


# Sympathetic cardiovascular and sudomotor functions are frequently affected in early multiple sclerosis

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## Abstract

**Objective** The aim of this study was to determine the prevalence of autonomic dysfunction using the composite autonomic scoring scale (CASS) and heart rate variability (HRV) in patients with clinically isolated syndrome (CIS) and to correlate autonomic dysfunction with other measures of MS disease activity.

**Methods** CASS, HRV and plasma catecholamines during supine and tilted phase were performed in 104 CIS patients. MRI findings were analyzed for total number of lesions and the presence of brainstem and cervical spinal cord lesions.

**Results** Autonomic dysfunction (CASS >1) was present in 59.8 % of patients, parasympathetic dysfunction in 5 %, sympathetic in 42.6 % and sudomotor in 32.7 % of patients. Patients with autonomic dysfunction on CASS had lower level of norepinephrine in the supine position compared to patients without autonomic dysfunction ( $1.06 \pm 0.53$  vs.  $1.37 \pm 0.86$ ,  $p = 0.048$ ). The CASS score showed positive correlation with s-HF ( $r = 0.226$ ,  $p = 0.031$ ), s-SDNN ( $r = 0.221$ ,  $p = 0.035$ ), t-HF ( $r = 0.225$ ,  $p = 0.032$ ), and t-HFnu ( $r = 0.216$ ,

$p = 0.04$ ), and a negative correlation with t-LF/HF ( $r = -0.218$ ,  $p = 0.038$ ). More patients with MRI brainstem lesions had a positive adrenergic index ( $p = 0.038$ ). Patients with MRI brainstem lesions also had a lower t-SDNN ( $26.2 \pm 14.2$  vs.  $32 \pm 13.3$ ,  $p = 0.036$ ) and a lower t-LF (median 415.0 vs. 575.5,  $p = 0.018$ ) compared to patients without these lesions. Patients with adrenergic index  $\geq 1$  had a significantly higher standing heart rate compared to patients with an adrenergic index of 0 ( $96 \pm 13.5$  vs.  $90 \pm 12$ ,  $p = 0.032$ ).

**Conclusion** Autonomic (primarily sympathetic) dysfunction is present in a large proportion of early MS patients and it seems to be related to brainstem involvement.

**Keywords** Multiple sclerosis · Autonomic nervous system · Composite autonomic scoring scale · Sudomotor function · Heart rate variability

## Abbreviations

ANS	Autonomic nervous system
CIS	Clinically isolated syndrome
MS	Multiple sclerosis
MRI	Magnetic resonance imaging
CASS	Composite autonomic scoring scale
HRV	Heart rate variability
QSART	Quantitative sudomotor axon reflex test
TFM	Task force monitor
HF	High frequency
LF	Low frequency
nu	Normalized units
HR	Heart rate
sBP	Systolic blood pressure
dBp	Diastolic blood pressure
BS	Brainstem

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## Introduction

The clinically isolated syndrome (CIS) is clinically indistinguishable from multiple sclerosis (MS) relapses, except that it is isolated (at least clinically) in time and in most patients in space [1]. More than two-thirds of patients with CIS will have further episodes of neurological dysfunction and convert to MS. Although a number of demographic, genetic, environmental and clinical factors have been identified that confer an increased risk of conversion to MS, the most robust predictor remains conventional MRI abnormalities [1].

Reports about frequency of autonomic dysfunction in MS vary notably, with cardiovascular autonomic dysfunction being present in up to 50 %, bladder dysfunction in up to 97 %, sexual dysfunction in more than 80 %, and bowel dysfunction in more than 50 % of MS patients, while the prevalence of sudomotor dysfunction in MS is still unknown [2]. Identifying autonomic dysfunction in patients with multiple sclerosis is relevant both from clinical (i.e., diagnosis and treatment) and research (i.e., identifying potential treatment targets) perspectives [3]. However, how to define and/or test autonomic dysfunction in multiple sclerosis remains an unanswered question. The composite autonomic scoring scale (CASS) is a 10-point scoring scale of autonomic function, which consists of the quantitative sudomotor axon reflex test (QSART), orthostatic blood pressure and heart rate response to tilt, heart rate response to deep breathing, the Valsalva ratio, and beat-to-beat blood pressure measurements during phases II and IV of the Valsalva maneuver, tilt, and deep breathing [4]. The maximum CASS is 10, with 4 points for adrenergic and 3 points each for sudomotor and cardiovagal failure. CASS has been useful in detecting and monitoring autonomic dysfunction in many conditions; however, it has not been investigated in MS. In a recently published study, we have compared the CASS between 24 CIS patients and 17 healthy controls and found that the proportion of CIS patients with a pathological adrenergic index was statistically significantly higher compared to healthy controls, while there was no difference in cardiovagal index between groups [5]. Furthermore, we found sudomotor dysfunction diagnosed with QSART in 31 % of patients (5 out of 16 patients with QSART available) [5]. With this study, we have shown that the CASS is able to detect autonomic nervous system dysfunction in CIS patients and have confirmed findings from previous studies that ANS dysfunction in active MS (i.e., relapses) is restricted to the sympathetic nervous system (both adrenergic and cholinergic), while the parasympathetic nervous system seems to be preserved [6].

