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Progressive multiple sclerosis patients have a higher burden of autonomic dysfunction compared to relapsing remitting phenotype

Ivan Adamec[1], Luka Crnošija[1], Anamari Junaković[1], Magdalena Krbot Skorić[1], Mario Habek[1,2]

[1] University Hospital Center Zagreb, Department of Neurology, Referral Center for Autonomic Nervous System Disorders, Zagreb, Croatia
[2] School of Medicine, University of Zagreb, Zagreb, Croatia

Corresponding author:
Mario Habek, MD, PhD
Department of Neurology, University Hospital Center Zagreb
Kišpatićeva 12
HR-10000 Zagreb
Croatia
Phone/Fax: +38512388033; e-mail: mhabek@mef.hr

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Abstract

Objective. To determine autonomic dysfunction (AD) differences in patients with relapsing remitting multiple sclerosis (pwRRMS) and progressive MS (pwPMS).

Methods. Composite autonomic scoring scale (CASS) and heart rate variability (HRV) were performed in 40 pwRRMS and 30 pwPMS.

Results. pwPMS had a significantly higher sudomotor index and total CASS score compared to pwRRMS ($p<0.001$ and $p<0.001$, respectively). Disease duration positively correlated with sudomotor index and total CASS ($r_s=0.409$, $p<0.001$ and $r_s=0.472$, $p<0.001$, respectively), while the Expanded Disability Status Scale (EDSS) positively correlated with sudomotor index and total CASS ($r_s=0.411$, $p<0.001$ and $r_s=0.402$, $p=0.001$, respectively) in all patients. Type of multiple sclerosis (pwRRMS or pwPMS) corrected for age, sex and disease duration, was a statistically significant predictor of CASS value ($B=1.215$, $p=0.019$). Compared to pwRRMS, pwPMS had a significantly lower standard deviation of NN intervals (SDNN), low frequency (LF), and high frequency (HF), during both the supine and tilt-up phases (all p-values <0.006). pwPMS had a significantly lower LF/HF ($p=0.008$) during tilt-up.

Conclusion. There is a significant difference in autonomic function in pwRRMS and pwPMS; with pwPMS having a higher burden of AD, which is particularly evident for sweating dysfunction.

Significance. Further research is needed to establish whether parasympathetic and sudomotor dysfunction may serve as markers of progressive MS.

Key words: multiple sclerosis, relapsing-remitting; multiple sclerosis, progressive; autonomic nervous system; composite autonomic scoring scale; heart rate variability.

Highlights

- MS disease type is an independent predictor of dysautonomia.
- There is a difference in pattern of dysautonomia in pwRRMS and pwPMS.
- Sweating dysfunction is common in MS, particularly in advanced disease.

Introduction

Multiple sclerosis (MS) is an idiopathic demyelinating disorder of the central nervous system. It most commonly affects young individuals, between 20 and 40 years-of-age and represents the leading cause of non-traumatic neurologic disability in young adults (Edmonds et al., 2010). Although the exact etiology is unknown, there is a complex interaction between several environmental factors and a distinct genetic susceptibility which results in demyelinating lesions, the pathological hallmark of MS (Compston and Coles, 2008). The pathogenesis of the disease is marked by the production of autoreactive lymphocytes that cross the blood-brain barrier and enter into the central nervous system causing demyelination, axonal loss and, ultimately, neurodegeneration (Wu and Alvarez, 2011).

