Lower cerebral arterial blood flow is associated with greater serum neurofilament light chain levels in multiple sclerosis patients

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Disclosures:

- **Brianna L Gibney, Karen Marr, Deepa P Ramasamy, and Niels Bergsland,** have nothing to disclose.
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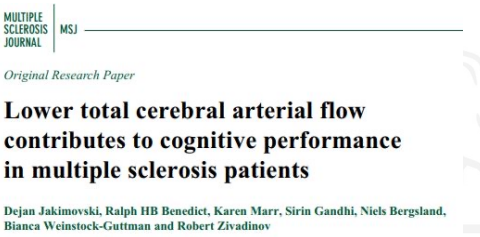
Background:

- Hypoperfusion, vascular pathology, and cardiovascular risk factors are associated with disease severity in MS.[1,2,3]
- Serum neurofilament light chain (sNfL) has been suggested as a promising MS biomarker with good prognostic value and treatment responsiveness.[4]


MULTIPLE SCLEROSIS JOURNAL | MSJ

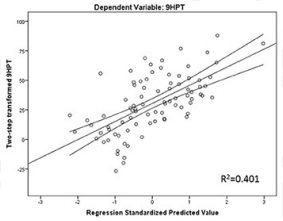
Original Research Paper

Lower total cerebral arterial flow contributes to cognitive performance in multiple sclerosis patients

Dejan Jakimovski, Ralph HB Benedict, Karen Marr, Sirin Gandhi, Niels Bergsland, Bianca Weinstock-Guttman and Robert Zivadinov

Objectives and hypothesis:

- To assess the relationships between CABF and sNfL in a heterogeneous group of MS patients and comparing it to age-matched HCs.
- We hypothesize that lower CABF would correlate with higher sNfL levels in MS patients, reflecting the relationship between cerebral hypoperfusion and increased axonal injury.
- We also hypothesize that If the vascular pathology is an independent MS factor, the aforementioned relationship will remain significant after controlling for common MS-based disease measure such as brain volume, lesion load and MS-based therapy.



MS – multiple sclerosis, HCs – healthy controls, sNfL – serum neurofilament light chain, CABF – cerebral arterial blood flow

1. Jakimovski D et al. Mult Scler 2020;26:201-209. 2. Jakimovski D, Front Neurol 2020;11:700. 3. Jakimovski D, Expert Rev Neurother 2019;19:445-458. 4. Jakimovski D. Mult Scler 2020;26:1670-1681.

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Methods:

Study population:

- The MS patients and HCs included in this analysis were part of a larger, prospective study that investigated the cardiovascular, environmental and genetic risk factors in MS (CEG-MS).[1]

Inclusion criteria:

1) Age of 18-75 years old.
2) Diagnosed with MS based on the 2010-revised McDonald criteria[2] or CIS patients.
3) Clinical visit, blood sampling, echo-color Doppler, and MRI examination within 30 days of each other.

Exclusion criteria:

1) Pregnant or nursing mothers.
2) Presence of known congenital morphological vascular pathology (such as Klippel–Trenaunay–Weber, Parkes Weber, Servelle–Martorell, or Budd–Chiari syndromes).
3) Contraindications prohibitive to performing a MRI exam.
4) Clinically-defined relapse or use of intravenous corticosteroids within 30 days of the study visit.

Serum neurofilament light chain (sNfL) quantification:

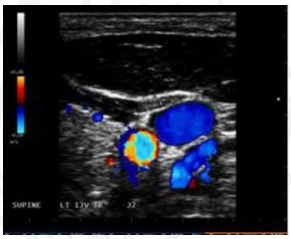
The sNfL levels were quantified using a previously validated Simoa assay and presented in picograms per milliliter (pg/mL).[3]

Ultrasound Doppler acquisition and analysis:

The total CABF was determined using an echo-color Doppler (Biosound MyLab 25 Gold; Esaote, Genoa, Italy) 7.5-10MHz transducer.[4]

- The blood flow of the bilateral CCA was measured at approximately 1.5cm below the bifurcation of the external and internal carotid artery.
- The blood flow of the bilateral VA was obtained at the C5-C6 level.