Therefore, the aim of the present study was to determine the prevalence and extent of ANS dysfunction using the

Composite Autonomic Scoring Scale (CASS) and heart rate variability analysis (HRV) in a large cohort of CIS patients and to correlate autonomic dysfunction with other measures of MS disease activity.

## Methods

### Design

This was a cross-sectional study which included consecutive patients diagnosed with the first clinical symptom of multiple sclerosis (CIS) from August 1st 2014 until the 1st of December 2015 at the Department of Neurology, University Hospital Center Zagreb—a tertiary medical center and a referral center for MS and ANS disorders. The diagnosis of CIS was made in a patient with acute or subacute development of neurological symptoms and/or signs lasting longer than 48 h in the absence of fever or infection and with at least one demyelinating lesion larger than 3 mm showing on the brain and/or spinal cord MRI. The exception was patients with optic neuritis who were included if the classical clinical triad was present (i.e., rapidly developing impairment of vision, dyschromatopsia and retro-orbital pain), accompanied by prolonged latencies of visual evoked potentials, irrespective of the presence of brain and/or spinal cord MRI lesions.

All patients were recruited within 6 months of the MRI scan and diagnosis of CIS. All autonomic tests were performed on the same day, at least 2 weeks after pulse corticosteroid therapy, if the patient received one.

Ethical committees of the University Hospital Center Zagreb and University of Zagreb, School of Medicine approved the study. All participants signed informed consent.

### MRI

All MRIs were performed on a 1.5T MRI scanner. Brain MRI was available for all patients. Cervical spinal cord MRI was analyzed if it was available. MRI sequences included multi-planar dual fast spin-echo PD and T2-WI, FLAIR and T1 postcontrast sequences. A neurologist with at least 5 years of experience with MS reviewed all MRIs for the presence of lesions.

### Autonomic nervous system testing

Autonomic nervous system testing was performed in the following order: first, Quantitative Sudomotor Axon Reflex Test (QSART) was performed with the Q-Sweat (WR Medical Electronics Co Maplewood, MN, USA) [7]; second, heart rate and blood pressure responses to Valsalva

maneuver; third, heart rate response to deep breathing; and finally, blood pressure response to passive tilt in the duration of 10 min (Task Force Monitor (TFM), CNSSystems Medizintechnik AG, Austria) [7, 8]. The results of the autonomic nervous system testing were interpreted in the form of the CASS score [4].

After all the tests were performed, patients were questioned by an experienced neurologist for symptoms of autonomic dysfunction, specifically, symptoms of orthostatic intolerance and symptoms of secretomotor, vasomotor, gastrointestinal, bladder or pupilomotor dysfunction.

Blood samples were collected directly from a peripheral vein catheter, in chilled tubes containing EGTA and reduced glutathione, for determination of plasma catecholamine levels in the 10th minute of the supine phase before the beginning the Valsalva maneuver and in the 10th minute of the tilted phase (Kabevette<sup>®</sup> N, Kabe Labortechnik GmbH). Plasma levels of catecholamine were measured on high-pressure liquid chromatography (HPLC Prominence; Shimadzu GmbH) with an electrochemical detector CLC 100 (Chromsystems GmbH, Germany) using a commercially available HPLC kit and a reverse-phase analytical column for HPLC analysis of catecholamines in plasma (Chromsystems GmbH, Germany).

### Heart rate variability analysis

Power spectral analysis of HRV was performed by Kubios HRV 2.2 software (Department of Applied Physics, University of Eastern Finland, Kuopio, Finland) using time- and frequency-domain methods. In spectral analysis of the frequency domain, the variables autoregressive spectral estimation method was used. Data used for the HRV analysis were recorded with TFM using a sampling frequency of 1000 Hz, and the data were subsequently inspected and edited for any missing data. The medium artefact correction option and Smoothness priors-based detrending approach (Lambda = 500) were used to ensure data quality [9].

