) adjusted for age, as appropriate. In particular, the $\chi^2$ was used to compare categorical variables including the sex ratios, disease phenotype (RRMS/PMS) and the differences in prevalence of the cardiovascular diseases, whereas Student's *t*-test was used to compare normally distributed numerical variables including age, disease duration, body mass index, years of education, CVLT-II, BVMT-R, SDMT, total CABF, and MRI-derived T2-LV and GMV. Due to the ordinal nature of the EDSS scale, Mann-Whitney U-test was used. The associations between the total CABF and the neuropsychological tests were derived using partial correlation, adjusted for age and years of education.

Furthermore, the significant associations from the partial correlation analysis, were only tested in linear regression models. The neuropsychological tests (SDMT, CVLT-II, and BVMTR) were used as dependent variables, whereas age, sex, years of education, T2-LV and GMV as independent variables. The stepping criteria for the second block used entry level of 0.05 and removal level of 0.1. F tests were used to assess R2 changes between models. Second confirmatory regression model utilized block 1 which consisted of forced demographic (sex, age and years of education) and MRI-derived variables (T2-LV and GMV) and in the second block the total CABF. After utilizing Benjamini-Hochberg procedure, the false discovery rate (FDR)corrected p-values (i.e. q-values) lower than 0.05 were considered statistically significant.

## Results

### Demographic and clinical characteristics

The demographic, clinical, neuropsychological, MRI and Doppler characteristics of both the MS patients and HCs are shown in Table 1. The MS patients were 53.5 years old, had 14.9 years of education, and had mean disease duration of 20.4 years. There were no differences in terms of age, sex ratio, and years of education between the MS patients and HCs. Additionally, 82 (62.1%) patients had RRMS and 50 (37.9%) had PMS. There were no significant differences in the prevalence of cardiovascular comorbidities between MS and HCs.

On the cognitive examination, as expected, the MS patients performed worse than the HCs in both BVMT-R and SDMT tests (21.9 vs. 26.5, p=0.001 and 49.5 vs. 55.5, p=0.029, respectively). There was no performance difference on the CVLT-II examination (51.9 vs. 54.9, p=0.222).

Similarly, the MS patients had on average lower GMV (732.3ml vs. 768.0ml, p=0.001) and higher T2-LV (13.5ml vs. 0.4ml, p<0.001) compared to HC. There were no differences in total arterial blood flow between the MS patients and the HCs (961.6ml/min vs. 967.5ml/min, p=0.822).

### Association between cognitive performance and total cerebral arterial flow

The partial correlations between the total CABF and the cognitive performance are shown in Table 2. The association between lower total CABF and the lower cognitive performance

was observed in the MS patients (r=0.318, q<0.001 for SDMT, and r=0.244, q=0.012 for BVMT-R, respectively), whereas there was no significant association between total CABF and CVLT-II. There were also no significant associations between total CABF and the lower cognitive performance among the HCs. Furthermore, the associations between the blood flow within specific arterial pathways (CCA vs VA) and the cognitive performance is shown in Supplement Table 1. The aforementioned significant associations between global arterial blood flow and SDMT/BVMT-R were confirmed regardless of the side of the measurement (right vs. left vessels) and regardless of the arterial pathway (CCA vs. VA).

In linear regression models, the total CABF remained as a significant predictor of variance within the neuropsychological test performances associated with processing information speed ($R^2$=0.295, t-statistics=2.538, standardized B=0.203, and q=0.020 for SDMT). In addition to the GMV prediction, the step-wise addition of total CABF contributed to the model (from adjusted $R^2$=0.261 to adjusted $R^2$=0.295). The model generation and the effect size (t-statistics and standardized beta) are shown in Table 3. Second confirmatory regression model confirmed the explanatory SDMT variance by total CABF (Supplement Table 2).

In similar regression models of the HC group, there were no significant associations between total CABF and the cognitive performance (results not shown).