The total CABF was derived as a sum of all four vessels and quantified in milliliters per minute (mL/min).



MS – multiple sclerosis, HCs – healthy controls, CIS – clinically isolated syndrome, MRI – magnetic resonance imaging, sNfL – serum neurofilament light chain, CCA – common carotid artery, VA – vertebral artery, CABF – cerebral arterial blood flow

1. Jakimovski D et al. Mult Scler 2020;26:322-332. 2. Polman CH et al. Ann Neurol 2011;69:292-302. 3. Disanto G et al. Ann Neurol 2017;81:857-870 4. Marr K et al. Ultrasound Med Biol 2018;44:1762-1769.

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Methods – continued:

MRI acquisition and analysis:

- Both the MS patients and HCs underwent an MRI examination on a 3.0T GE Signa Excite scanner (Milwaukee, WI, USA) with eight-channel head and neck coil.
- The MRI protocol included an axial 3D SPGR T1-WI with TE/TI/TR/ of 2.8/900/5.9ms, flip angle of 15 degrees, FOV of 265 x 192 and isometric voxel size of 1x1x1mm with no gap and an axial 2D T2-WI FLAIR sequence with TE/TI/TR of 120/2100/8500ms, FOV of 265x192, slice thickness of 3mm (voxel size of 1x1x3mm) with no gap.
- T2 and T1-LV were derived using semi-automated, threshold and contour segmentation.
- WBV was obtained with the cross-sectional package from SIENAX (version 2.6, Oxford Centre for Functional Magnetic Resonance Imaging of the Brain, Oxford, UK).[1]

Statistical analyses:

- All statistical analyses were performed on SPSS version 26.0 (IBM, Armonk, NY, USA). Data distributions were evaluated using the Kolmogorov-Smirnov test and visual inspection of the Q-Q plots.
- The demographic and clinical variables were compared by χ^2 (for categorical variables), Student's t-test (parametric continuous variable comparison), Kruskal-Wallis H-test and Mann-Whitney U-test (non-parametric continuous variable comparisons).
- Multivariable linear regression models were utilized to determine the effect of total CABF with inclusion of demographic characteristics such as sex, age and BMI. A step-wise inclusion of CABF, T2-LV, WBV, presence of CVD and DMT was also utilized.
- For 80% study power ($\beta = 0.2$) and 0.05 threshold probability to reject the null hypothesis ($\alpha = 0.05$), a study with an expected correlation coefficient of at least 0.25 requires a sample size of 123 participants.

MS – multiple sclerosis, HCs – healthy controls, LV – lesion volume, WBV – whole brain volume, BMI – body mass index, CABF – cerebral arterial blood flow, CVD – cerebrovascular disease, DMT – disease modifying therapy, FOV – field of view, SPGR - spoiled gradient recalled, WI – weighted image, TE – echo time, TI – inversion time, TR – repetition time, FLAIR - Fluid Attenuated Inversion Recovery, SIENAX - Structural Image Evaluation, using Normalisation, of Atrophy

1. Smith SM et al. Neuroimage 2002;17:479-489.

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Results:

Demographic, clinical and MRI characteristics	MS (n=137)	CIS/RRMS (n=86)	PMS (n=51)	HCs (n=48)	One-way ANOVA or Kruskal Wallis p-value	MS vs. HCs p-value	RRMS vs. PMS p-value
Females, n (%)	100 (73)	61 (70.9)	39 (76.5)	35 (72.9)	0.498[c]	1.000[a]	0.553[a]
Age, mean (SD)	53.8 (11.1)	49.6 (10.7)	61.0 (7.3)	50.4 (15.2)	**<0.001**[d]	0.153[b]	**<0.001**[b]
BMI, mean (SD)	27.6 (6.0)	27.8 (6.3)	27.2 (5.5)	26.1 (5.6)	0.271[d]	0.141[b]	0.539[b]
Disease duration, mean (SD)	20.4 (10.7)	16.4 (9.0)	27.1 (9.9)	-	-	-	**<0.001**[b]
EDSS, median (IQR)	3.0 (1.5-6.0)	2.0 (1.5-3.0)	6.0 (4.0-6.5)	-	-	-	**<0.001**[c]
Hypertension, n (%)	25 (18.2)	12 (14.0)	13 (25.5)	10 (20.8)	0.231[c]	0.674[a]	0.111[a]
Hyperlipidemia, n (%)	29 (21.2)	17 (19.8)	12 (23.5)	7 (14.6)	0.529[c]	0.4[a]	0.667[a]
Heart disease, n (%)	22 (16.1)	16 (18.6)	6 (11.8)	4 (8.3)	0.211[c]	0.232[a]	0.341[a]
sNfL, median (IQR)	23.5 (15.0-32.4)	20.6 (13.1-25.9)	30.2 (21.2-42.7)	16.65 (8.1-23.5)	**<0.001**[d]	**0.002**[c]	**<0.001**[c]
Total CABF, mean (SD)	954 (260)	963 (249)	939 (279)	974 (295)	0.831[d]	0.664[b]	0.606[b]
T2-LV, mean (SD)	14.7 (19.0)	10.4 (15.3)	22.6 (22.6)	0.59 (1.3)	**<0.001**[d]	**<0.001**[b]	**0.002**[b]
WBV, mean (SD)	1448 (94.2)	1483 (80.4)	1386 (84.5)	1522 (102.8)	**<0.001**[d]	**<0.001**[b]	**<0.001**[b]
DMT, n (%)					-	-	0.762[a]
No therapy	31 (22.6)	18 (20.9)	13 (25.5)	-			
Medium potency DMTs	93 (67.9)	59 (68.6)	34 (66.7)	-			
High potency DMTs	13 (9.5)	9 (10.5)	4 (7.8)	-			

Legend: MS – multiple sclerosis, CIS – clinically isolated syndrome, RR – relapsing-remitting multiple sclerosis, PMS – progressive multiple sclerosis, HCs – healthy controls, BMI – body mass index, EDSS – Expanded Disability Status Scale, sNfL – serum neurofilament light chain, CABF – cerebral arterial blood flow, MRI – magnetic resonance imaging, T2-LV – T2 lesion volume, WBV – whole-brain volume, DMT – disease modifying therapy, SD – standard deviation, IQR – interquartile range,

Data is compared with χ^2 test (a), Student's t-test (b), and Mann-Whitney U-test (c), one-way analysis of variance (ANOVA) (d), and Kruskal Wallis H test (e), as appropriately. Age and disease duration are shown in years, sNfL is shown in picograms per milliliter, CABF is shown as milliliters per minute and T2-LV/WBV are shown as milliliters. P-value lower than 0.05 was considered statistically significant and shown in bold.

The DMT was not recorded for 2 RRMS patients. The classification and potency of the DMT were based on previously published European Medicines Agency (EMA) classification and as follows: interferon-β, teriflunomide, dimethyl fumarate and glatiramer acetate were considered as medium potency DMT, whereas natalizumab, fingolimod, alemtuzumab, cladribine and ocrelizumab were considered as high potency DMT. All off-label medications were considered as with medium potency.