HRV was analyzed in 5-min intervals of beat-to-beat data recorded during the testing [10]. HRV analysis done on the supine phase dataset was performed on the most stable 5-min interval for every patient ('s' variables) and most stable 5-min interval between the 1st and 9th minute was used for the analysis of HRV during the tilted phase ('t' variables). High-frequency (0.15–0.4 Hz) and low-frequency (0.04–0.15 Hz) power of RR interval expressed in absolute units (HF and LF) were used as an index of cardiovagal activity and combined sympathetic and parasympathetic cardiac activity, respectively [11]. HF expressed in normalized units (HFnu) was used an index of

modulation of the parasympathetic branch of the ANS [10]. Low to high frequency ratio (LF/HF) was used as a marker of the sympathovagal balance [7]. The time domain analysis parameter SDNN was used as a marker of overall HRV [10].

### Outcomes

The primary outcome was to determine the prevalence and the extent of the autonomic dysfunction in the studied cohort. This was assessed by: (1) the proportion of patients with pathological CASS; (2) the proportion of patients with pathological adrenergic, cardiovagal and sudomotor index of the CASS; and (3) the correlation between autonomic dysfunction measured with the CASS and with the plasma catecholamine levels.

Secondary outcomes were to investigate the possible correlates of autonomic dysfunction in the studied cohort. Widely used measures of disease activity [expanded disability status scale (EDSS) and MRI] were tested with this aim. Furthermore, we wanted to see whether pathological CASS and its indices have clinical consequences. This was assessed by comparing differences in supine heart rate, standing heart rate, systolic blood pressure and diastolic blood pressure in patients with different autonomic dysfunctions. Taking into account that cardioregulatory centers are located in the brainstem, we wanted to see whether lesion location could have influenced heart rate variability. Furthermore, we wanted to determine if HRV parameters could be related to the ANS testing results interpreted in the form of CASS.

### Statistical analysis

Statistical analysis was performed using the IBM SPSS software, version 20. The Kolmogorov–Smirnov test was applied to test whether the data have a normal distribution. Differences in the distribution of qualitative variables were determined with the  $\chi^2$  test, while the differences in quantitative variables were determined with the use of a parametric *t* test and nonparametric Mann–Whitney and Kruskal–Wallis test. To determine the correlation between the variables, the Spearman correlation method was used. *P* values less than 0.05 were considered as significant.

## Results

### Patients

During the study period, 104 consecutive patients were included (31 males) with a mean age of  $32.3 \pm 8.9$  years. There were 32 cases of optic neuritis, 32 of incomplete

**Table 1** Frequency of function-specific symptoms of autonomic dysfunction

Reported symptoms	%
Orthostatic intolerance	61.2
Vasomotor dysfunction	16.3
Secretomotor dysfunction	31.6
Gastrointestinal symptoms	75.5
Bladder dysfunction	32.7
Pupillomotor dysfunction	64.3
Any symptom	86.7

transverse myelitis, 27 brainstem/cerebellar cases, 10 cases of hemispherical and 3 cases of multifocal type CIS. Median EDSS was 1.0 (range 0–3.5).

Brain MRI was available for all patients and cervical spinal cord MRI was available for 86 (82.7 %) patients. Ninety-nine patients (95.2 %) had lesions present on brain MRI, 42 (40.4 %) patients had brainstem lesions (15 in the midbrain, 32 in the pons and 15 in the medulla oblongata) and 41 (47.7 %) patients had lesions in the cervical spinal cord. Median number of lesions was 11 (0–84). None of the patients received any MS-specific medications or any other medications which could influence the autonomic nervous system at the time of testing. Table 1 shows the frequency of symptoms related to specific autonomic nervous system function.

### Primary outcomes

Total CASS was available for 92 participants; three patients could not perform the Valsalva maneuver so adrenergic and cardiovagal indices could not be calculated; and, for 9 patients the sudomotor index could not be calculated due to technical difficulties during the testing. The prevalence of sympathetic, parasympathetic and sudomotor dysfunction in the form of CASS and its indices is presented in Fig. 1. In total, 59.8 % of patients had some form of autonomic dysfunction (CASS >1); parasympathetic dysfunction was present in 5 %, sympathetic in 42.6 % and sudomotor in 32.7 % patients. All parameters used to calculate adrenergic and cardiovagal indices are presented in Tables 2 and 3. The prevalence of abnormal QSART responses is presented in the Table 4 and QSART variables (sweat output and latency) on each tested site (forearm, proximal and distal leg, and foot) are presented in Table 5. Heart rate, blood pressure, catecholamine values and HRV analysis data in the supine and upright positions are presented in Table 6.