### Clinical, MRI and total cerebral arterial differences between cognitively-impaired and cognitively-preserved MS patients

The demographic, clinical, neuropsychological, MRI and Doppler characteristics of the CI and CP MS patients are shown in Table 4. The CI MS patients were more disabled (median EDSS score 3.5 vs. 2.5, p=0.004) and had higher prevalence of PMS diagnosis when compared to CP MS patients (p=0.029). As expected by their classification, the CI MS patients had worse performance on all neuropsychological examinations (q<0.001). CI MS patients also had higher T2-LV (18.1ml vs. 10.1ml, q=0.015), lower GMV (721.1ml vs. 746.6ml, q=0.039), and lower total CABF (884.5ml/min vs. 1020.2ml/min, q=0.008) compared to CP ones.

## Discussion

This study describes an association between lower total CABF at the level of extracranial arteries and abnormal cognitive performance within MS population. MS patients that were classified as CI had lower GMV, lower total CABF and higher T2-LV. This association of the CABF and cognitive performance was not observed in the matched HC group.

The decreased total CABF has been implicated as a silent substrate contributing to progression of CI in patients with amnestic mild CI, Alzheimer's disease, vascular dementia, and other neurodegenerative diseases. [23] In addition to the previously established negative effect of the cardiovascular diseases, the process of aging itself has been associated with lower CABF, decreased cardiac output, and cognitive changes. [24] For example, the cerebral blood flow in an aging population becomes increasingly dependent on the cardiac output irrespective of the functionality of the cerebral autoregulation. [25, 26] As a consequence of

potential hypoperfusion in subcortical structures associated with cognition, lower cardiac output has been additionally associated with lower executive function in healthy aging. [27] Therefore, the significant CABF changes seen in our numerically older CI MS group may be further explained by a potential aging interaction. However, the flow disparity remained significantly different despite adjusting for age in the statistical analysis. Furthermore, cerebral small vessel disease has been shown as a strong pathologic correlate responsible for appearance of WM hyperintensities in otherwise healthy and aging population. Therefore, vascular brain injury can promote neurodegenerative changes and contribute to the cognitive decline commonly seen in aging population. [23] Although the etiology of the T2 hyperintensities can be ambivalent, the CI MS patients in this study had significantly higher T2-LV. The contribution of arteriolosclerosis-associated pathology, especially within areas that are commonly affected by this process, can be difficult to distinguish from the presence of demyelinating MS-derived lesions. [28] As the average age of the MS population continues to increase, the cerebrovascular small vessel disease may provide additional confounding factor in MS lesion presentation.

The lower CABF may influence the extent and capability of the repair mechanisms that are counteracting the acute demyelinating processes. The inflammation which is associated with development of new T2 lesions has been correlated with an initial and acute increase of perfusion rates, as these changes within the perfusion can even be detected before the disruption of the blood-brain barrier and precede the formation of the gadolinium-enhancing lesions. [29] However, persistently active chronic plaques tend to be more prevalent within WM regions with relatively lower perfusion ratios. [30] It has been shown that regional perfusion deficit is associated with decreased lesion repair mechanisms, progression into chronic lesions, formation of T1-hypointense (black holes) lesions, and neurodegeneration. [31, 32] In particular, older and more disabled MS patients have lower cerebral blood flow within the cortical GM when compared to younger, less disabled RRMS patients. [33] and T1-black hole lesions are clustering in regions that are associated with lower perfusion rates. [31] In addition, the cortical cerebral blood flow is strongly associated with accumulation of cortical and WM lesions and with longer disease duration. [32] Even in cases where there is a lack of cerebral structural differences, the decreased cortical cerebral blood flow and cerebral blood volume have been associated with cortical pathology and poorer cognitive performance in MS patients. [34]