The natural history of MS seems to be divided into two distinct phases. First is the relapsing-remitting phase, characterized by bouts of acute exacerbation of disease activity. Pathologically, this is correlated with central nervous system (CNS) inflammation. The second phase is determined by a slow but steady progression in neurologic deficit, associated with CNS degeneration (Compston and Coles, 2008). The differentiation between these two phases of the disease (on an individual level) can sometimes be difficult. It is based on a temporal relationship between relapses from which patients typically experience partial or complete recovery, while simultaneously undergoing a progression of irreversible central nervous system dysfunction. Deciding whether increased disability is a consequence of a partially recovered relapse or a sign of the progressive form of the disease is still a troublesome task for the clinician. Onset of a progressive disease course in MS is defined by the onset of insidiously worsening and irreversible decline in neurologic function, regardless of the absence or presence of relapses; and, which cannot be explained purely with a step-wise worsening, associated with ongoing relapses (Tutuncu, 2012). Although somewhat simplistically dichotomized, this distinction between relapsing-remitting MS (RRMS) and progressive MS (PMS) does reflect disease evolution in a real life setting. In most patients, MS will begin with a relapsing-remitting course, with a smaller number of patients having progressive disease from the start, primary progressive MS (PPMS). Approximately 50% of RRMS patients will go on to develop secondary progressive MS (SPMS), in about nineteen years' time (Confavreux and Vukusic, 2006). Altogether, 80% of RRMS patients will ultimately develop SPMS after an average of 25 years. About 20% of patients will remain in the relapsing-remitting form of the disease, ultimately experiencing a reduced number of relapses as time passes (Kremenchutzky et al., 2006). It is not clear which patients will eventually progress to SPMS, but frequent relapses and the number of demyelinating lesions seem to carry a certain risk for future progression (Bhsteh et al., 2016).

When the progressive phase occurs, there are many clinical similarities in patients with SPMS and PPMS, leading to a unifying theory that SPMS and PPMS can be considered as a distinct disease entity when compared to RRMS. This observation is mainly related to patients' age and the time it takes them to reach

certain disability milestones, such as impaired walking or walking with a cane, referenced to the time that passed from one particular milestone to the other. The expanded disability status scale (EDSS), a standardized tool for neurologic disability assessment in MS, reflects this. Specifically, it takes patients with SPMS and PPMS about the same amount of time to reach EDSS 6 from EDSS 4, around 12 years. Bearing this in mind, RRMS can be regarded as a 'younger' disease that has not yet had time do develop into the progressive type; while SPMS and PPMS represent disease which 'got older' or was, in fact, 'old' to begin with, respectively (Confavreux and Vukusic, 2006). The diagnosis of RRMS in clinical practice begins with the clinically isolated syndrome (CIS), which represents the first clinical episode suggestive of MS. The course of the disease is marked by an acute exacerbation and periods of clinical stability, characterized as relapsing-remitting. On the other hand, when the disease progresses after an initial relapsing-remitting period, the disease is characterized as secondary progressive. Lastly, when there is progression of neurologic disability from the start, the disease is considered primary progressive in its nature. Therefore, the diagnosis of PMS is actually made retrospectively and the difference between RRMS and PMS is based on clinical evidence.

Little is known about how different disease courses affect different non-motor symptoms of MS, impeding prognosis and disease management. In a recent meta-analysis, for example, it has been shown that cognitive impairment significantly differs between RRMS and PMS (Johnen et al., 2017). These results imply that patients with PMS (pwPMS) display severe degrees of cognitive impairment and need more specialized disease management than patients with RRMS (pwRRMS).

Knowing that autonomic dysfunction (AD) in MS can affect virtually every end organ that the autonomic nervous system (ANS) innervates, the lack of studies on AD in MS – in particular studies investigating differences between RRMS and PMS – is surprising (Adamec and Habek, 2013). The most extensively investigated part of the ANS is the cardiovascular autonomic system, due to its convenience for testing. In general, ANS research can be divided into research regarding patient reported symptoms (usually using a variety of questionnaires) and assessment of ANS function/dysfunction in a controlled setting. In structural disorders of the ANS (dysautonomia caused by different pathological processes in the central or peripheral nervous system), a great discrepancy between patient reported symptoms and laboratory findings can be observed. One study has shown that even patients with severe sympathetic dysfunction (orthostatic hypotension with a decrease in systolic blood pressure more than 60 mm Hg from baseline during a head-up tilt table test) can be completely asymptomatic during the head-up tilt table test in up to one third of cases (Arbogast, 2009). Therefore, in patients with structural ANS disorders, like MS, autonomic dysfunction should actively be searched for with laboratory tests.