Plasma catecholamine concentrations were available for 95 patients in the supine position and for 94 patients in standing position. The distribution of both epinephrine and

norepinephrine values in the supine and standing positions was the same across all CIS subtypes (all  $p > 0.05$ ). Patients with a CASS  $\geq 1$  had a lower level of norepinephrine in the supine position compared to patients with a CASS of 0 ( $1.06 \pm 0.53$  vs.  $1.37 \pm 0.86$ ,  $p = 0.048$ ). When we looked at the CASS subscores, there was no difference in plasma catecholamine concentrations between groups of patients with adrenergic, cardiovagal or sudomotor indices  $\geq 1$  vs. 0.

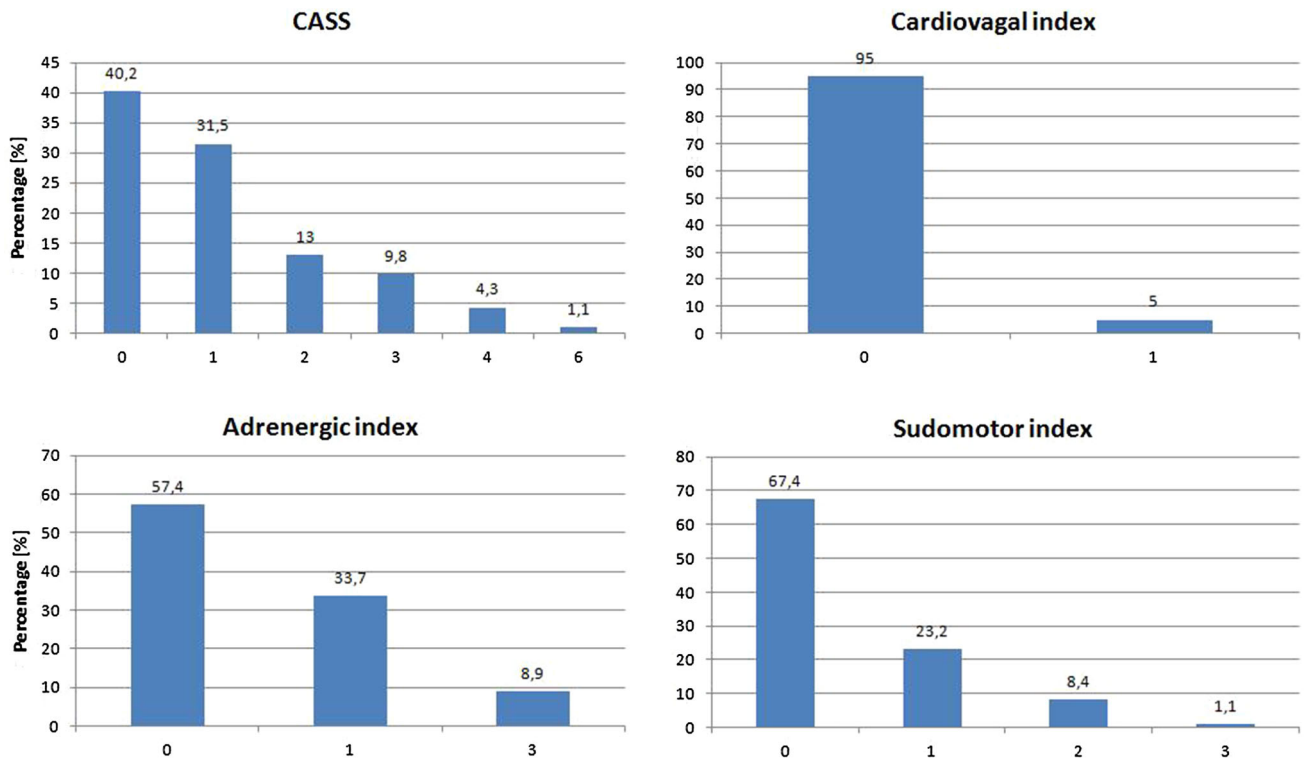
There was no difference in the CASS, adrenergic, cardiovagal or sudomotor indices between different CIS types ( $p = 0.256$ ,  $p = 0.745$ ,  $p = 1.000$ ,  $p = 0.075$ , respectively).

### Secondary outcomes

With regard to correlation between autonomic dysfunction and widely used measures of MS disease activity, there was no correlation between autonomic dysfunction (CASS, adrenergic, cardiovagal or sudomotor indices) and the EDSS ( $p = 0.055$ ,  $p = 0.189$ ,  $p = 0.182$ ,  $p = 0.190$ , respectively). Similarly, there were no differences with regard to the presence of autonomic dysfunction (CASS, cardiovagal or sudomotor indices  $\geq 1$ ) between patients with and without brainstem or cervical spinal cord lesions, except for positive adrenergic index and the presence of brainstem lesions ( $p = 0.038$ ) (Table 7). There was no correlation between CASS, adrenergic, cardiovagal or sudomotor indices and the total number of T2 lesions ( $p = 0.525$ ,  $p = 0.909$ ,  $p = 0.744$ ,  $p = 0.403$ , respectively).