The perfusion changes that coincide with active inflammation, black hole formation, and neurodegeneration can be additionally influenced by the overall cardiovascular health. A recent UK-wide neuropathological study showed that the cerebrovascular pathology, which included arteriolosclerosis, perivascular space dilatation, and myelin loss, was able to predict the presence of CI. [35] The study recommended that neuropathologists should start reporting the likelihood of cerebrovascular disease and the extent of their contribution to CI. [35] Therefore, cardiovascular comorbidities, mediated through atherosclerosis, cerebral small vessel disease, and lowering of the CABF may contribute to increase of MS disability, development of brain atrophy, and ultimately poorer cognitive performance. [13, 20] As previously mentioned, a recent large casecontrolled study demonstrated that MS patients had lower arterial cross-sectional CCA and VA area when compared to age-, sex-, and CVD-matched HCs. [10] These alterations remained significant even after removing the portion of

MS patients with diagnosis of cardiovascular disease (hypertension and heart disease), suggesting potentially an MS-specific change. [10] In addition, over 5 years, MS patients showed significant cross-sectional decrease of all major neck vessels, regardless of the disease course and cardiovascular status. [11] In a similar fashion, decreased total CABF within this analysis was not accompanied with higher prevalence of cardiovascular diseases in MS population.

Although in this study we did not demonstrate difference in the overall CABF between the MS and HC groups, the differences were evident when we compared the CI with the CP MS patients (lower total CABF in CI MS<CP MS≈HC). Due to global processing network changes seen early in the MS disease, the task of proper cognitive functioning would require increase in total activated brain areas, larger energy expenditure, and larger blood flow recruitment. [36] However, aging MS patients may eventually exhaust this compensatory potential and present with lower cerebrovascular reactivity (responsiveness of the cerebral vasculature under vasoactive stimuli), which in turn, would lead to decreased recruitment of the required additional blood flow. [37] More comprehensive review of the cerebrovascular reactivity, its effect on cognition, and the role of cardiovascular risk factors as their mediating factor has been published elsewhere. [38]

The limitations of the study include the lack of cortical lesion detection and quantification. Cortical lesions have been shown as one of the major structural changes that contribute to cognitive impairment in RRMS patients. [39] However, this study has been strengthened by its multimodal nature, utilizing both Doppler, neuropsychological, and MRI examinations, which were prospectively collected for both the MS patients and the HCs. Future MS studies should demonstrate the associations between the CI and arterial flow, measured within the main arterial blood vessels, the changes in cortical thickness, the effect on cerebral perfusion, lesion accrual, and overall myelin content. Additionally, there is a need of longitudinal studies that would examine the contribution of successful hypertension and hyperlipidemia control on overall MS disease progression and cognitive performance.

Although minimally, the total CABF was associated with altered cognitive performance in MS patients. However, the lower CABF may also be explained by the decreased metabolic rate. Further longitudinal investigations should investigate the temporal sequence of the CABF changes and the multilayered factors that may contribute to the neurodegeneration and CI within the aging MS population.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## MS Journal Appendix for MRI methodology

| **Hardware** | |
|---|---|
| Field strength | 3T |
| Manufacturer | General Electric |
| Model | Signa Excite |
| Coil type (e.g. head, surface) | Multi-channel Head and Neck Coil |
| Number of coil channels | 8 |

| **Acquisition sequence** | |
|---|---|
| Type (e.g. FLAIR, DIR, DTI, fMRI) | 3D T1-WI (IR-FSPGR) |
| Acquisition time | 9:18 |
| Orientation | Oblique |
| Alignment (e.g. anterior commissure/poster commissure line) | anterior commissure/poster commissure line |
| Voxel size | 1×1×3 |
| TR | 6.6ms |
| TE | 2.8ms |
| TI | 900ms |
| Flip angle | 10 |
| NEX | 1 |
| Field of view | 25.6cm × 19.2cm |
| Matrix size | 256 × 256 |
| Parallel imaging | Yes **No** |
| If used, parallel imaging method: (e.g. SENSE, GRAPPA) | |
| Cardiac gating | Yes **No** |
| If used, cardiac gating method: (e.g. PPU or ECG) | |
| Contrast enhancement | Yes **No** |
| If used, provide name of contrast agent, dose and timing of scan post-contrast administration | |
| Other parameters: | |