In recent years there has been an upsurge in cardiovascular ANS laboratory investigations, involving patients with MS. It has been demonstrated that AD is frequent in MS and is present even in the earliest stages of the disease (CIS) with parasympathetic dysfunction present in 5%, sympathetic in 42.6% and

sudomotor in 32.7% of patients (Habek et al., 2016). Furthermore, there is emerging evidence suggesting that certain ANS disorders, like postural orthostatic tachycardia syndrome, may serve as significant predictors of early conversion from CIS to MS (Habek et al., 2017). Several studies, using standardized tests of cardiovascular autonomic function (heart rate and blood pressure responses to Valsalva maneuver and heart rate response to deep breathing), have suggested a distinct pattern of AD in different phases of the disease. In the CIS stage there is predominant sympathetic dysfunction (both adrenergic and cholinergic), with sparing of the parasympathetic system (Crnošija et al., 2016). A similar finding was observed in pwRRMS, where adrenergic sympathetic dysfunction was higher in patients with active MS compared to healthy controls or stable patients (Flachenecker et al., 2001). In contrast, parasympathetic, but not sympathetic dysfunction, increases with disease duration significantly correlating with an increase in clinical disability (Flachenecker et al., 2001). In order to confirm this distinct pattern of autonomic involvement in MS, and due to lack of studies specifically assessing the difference in autonomic function in relapsing-remitting and progressive stages of the disease, we aimed to to determine differences in AD in pwRRMS and pwPMS.

Materials and methods

Patients

This was a prospective study performed from September 2015 to September 2016 that included consecutive patients diagnosed with RRMS and PMS; with the PMS group including patients with both PPMS and SPMS. The patients were recruited during their regular follow-up visits at the Outpatient Clinic of the Department of Neurology, University Hospital Center Zagreb – a tertiary medical center and a referral center for autonomic nervous system disorders. Patients were diagnosed with RRMS and PPMS based on the 2010 revision of the McDonald criteria (Polman et al., 2011). SPMS was defined based on the criteria by Lublin et al. (2014). The patients were examined by two of the authors (MH and IA), neurologists with more than five years of experience dealing with individuals with MS, and they performed the EDSS examinations. The EDSS is a standard tool used to evaluate neurologic disability in patients with MS (Kurtzke, 1983).

Exclusion criteria included significant cardiac or pulmonary disease and medication with known influence on the autonomic nervous system (anticholinergics, antihypertensives, beta blockers, diuretics, antiarrhythmics, sympathomimetics, parasympathomimetics).

The ethical committee of the University Hospital Center Zagreb approved the study. All participants signed informed consent.

Autonomic nervous system testing

ANS testing was performed in a quiet and dimly lit room. Quantitative Sudomotor Axon Reflex Test (QSART) was performed with the Q-Sweat (WR Medical Electronics Co Maplewood, MN, USA) (Novak, 2011). Afterwards, heart rate and blood pressure responses to the Valsalva maneuver were measured, followed by heart rate response to deep breathing (Novak, 2011). Finally, the tilt table test was performed, measuring the blood pressure response to passive tilt with a duration of 10 minutes (Task Force Monitor (TFM), CNSystems Medizintechnik AG, Austria) (Freeman, 2006). The Composite Autonomic Scoring Scale (CASS) was utilized to quantify AD (Low, 1993). The CASS is a score that is further divided into three parts or indices – adrenergic, cardiovagal and sudomotor. Blood pressure response to the Valsalva maneuver and passive tilting determine the adrenergic index. The Valsalva index, heart rate response to Valsalva maneuver, and heart rate response to deep breathing determine the cardiovagal index. Finally, QSART results give rise to the sudomotor index. The adrenergic, cardiovagal and sudomotor indices of the CASS score are useful in identifying adrenergic, cardiovagal and sudomotor disturbances of the ANS, respectively. This approach enables the diagnosis of limited or restricted forms of the autonomic failure, beside the generalized autonomic failure alone (Low et al., 2013). Autonomic dysfunction is defined as a CASS score greater than 0. Grading of AD is based on the severity of findings in each index. The adrenergic index ranges from 0 to 4; the cardiovagal index from 0 to 3; and the sudomotor index from 0 to 3. When the three indices are tallied, the total CASS score may range from 0 to 10. Results are interpreted as normal (total CASS score = 0) or abnormal (total CASS > 0). The abnormalities can range from mild autonomic failure (total CASS score 1–3), moderate (total CASS score 4–6), or severe (total CASS score 7–10) (Low, 1993).