To investigate whether pathological CASS and its indices have clinical consequences, we compared differences in supine and standing heart rate (HR), systolic (sBP) and diastolic blood pressure (dBP) in patients with different autonomic dysfunctions, and found that patients with an adrenergic index of  $\geq 1$  had significantly higher standing heart rate compared to patients with an adrenergic index of 0 ( $96 \pm 13.5$  and  $90 \pm 12$ , respectively,  $p = 0.032$ ). No differences were found regarding sBP and dBP ( $p > 0.05$ ). In addition, no differences in HR, sBP or dBP in relation to cardiovagal or sudomotor indices or CASS were found ( $p > 0.05$ ). We compared HR, sBP and dBP values between patients with and without BS lesions on brain MRI and found that patients with BS lesions had significantly higher supine sBP ( $116.2 \pm 12.5$  vs  $110.6 \pm 10.4$  mmHg,  $p = 0.016$ ) and dBP values ( $73.4 \pm 8.9$  vs  $69.7 \pm 7.9$  mmHg,  $p = 0.032$ ). There were no other significant differences in HR or blood pressure values in the supine or tilted phases of testing.

We included 103 patients in the HRV analysis; one patient was excluded because of the inadequate length of the recording period due to the development of vasovagal



**Fig. 1** Prevalence of autonomic, parasympathetic, sympathetic and sudomotor dysfunction in the studied cohort measured with CASS, cardiovagal index, adrenergic index and sudomotor index, respectively. The CASS consists of quantitative sudomotor axon reflex test, orthostatic blood pressure and heart rate response to tilt, heart rate response to deep breathing, the Valsalva ratio and beat-to-beat blood pressure measurement during the Valsalva maneuver, tilt and deep

breathing. The grading of the autonomic dysfunction is divided into three indexes: the sudomotor index 1, 2 and 3, the adrenergic index 1, 2, 3 and 4 and the cardiovagal index 1, 2 and 3. The minimal CASS is 0 and maximum 10. Results are referred to as normal (CASS total score = 0) or abnormal. The abnormalities range from mild generalized autonomic failure (total CASS score 1–3), to moderate (total CASS score 4–6), or severe (total CASS score 7–10)

**Table 2** Frequencies of normal or pathological responses to deep breathing test, Valsalva maneuver and tilt-up test in the whole cohort of CIS patients

Parameter (abnormal value)	Normal (%)	Abnormal (%)
Deep breathing test (*)	100 (96.15)	4 (3.85)
Valsalva ratio (*)	99 (99)	1 (1)
Drop of mBP during phase II of the VM (>20 mmHg)	98 (97.03)	3 (2.97)
Recovery of mBP at the end of late phase II of the VM	92 (91.09)	9 (8.91)
Overshoot of mBP in phase IV of the VM	101 (100)	0 (0)
Maximal PP drop during phase 2 of the VM (<50 %)	73 (72.28)	28 (27.72)
Blood pressure response to tilt-up test (OH)	96 (92.31)	8 (7.69)

\* Results are compared to age and gender-adjusted reference values. Note that three patients could not perform VM due to facial muscle weakness and in one patient the Valsalva ratio could not be calculated because VM provoked ventricular extrasystoles

VM valsalva maneuver, mBP mean blood pressure, PP pulse pressure, OH orthostatic hypotension

**Table 3** Mean and median values of quantifiable parameters of deep breathing test (RSA) and heart rate and blood pressure response to the Valsalva maneuver

Parameter	Mean	Median	St. dev.	Min	Max
RSA (bpm)	23.65	23.00	8.31	7.00	43.00
Valsalva ratio	2.17	2.18	0.39	1.50	3.33
Maximal PP drop during phase 2 (mmHg)	58.17	59.00	17.30	8.00	97.00
sBP recovery time (s)	0.39	0.00	0.75	0.00	3.00

RSA respiratory sinus arrhythmia represents an average of six best responses during deep breathing test, PP pulse pressure, sBP systolic blood pressure

**Table 4** Prevalence of specific types of responses for each tested site in the studied cohort

Site tested	Response			
	Normal (%)	Hypohydrosis (%)	Hyperhydrosis (%)	Anhydrosis (%)
Forearm	76 (80.0)	11 (11.6)	7 (7.4)	1 (1)
Proximal leg	82 (86.3)	9 (9.5)	4 (4.2)	0 (0)
Distal leg	85 (89.5)	4 (4.2)	6 (6.3)	0 (0)
Foot	88 (92.6)	7 (7.4)	0 (0)	0 (0)