| **Acquisition sequence** | |
|---|---|
| Type (e.g. FLAIR, DIR, DTI, fMRI) | FLAIR |
| Acquisition time | 5:16 |
| Orientation | Axial-oblique |

| **Acquisition sequence** | |
|---|---|
| Alignment (e.g. anterior commissure/poster commissure line) | parallel to the sub-callosal line |
| Voxel size | 1 × 1 × 3 |
| TR | 8500ms |
| TE | 120ms |
| TI | 2100ms |
| Flip angle | 90 |
| NEX | 1 |
| Field of view | 25.6cm × 19.2cm |
| Matrix size | 256 × 192 |
| Parallel imaging | Yes **No** |
| If used, parallel imaging method: (e.g. SENSE, GRAPPA) | |
| Cardiac gating | Yes **No** |
| If used, cardiac gating method: (e.g. PPU or ECG) | |
| Contrast enhancement | Yes **No** |
| If used, provide name of contrast agent, dose and timing of scan post-contrast administration | |
| Other parameters: | |

| **Image analysis methods and outputs** | |
|---|---|
| **Lesions** | |
| Type (e.g. Gd-enhancing, T2-hyperintense, T1-hypointense) | T2 hyperintense lesions |
| Analysis method | Semi-automated edge detection contouring/ thresholding technique |
| Analysis software | JIM version 6.0 |
| Output measure (e.g. count or volume [ml]) | mL |
| **Tissue measures (e.g. MTR, DTI, T1-RT, T2-RT, T2*, T2', $^{1}$H-MRS, perfusion, Na)** | |
| Type (e.g. whole brain, grey matter, white matter, spinal cord, normal-appearing grey matter or white matter) | Gray matter volume |
| Analysis method | SIENAX |
| Analysis software | FSL |
| Output measure | mL |

## Abbreviations

| | |
|---|---|
| **MS** | multiple sclerosis |
| **HC** | healthy controls |
| **SDMT** | Symbol Digit Modalities Test |
| **CVLT-II** | California Verbal Learning test – Second Edition |

| | |
|---|---|
| **BVMT-R** | Brief Visuospatial Memory Test – Revised |
| **CCA** | Common carotid artery |
| **VA** | Vertebral artery |
| **CABF** | Cerebral arterial blood flow |
| **GMV** | Gray matter volume |
| **T2-LV** | T2-lesion volume |
| **CI** | Cognitively impaired |

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**Table 1.**

Demographic, clinical, MRI and Doppler characteristics of the multiple sclerosis patients and the healthy controls.

| Group characteristics | MS (n=132) | HC (n=47) | p-value* | Cohen's d | ANCOVA** |
|---|---|---|---|---|---|
| Female/male, n (females %) | 92/40 (69.7) | 34/13 (72.3) | 0.761 | - | - |
| Age, mean (SD) | 53.5 (11.3) | 50.8 (12.8) | 0.184 | - | - |
| Disease duration, mean (SD) | 20.4 (10.5) | - | - | - | - |
| EDSS, median (IQR) | 3.0 (2.0–6.0) | - | - | - | - |
| RRMS vs. PMS | 82/50 | - | - | - | - |
| Body mass index, mean (SD) | 27.7 (5.9) | 26.4 (5.7) | 0.230 | - | - |
| Hypertension, n (%) | 25 (18.9) | 12 (25.5) | 0.402 | - | - |
| Hyperlipidemia, n (%) | 27 (20.5) | 11 (23.4) | 0.681 | - | - |
| Heart Disease, n (%) | 23 (17.4) | 4 (8.5) | 0.232 | - | - |
| Years of education, mean (SD) | 14.9 (2.3) | 14.7 (2.5) | 0.639 | - | - |
| CVLT-II total, mean (SD) | 51.9 (12.6) | 54.9 (9.9) | 0.107 | 0.262 | 0.222 |
| BVMT-R total, mean (SD) | 21.9 (7.9) | 26.5 (6.5) | **0.001** | 0.625 | **0.001** |
| SDMT total, mean (SD) | 49.5 (12.5) | 55.5 (12.6) | **0.013** | 0.442 | **0.029** |
| Total CABF (ml/min) | 961.6 (257.8) | 967.5 (292.0) | 0.897 | 0.026 | 0.822 |
| T2-LV, mean (SD) | 13.5 (16.0) | 0.4 (1.1) | **<0.001** | 1.149 | **<0.001** |
| GMV, mean (SD) | 732.3 (60.5) | 768.0 (58.1) | **0.003** | 0.544 | **0.001** |