Heart rate variability analysis

Heart rate variability (HRV) analysis was performed as previously described (Habek et al., 2016). Power spectral analysis of HRV was performed with the Kubios HRV 2.2 software (Department of Applied Physics, University of Eastern Finland, Kuopio, Finland) using time and frequency-domain methods. The variables autoregressive spectral estimation method was used in spectral analysis of the frequency domain. Data that were used for the HRV analysis were recorded with the TFM, with a sampling frequency of 1000 Hz. The data were subsequently inspected and edited for any missing data. Data quality was ensured by using the medium artefact correction option and Smoothness priors-based detrending approach (Lambda = 500) (Tarvainen et al., 2014). Heart rate variability was analyzed in 5-minute intervals of beat-to-beat data recorded during the testing (Malik et al., 1996). HRV analysis of the supine phase data set was performed on the most stable 5-minute interval for every patient ('s' variables). HRV analysis of the tilted data set was performed on the most stable 5-minute interval between the 1st and 9th minutes of testing ('t' variables). High-frequency (HF) (0.15–0.4 Hz) power of RR intervals, expressed in absolute units, was used

as a cardiovagal activity index (Sztajzel 2004). Low frequency (LF) (0.04–0.15 Hz) power of RR intervals, expressed in absolute units, was used as an index of combined sympathetic and parasympathetic cardiac activity (Sztajzel 2004). HF expressed in normalized units (HFnu), was utilized as an index of parasympathetic branch modulation of the ANS (Malik et al., 1996). Low to high frequency ratio (LF/HF) was utilized as a marker of sympathovagal balance (Malik et al., 1996). The time domain analysis parameter, standard deviation of NN intervals (SDNN), was utilized as a marker of overall HRV (Malik et al., 1996).

Outcomes

The primary aim was to determine differences in AD between pwRMMS and pwPMS. Specifically, differences in total CASS, as well as adrenergic, cardiovagal and sudomotor indices, were assessed for the two groups.

In addition, a correlation analysis was performed to see the level of association between clinical parameters (EDSS and disease duration) and ANS parameters (CASS indices). Finally, a multiple linear regression model was used in order to examine the influence of sex, age, disease duration and MS type (RRMS or PMS) on the likelihood that pwMS will have AD measured with the total CASS.

The secondary outcome was to determine differences in neural regulation of heart rate in pwRRMS and pwPMS by assessing HRV parameters.

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 χ^2 test (sex, frequency of QSART response, frequency of orthostatic hypotension), while the differences in quantitative variables were determined with the use of a parametric *t*-test (age, RSA, Valsalva index) or a non-parametric Mann–Whitney test (disease duration, EDSS, CASS). To determine the correlation between the variables, the Spearman correlation method was used (disease duration, EDSS, CASS). A multiple linear regression model, based on four predictors (age, sex, disease duration and MS type (RRMS or PMS)), was used in order to determine significant predictors for the presence of AD, measured with the total CASS score. The multiple regression model, based on four predictors (age, sex, disease duration and MS type (RRMS or PMS)), was also used in order to determine significant predictors for HRV variables. For the predictors in the multiple regression models, p values less than 0.05 were considered as significant. For analysis that included multiple comparisons on the same

data set, p values corrected with the Bonferroni correction were considered as significant (number of comparisons=4, Bonferroni corrected p-value=0.05/4=0.0125).

Results

Patients

During the study period, 40 pwRRMS and 30 pwPMS were enrolled. There were 6 patients with PPMS and 24 patients with SPMS. Patients' characteristics are presented in Table 1. Patients in the RRMS group were significantly younger ($p<0.001$), and the RRMS group had a shorter disease duration ($p<0.001$) and lower EDSS values ($p<0.001$).

Autonomic testing results

Total CASS was available for 39 pwRRMS. In one patient, the adrenergic index could not be calculated due to technical difficulties during testing. The CASS results for pwRRMS are presented in Table 2. Autonomic nervous system dysfunction, a CASS score greater than 0, was present in 59.0% of pwRRMS. Sympathetic dysfunction was noted in 35.9%, cardiovagal in 2.5% and sudomotor in 35.0% of patients (Fig. 1).

In pwPMS, the total CASS score was available for 23 patients, as the blood pressure values in 7 patients during the Valsalva maneuver could not be properly evaluated, due to artifacts. The CASS results for pwPMS are presented in Table 2, with frequency of specific systems involved presented in Figure 1. ANS dysfunction, a CASS score greater than 0, was present in 91.3% of patients. Sympathetic dysfunction was noted in 60.9%, cardiovagal in 20.0% and sudomotor in 73.3% of patients (Fig. 1).