**Table 5** Quantitative sudomotor axon reflex test (QSART) variables (sweat output and latencies) for each tested site in the studied cohort

QSART	Mean	Median	St. dev.	Min	Max
Volume ( $\mu$ l)					
Forearm	0.792	0.563	0.724	0.000	3.768
Proximal leg	0.933	0.838	0.568	0.075	3.436
Distal leg	1.097	0.983	0.657	0.086	3.299
Foot	0.462	0.393	0.311	0.058	1.371
Latency (s)					
Forearm	105.085	99.500	38.277	36.000	236.000
Proximal leg	80.432	80.000	37.000	3.000	206.000
Distal leg	82.011	76.000	36.273	2.000	194.000
Foot	138.516	128.000	47.981	68.000	267.000

syncope upon head-up tilting. We investigated possible differences in HRV parameters when comparing certain CIS groups with the rest of the sample. We found

differences only in the BS CIS group; these patients had a higher t-HFnu ( $26 \pm 15.4$  vs.  $18.6 \pm 10.1$ ,  $p = 0.026$ ) and a lower t-LF/HF (median 2.77 vs. 4.79,  $p = 0.021$ ). Disregarding the CIS type, patients with BS lesions evident on brain MRI had lower t-SDNN ( $26.2 \pm 14.2$  vs.  $32 \pm 13.3$ ,  $p = 0.036$ ) and lower t-LF (median 415 vs. 575.5,  $p = 0.018$ ) compared to patients without those lesions.

We also wanted to investigate whether HRV results could be related to the findings of ANS testing expressed in the form of the CASS. We found that patients with a positive total CASS score had significantly higher s-HF (median 729 vs. 321.5,  $p = 0.006$ ), s-HFnu ( $49.1 \pm 18.3$  vs.  $41.1 \pm 12.7$ ,  $p = 0.017$ ), and s-SDNN ( $41.4 \pm 18.8$  ms vs.  $33.1 \pm 13.7$  ms,  $p = 0.025$ ). These patients also had significantly lower t-LF/HF (median 3.76 vs. 4.82,  $p = 0.018$ ). Furthermore, total CASS score showed positive correlation with s-HF ( $r = 0.226$ ,  $p = 0.031$ ), s-SDNN ( $r = 0.221$ ,  $p = 0.035$ ), t-HF ( $r = 0.225$ ,

**Table 6** Heart rate, blood pressure, catecholamine values and HRV analysis data in the supine and upright positions

	Mean	Median	St. dev.	Min	Max
Supine					
HR (bpm)	71.69	70.80	10.40	49.40	104.70
sBP (mmHg)	112.86	111.30	11.55	88.20	146.20
dBp (mmHg)	71.15	70.70	8.46	51.10	92.70
Epinephrine (nmol/L)	0.18	0.14	0.12	0.08	0.68
Norepinephrine (nmol/L)	1.18	1.06	0.71	0.13	4.20
LF	785.58	538.00	734.39	22.00	4205.00
HF	762.22	533.00	822.36	24.00	4787.00
LF/HF	1.63	1.17	1.76	0.21	13.70
HFnu	45.53	46.10	16.78	1.39	82.60
SDNN	38.77	36.80	17.03	12.60	96.90
Upright					
HR (bpm)	92.35	92.50	12.99	66.60	123.10
sBP (mmHg)	110.14	108.40	14.22	81.20	157.20
dBp (mmHg)	74.32	73.90	9.87	51.80	95.00
Epinephrine (nmol/L)	0.32	0.25	0.27	0.08	1.86
Norepinephrine (nmol/L)	2.59	2.35	1.21	0.33	6.49
LF	793.40	481.00	992.37	57.00	6820.00
HF	179.48	113.00	209.60	13.00	1251.00
LF/HF	5.94	4.45	4.72	0.36	27.38
HFnu	20.55	18.40	12.08	3.50	73.40
SDNN	29.69	27.40	13.89	9.90	88.00