Legend: MS – multiple sclerosis, HC – healthy controls, IQR – interquartile range EDSS – Expanded Disability Status Scale, RRMS – relapsing-remitting multiple sclerosis, PMS – progressive multiple sclerosis, CVLT-II- California Verbal Learning Test - Second Edition, BVMT – Brief Visual-Spatial Memory Test, SDMT – Symbol Digit Modalities Test, CABF – cerebral arterial blood flow, GMV – gray matter volume, LV – lesion volume.

All neuropsychological test are shown as raw scores. P-values <0.05 was considered significant and are shown in bold.

* $\chi^2$ test, Mann-Whitney ran-sum test and Student's *t*-test were used, as appropriate.

** Analysis of Covariance (ANCOVA) adjusted for age was used.

**Table 2.**

Correlation between the total cerebral arterial blood flow and neuropsychological tests in multiple sclerosis patients and healthy controls.

| Correlations between arterial blood flow and neuropsychological tests | | | | SDMT | CVLT-II | BVMT-R |
|---|---|---|---|---|---|---|
| HC | N=47 | Total CABF | r-value | 0.065 | −0.002 | 0.151 |
| | | | q-value | 0.716 | 0.989 | 0.369 |
| MS | N=132 | Total CABF | r-value | **0.318** | 0.094 | **0.244** |
| | | | q-value | **0.001** | 0.357 | **0.012** |

Legend: MS – multiple sclerosis, HC – healthy controls, CVLT-II - California Verbal Learning Test, BVMT – Brief Visual-Spatial Memory Test, SDMT – Symbol Digit Modalities Test, CABF – cerebral arterial blood flow.

Partial correction adjusted for age and years of education was used. False discovery rate was adjusted using Benjamini-Hochberg procedure and q-values are reported. Q values <0.05 were considered significant and are shown in bold.

**Table 3.**

Regression models analyzing the explanatory and predictive value of sex, age, years of education, total arterial cerebral blood arterial flow, lesion volume, and gray matter brain volume on the neuropsychological performance in multiple sclerosis patients.

| | SDMT | | | | |
|---|---|---|---|---|---|
| | R2 | Adj $R^2$ | t-statistics | Standardized B | P-value |
| Block 1 | 0.149 | 0.127 | | | |
| Sex | | | −0.781 | −0.062 | 0.437 |
| Patients' age | | | −1.457 | −0.138 | 0.148 |
| Years of education | | | 1.064 | 0.083 | 0.289 |
| Block 2 | | | | | |
| Step 1: GMV | 0.256 | 0.230 | 2.714 | 0.279 | **0.016*** |
| Step 2: GMV + T2-LV | 0.292 | 0.261 | −2.666 | −0.223 | **0.016*** |
| Step 3: GMV + T2-LV + total CABF | 0.331 | 0.295 | 2.538 | 0.203 | **0.020*** |
| | BVMT-R | | | | |
| | R2 | Adj $R^2$ | t-statistics | Standardized B | p-value |
| Block 1 | 0.099 | 0.075 | | | |
| Sex | | | 1.008 | 0.086 | 0.316 |
| Patients' age | | | −0.793 | −0.081 | 0.429 |
| Years of education | | | 1.097 | 0.093 | 0.275 |
| Block 2 | | | | | |
| Step 1: GMV | 0.174 | 0.145 | 3.006 | 0.313 | **0.008*** |
| Step 2: GMV + total CABF | 0.204 | 0.169 | 2.064 | 0.179 | 0.055* |