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Results - continued:

- After adjustment for demographic factors (model 1), the first stepwise additions (R^2 change from 0.177 to 0.206 for model 2) of T2-LV as significant predictor of sNfL levels (standardized β=0.18, p=0.034).
- Moreover, second stepwise addition (R^2 change from 0.206 to 0.232 for model 3) also kept the total CABF variable with significant effect on sNfL levels (standardized β=-0.169, p=0.049).

sNfL regression model	Explanatory variable	Beta	R^2	Standardized β	t-statistics	VIF	p-value
Model 1 (n=137)	Sex	-2.81	0.177	-0.060	-0.719	1.04	0.474
	Age	0.505		0.268	3.191	1.06	**0.002**
	BMI	-0.683		-0.196	-2.342	1.05	**0.021**
Model 2	T2-LV	0.001	0.206	0.180	2.142	1.07	**0.034**
Model 3	Total CABF	-0.013	0.232	-0.169	-1.990	1.09	**0.049**

Legend: MS – multiple sclerosis, BMI – body mass index, sNfL – serum neurofilament light chain, CABF – cerebral arterial blood flow. T2-LV – T2 – lesion volume, VIF – variance inflation factor.
In the first model, sex, age, and BMI were entered regardless if they significantly explain the sNfL levels. In a second step-wise model, CABF, WBV, and/or T2-LV were only added if they were identified as significant factors. P-value lower than 0.05 was considered statistically significant and shown in bold.
In the regression model, sNfL levels were considered as a dependent variable, whereas age, sex, BMI, total CABF, T2-LV and WBV were added as independent variables (predictors). The demographic variables of age, sex and BMI are always entered in the model. With the step-wise inclusion of independent predictors (CABF, T2-LV and WBV) the model is created only if the predictors are significant.

Model that included DMT category as an additional predictor remained the same and the DMT variable was not kept in the final model as an independent predictor of sNfL.

Lastly, a model that included a variable that represented presence of at least two different CVD. Showed that both CVD and CABF are independent predictors of sNfL levels.

LV – lesion volume,, CABF – cerebral arterial blood flow, CVD – cerebrovascular disease, DMT – disease modifying therapy., sNfL – serum neurofilament light chain

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Results - continued:

- By adding CABF as predictor in the CIS/RRMS models showed that the CABF variable additionally explained 4.8% of the sNfL variance.
- The total CABF was not kept in the final model as an additional statistically significant variable in the sNfL model for the PMS subpopulation nor in the HCs.
- The discrepancies in the amount of sNfL variance explained by the demographic factors in HCs and MS patients indirectly confirm the presence of age-independent brain pathology. (R^2 of 0.538 vs. 0.137 and 0.223).

sNfL regression models	Explanatory variable	Beta	R^2	Standardized β	t-statistics	VIF	p-value
HCs (n=48) Model 1	Sex	0.432	0.538	0.014	0.126	1.00	0.901
	Age	0.611		0.648	5.995	1.01	**<0.001**
	BMI	-1.083		-0.418	-3.871	1.01	**<0.001**
CIS/RRMS subgroup (n=86) Model 1	Sex	-2.006	0.089	-0.057	-0.525	1.05	0.601
	Age	0.184		0.124	1.160	1.03	0.249
	BMI	-0.644		-0.251	-2.370	1.02	**0.020**
Model 1+CABF	Total CABF	-0.014	0.137	-0.225	-2.077	1.06	**0.041**
PMS subgroup (n=51) Model 1	Sex	1.864	0.223	0.033	0.248	1.024	0.805
	Age	1.421		0.415	3.105	1.036	**0.003**
	BMI	-0.847		-0.191	-1.443	1.012	0.156

Legend: HCs – healthy controls, CIS – clinically isolated syndrome, RRMS – relapsing-remitting multiple sclerosis, BMI – body mass index, sNfL – serum neurofilament light chain, CABF – cerebral arterial blood flow, VIF – variance inflation factor.

The multivariable regression models were performed for each subgroup. In the first model, sex, age, and BMI were entered regardless if they significantly explain the sNfL levels. In a second step-wise model, CABF was added if it was significant factor. P-value lower than 0.05 was considered statistically significant and shown in bold.

In all regression models, sNfL levels were considered as a dependent variable, whereas age, sex, BMI and total CABF were added as independent variables (predictors). The demographic variables of age, sex and BMI are always entered in the model. With the step-wise inclusion of independent predictors (CABF) the model is created only if the predictors are significant. For example, the CABF was not a significant predictor of sNfL in the HCs, thus this model was not created.