**Table 7** Differences in composite autonomic scoring scale (CASS) and its indices with regard to the MRI findings

Autonomic function	Lesions present on the MRI		<i>p</i> value
	N	P	
<b>Brainstem</b>			
CASS	N	25	0.279
	P	12	
Adrenergic index	N	41	0.038*
	P	17	
Cardiovagal index	N	58	0.646
	P	4	
Sudomotor index	N	40	0.268
	P	15	
<b>Midbrain</b>			
CASS	N	33	0.551
	P	46	
Adrenergic index	N	52	0.258
	P	35	
Cardiovagal index	N	82	0.607
	P	5	
Sudomotor index	N	56	0.538
	P	25	
<b>Pons</b>			
CASS	N	27	0.499
	P	36	
Adrenergic index	N	44	0.189
	P	27	
Cardiovagal index	N	66	0.318
	P	5	
Sudomotor index	N	44	0.816
	P	20	
<b>Medulla oblongata</b>			
CASS	N	33	0.390
	P	45	
Adrenergic index	N	50	0.782
	P	36	
Cardiovagal index	N	82	1.000
	P	4	
Sudomotor index	N	57	0.215
	P	24	
<b>Cervical spinal cord</b>			
CASS	N	13	0.640
	P	26	
Adrenergic index	N	20	0.083
	P	24	
Cardiovagal index	N	40	0.363
	P	4	
Sudomotor index	N	27	0.642
	P	13	

Pearson Chi Square test, Exact Sig  
*N* negative, *P* positive

$p = 0.032$ ), and  $t$ -HFnu ( $r = 0.216$ ,  $p = 0.04$ ), and a negative correlation with  $t$ -LF/HF ( $r = -0.218$ ,  $p = 0.038$ ). We also found that patients with a positive adrenergic CASS index had significantly higher  $s$ -SDNN ( $42.9 \pm 20.3$  vs.  $35.5 \pm 13.8$  ms,  $p = 0.043$ ),  $s$ -LF ( $968.7 \pm 938.8$  vs.  $647.3 \pm 514.6$  ms<sup>2</sup>,  $p = 0.047$ ), and a higher  $t$ -HFnu ( $23 \pm 12.1$  vs.  $18.2 \pm 11.5$ ,  $p = 0.047$ ). The adrenergic CASS score positively correlated with  $t$ -HFnu ( $r = 0.219$ ,  $p = 0.029$ ) and negatively with  $t$ -LF/HF ( $r = -0.220$ ,  $p = 0.028$ ). No differences or correlations were found regarding the cardiovagal CASS score.

To investigate the possible influence of supine or standing NA on HRV results, we performed a correlation analysis. This resulted in a negative correlation between supine NA values and  $s$ -HF ( $r = -0.242$ ,  $p = 0.019$ ) and a positive correlation with  $s$ -LF/HF ( $r = 0.412$ ,  $p < 0.001$ ). Standing NA values showed negative correlation with  $s$ -HF ( $r = -0.206$ ,  $p = 0.048$ ) and  $t$ -HF ( $r = -0.256$ ,  $p = 0.013$ ) and a positive correlation with  $s$ -LF/HF ( $r = 0.338$ ,  $p = 0.001$ ).

### Discussion

The present study has shown that sympathetic cardiovascular function is affected in 42,6 % and sudomotor function in 32,6 % of CIS patients. This is further supported with the finding of lower norepinephrine levels in the supine position in patients with ANS dysfunction. Furthermore, sympathetic cardiovascular dysfunction is probably the consequence of brainstem lesions.

Our study has confirmed previous findings which indicate that the sympathetic nervous system is affected in the acute stages of MS (relapses), with the parasympathetic nervous system being spared in the initial stages of MS [5, 6, 12]. Furthermore, it has been shown that brainstem MS lesions account for most of the cardiovascular abnormalities in MS patients [13]. This is especially interesting since damage to the locus coeruleus, which is located in the brainstem and is the principal site for brain norepinephrine synthesis, could lead to MS sympathetic dysfunction [15].

This notion is further supported by the results of HRV analysis. Results indicate that BS CIS patient groups have lower sympathetic cardiac activity in response to tilt when compared to the rest of CIS patients indicated by lower  $t$ -LF/HF and higher  $t$ -HFnu. Also, when we grouped patients into those with BS lesions and those without them, disregarding the CIS group affiliation, patients with BS lesions had lower cardiac sympathetic activity as indicated by the lower LF values during tilt. However, this was not reflected in the lower HR values during tilt in these