Legend: CABF – cerebral arterial blood flow, SDMT – Symbol Digit Modalities Test, BVMT – Brief Visuospatial Memory Test - Revised, GMV – gray matter volume, LV – lesion volume.

The regression model is composed of two blocks. The first block forced entry of variables regardless of whether or not they provided significant contributions in the model. Furthermore, the block 2 utilizes step-wise addition of the hypothesized variables. The block 2 orders and includes the variables based on their explanatory power and significance.

*-Benjamini-Hochberg procedure for multiple comparison was used and q-values are reported. Qvalues <0.05 were considered significant and are shown in bold and demonstrate the significance of the contributing variable in the regression model.

**Table 4.**

Demographic, clinical, MRI and Doppler differences between cognitively impaired versus cognitively preserved multiple sclerosis patients.

| | MS patients | | |
|---|---|---|---|
| | **CI (n=57)** | **CP (n=75)** | **P-value** |
| Female n (%) | 39 (68.4) | 53 (70.7) | 0.849 |
| Age, mean (SD) | 55.6 (11.1) | 51.7 (11.2) | 0.057 |
| Disease duration, mean (SD) | 21.8 (11.2) | 19.3 (9.9) | 0.180 |
| EDSS, median (IQR) | 3.5 (2.5–6.5) | 2.5 (1.5–4.88) | **0.004** |
| RRMS vs. PMS | 29/28 | 53/22 | **0.029** |
| Body mass index, mean (SD) | 27.2 (6.1) | 27.9 (5.9) | 0.495 |
| Hypertension, n (%) | 10 (17.5) | 15 (20.0) | 0.824 |
| Hyperlipidemia, n (%) | 11 (19.3) | 16 (21.3) | 0.830 |
| Heart Disease, n (%) | 5 (8.8) | 18 (24.0) | **0.035** |
| Years of education, mean (SD) | 14.6 (2.5) | 15.2 (2.2) | 0.189 |
| Raw CVLT-II total, mean (SD) | 43.5 (12.1) | 58.2 (8.6) | **<0.001*** |
| Raw BVMT-R total, mean (SD) | 15.7 (6.7) | 26.5 (5.1) | **<0.001*** |
| Raw SDMT total, mean (SD) | 40.2 (14.5) | 56.6 (10.2) | **<0.001*** |
| Total CBAF (ml/min) | 884.5 (234.3) | 1020.2 (260.9) | **0.008*** |
| T2-LV, mean (SD) | 18.1 (17.9) | 10.1 (13.6) | **0.015*** |
| GMV, mean (SD) | 721.1 (67.7) | 746.6 (50.5) | **0.039*** |

Legend: CI –cognitively impaired, CP – cognitively preserved, EDSS – Expanded Disability Status Scale, RRMS – relapsing-remitting multiple sclerosis, PMS – progressive multiple sclerosis, IQR – interquartile range, SD – standard deviation, SDMT – Symbol Digit Modality Test, BVMT-R – Brief Visuospatial Memory Test - Revised, CVLT-II – California Verbal Learning Test - Second Edition, CBAF – cerebral blood arterial flow, GMV – gray matter volume, LV – lesion volume

$\chi^2$ test, Mann-Whitney rank-sum test and Student's *t*-test and analysis of covariance (ANCOVA) adjusted for age were used, as appropriate.

*FDR-corrected q-values.

Q-values <0.05 were considered significant and are shown in bold.