MS – multiple sclerosis, HCs – healthy controls, CIS – clinically isolated syndrome, RRMS – relapsing-remitting multiple sclerosis, PMS – progressive multiple sclerosis, CABF – cerebral arterial blood flow, sNfL – serum neurofilament light chain

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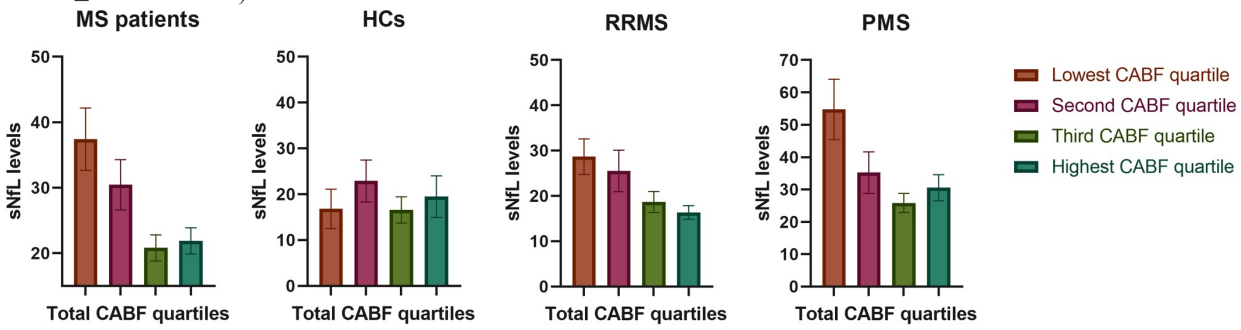
Results - continued:

- MS patients within the lowest CABF quartile (total CABF ≤761mL/min) had significantly higher sNfL levels (estimated marginal means of 34.6pg/mL versus 23.9pg/mL, age and BMI-adjusted p=0.042) when compared to patients within the highest CABF quartile (total CABF ≥1130.6mL/min).
- No such relationship was seen in the HCs.



Legend: MS – multiple sclerosis, RRMS – relapsing remitting multiple sclerosis, PMS – progressive multiple sclerosis, HCs – healthy controls, sNfL – serum neurofilament light chain, CABF – cerebral arterial blood flow, BMI – body mass index. The comparisons are performed with age and BMI-adjusted analysis of covariance (ANCOVA). The values in the figure are shown as absolute sNfL levels in pg/mL.

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Summary:

- Firstly, and confirming our hypothesis, CABF was associated with the amount of axonal injury, measured as released sNfL levels, in MS patients but not in age and sex-matched HCs.
- Secondly, this relationship remained significant in the CIS/RRMS after adjusting for age, sex, BMI and T2-LV effects.
- The presence of at least two CVD disease was a significant predictor of higher sNfL levels. Both CVD and total CABF exerted an independent effect on the sNfL measure.

MS – multiple sclerosis, HCs – healthy controls, CIS – clinically isolated syndrome, RRMS – relapsing-remitting multiple sclerosis, BMI – body mass index, sNfL – serum neurofilament light chain, LV – lesion volume, CABF – cerebral arterial blood flow, CVD – cerebrovascular disease

Limitations:

- A main limitation of this study is in its cross-sectional nature.
- The same relationship can also be driven by primary neurodegenerative process and brain atrophy (initial release of sNfL) which would have lower metabolic and perfusion demand (secondary reduction in blood flow).
- However, the CABF measure used in our study is more representative of the cardiac output and systemic blood flow than blood flow at the cerebral capillary levels. Therefore, we do not expect that the reduction in overall brain volume would necessarily result in blood flow decrease.

Conclusions:

- Lower CABF, a measures of systemic blood flow is associated with higher sNfL levels in MS patients. This relationship is independent of MS-based lesional and neurodegenerative pathology.

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Thank you!

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