patients. On the contrary, we found that patients with BS lesions had higher supine sBP and dBP, which is somewhat confounding. It has been found that reducing central nervous system sympathetic outflow, using clonidine, causes up-regulation of  $\beta$ -adrenergic receptors on peripheral blood mononuclear cells [14]. Perhaps, a similar adaptation of the blood vessel's smooth muscle cells to the diminished sympathetic output in patients with BS lesions might explain higher supine blood pressure in these patients compared to those without BS lesion. Interestingly, we also found that the standing heart rate is higher in CIS patients with sympathetic dysfunction. Moreover, studies on MS patients showed reduced brain norepinephrine and reduced intracellular catecholamine concentrations in the peripheral blood lymphocytes, suggesting the dysfunction of both central and peripheral sympathetic functions [15, 16]. Interestingly, we found a positive correlation between the supine and tilted phase NA values and the index of cardiac sympathovagal balance during supine phase of testing (i.e., s-LF/HF). Thus, the results of the present study might support these findings. Patients with a positive adrenergic CASS score also had higher parasympathetic (i.e., lower sympathetic) cardiac activity during tilt, indicated by t-HFnu. Moreover, both total CASS and adrenergic CASS scores correlated negatively with the LF/HF ratio, the index of cardiac sympathovagal balance, during tilt, while both correlated positively with the index of parasympathetic activity (HFnu) during tilt. Taken together, these results suggest an impairment of sympathovagal balance (i.e., diminished central sympathetic output) in response to various stresses demanding a response of sympathetic nervous system, such as orthostasis or blood pressure response to the Valsalva maneuver. We also found that patients with a positive total CASS score exhibited higher cardiac parasympathetic (i.e., lower sympathetic) activity when supine, which may indicate that sympathovagal balance is disrupted not only during stress, but also during periods of rest (i.e., supine phase of ANS testing). Patients with BS lesions showed lower overall heart rate variability during tilt, most probably caused by impaired activation of the sympathetic nervous system during tilt, as indicated by lower t-LF. Although reduction of overall HRV during tilt is considered as a normal response [10], previous studies also suggest that in MS patients this reduction is caused by diminished activation of the sympathetic system [17]. Therefore, lower-than-normal sympathetic output seems to be the main cause for sympathovagal imbalance in MS patients and this has already been argued for [17].

Unlike cardiovascular reflexes, research on the sweating response in early MS is scarce. QSART has rarely been studied in MS, mainly because it is considered that an absent response indicates a lesion of the postganglionic axon. However, it can become abnormal even if the

preganglionic lesion is present, which has been nicely shown in a study investigating cholinergic sweating responses with pilocarpine iontophoresis. The authors observed diminished peripheral sweating responses as a consequence of impairments in autonomic control of sudomotor function [18]. This has been confirmed both with our previous work and with the present study, which showed a sudomotor index  $\geq 1$  in one-third of CIS patients [5].

Results of the present study are important for several reasons. The first one is that dysregulation of the ANS system can lead to arrays of clinical symptoms often observed in MS patients, including cognitive dysfunction, sleep disorders, depression and migraine [17]. Especially interesting are findings of ANS testing in migraine patients, concerning the higher prevalence of migraine in MS patients compared to healthy controls. Several studies have found abnormalities in autonomic nervous system testing, low levels of plasma norepinephrine, and an impaired sweating function in migraine patients during headache free intervals, findings which are very similar to the findings observed in MS patients [19, 20]. Despite all of this, studies of the association of ANS dysfunction with cognitive dysfunction, sleep disorders, depression and migraine in MS are lacking.

The second reason is the finding of a connection between dysfunction of autonomic cardiovascular reflexes and development of cardiac side effects of several drugs that are used in MS treatment. MS relapses are treated with high-dose intravenous methylprednisolone, and cardiac complications including sinus bradycardia, supraventricular tachycardia, atrial fibrillation, ventricular tachycardia and myocardial infarction have all been reported in association with this treatment. These side effects have been additionally associated with autonomic dysfunction in MS patients [21]. Furthermore, fingolimod, an SP1 receptor modulator, which is used in the treatment of highly active MS, may cause prolonged or more prominent HR slowing in some MS patients [22, 23].

Finally, the third reason is the finding that cardiovascular and thermoregulatory autonomic dysfunctions in MS have considerable potential to adversely affect exercise [24]. Blunted heart rate responses to isometric handgrip exercise, the inability to increase arterial pressure during handgrip exercise, attenuated elevations in heart rate and systolic blood pressure in MS patients during graded arm ergometry, and blunted heart rate and systolic blood pressure responses to graded cycling have all been found in MS patients compared to healthy controls [25–28]. Furthermore, the change in rectal temperature following 60 min of cycling was almost doubled in the MS patients compared to the healthy control group in one study [29]. Collectively, these data argue that cardiovascular and thermoregulatory



autonomic dysfunction impacts exercise tolerance and capacity; however, little is known and it remains to be investigated whether ANS dysfunction measured with CASS can predict this exercise intolerance.

In conclusion, the results of this study strongly suggest a positive correlation between brainstem involvement and autonomic (primarily sympathetic) dysfunction in early MS patients. Also, since there is growing evidence indicating the importance of autonomic dysfunction in a number of different aspects of this disease (i.e., pathophysiology, comorbidities, treatment response, quality of life, etc.), ANS function testing may prove to be a valuable tool in the assessment and treatment of MS patients.

#### Compliance with ethical standards

**Financial and competing interest disclosure** None of the authors have relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

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