Primary outcome

pwPMS had a significantly higher sudomotor index and total CASS compared to pwRRMS ($p<0.001$ and $p<0.001$, respectively). Furthermore, disease duration positively correlated with sudomotor index and total CASS in all patients ($r_s=0.409$, $p<0.001$ and $r_s=0.472$, $p<0.001$, respectively). The correlation between total CASS and disease duration was moderate. The EDSS positively correlated with sudomotor index and total CASS in all patients ($r_s=0.411$, $p<0.001$ and $r_s=0.402$, $p=0.001$, respectively). The correlation between total CASS and EDSS was moderate. Although differences in CASS between pwRRMS and pwPMS were found, all scores were compatible with mild autonomic failure (Low, 1993).

When comparing results of individual autonomic tests, between the two groups, pwPMS had significantly lower respiratory sinus arrhythmia (RSA) compared to pwRRMS (14.33±6.06 vs. 22.40±7.36, respectively; $p<0.001$). Also, pwPMS had significantly more pathological sweating responses on QSART compared to

pwRRMS (Table 3). There was no significant difference between the two groups regarding Valsalva index values and frequency of orthostatic hypotension on tilt-up (p=0.246 and p=0.129, respectively).

A multiple regression model was used to predict the presence of AD measured with the total CASS, based on age, sex, disease duration and MS type (RRMS or PMS). The multiple regression model statistically significantly predicts the total CASS variable (F=7.792, p<0.001), with a R^2=0.354. MS type (RRMS or PMS), corrected for age, sex and disease duration, was a statistically significant predictor for the presence of AD measured with the total CASS (B=1.215, p=0.019). Age, sex and disease duration were not identified as independent predictors for the presence of AD measured with the total CASS (p=0.167, p=0.718 and p=0.089 respectively).

Secondary outcome

We included 40 pwRRMS and 28 pwPMS in the HRV analysis. Two pwPMS were not included in the analysis, as they were in the tilted position less than five minutes due to development of orthostatic symptoms. Values of HRV parameters for each group are presented in Table 4.

Compared to pwRRMS, pwPMS had significantly lower SDNN, LF, and HF during both supine and tilt-up phases (all p-values <0.006). There were no significant differences in LF/HF when supine, but pwPMS had significantly lower LF/HF (3.18±2.63 vs. 5.65±4.71, p=0.008) during tilt-up. In order to see which parameters (age, sex, disease duration, MS type (RRMS or PMS)) are possibly significant predictors for HRV variables, we performed a multiple regression analysis, the results of which are presented in supplementary Table 1.

Discussion

The results of this study have revealed two important aspects of AD in MS: 1) there is a difference in pattern of AD in pwRRMS and pwPMS; and 2) sweating dysfunction is common in MS, particularly in advanced disease.

The first important finding is the difference in pattern of AD in pwRRMS and pwPMS. The pwPMS had significantly higher CASS scores when compared to pwRRMS. This signifies a more pronounced ANS involvement in pwPMS, as a higher CASS score conveys greater involvement of the ANS. Previous studies have shown AD to be present in 60% of patients with early MS (Crnošija et al., 2016; Habek et al., 2016). Specifically, sympathetic dysfunction was most commonly present in patients with CIS, followed by sudomotor dysfunction with parasympathetic dysfunction present in just about 5% of patients (Habek et al., 2016). The present study has shown similar results for pwRRMS, with autonomic involvement being present in the majority of patients (Fig. 1). Sympathetic dysfunction was noted in 35.9% and cardiovagal in 2.5% of patients. These results suggest that in the active, inflammatory stage of the disease, the sympathetic system is most commonly affected with a relative sparing of the parasympathetic system.

Previous studies associated this preferential involvement of one arc of the ANS with the presence of demyelinating lesions in the brainstem and involvement of the locus coeruleus, resulting in disruption of norepinephrine synthesis (Polak et al., 2011). Another possible explanation was provided by Flachenecker et al. (2001), who argued that the sympathetic nervous system may be closely related with a disorder of immune regulation, which is the pathophysiological basis of MS, while the parasympathetic dysfunction may be caused by the disease itself. This interesting hypothesis is in line with the results of our study, showing predominant adrenergic affection in pwRRMS; with a similar pattern observed in a previous study on patients with CIS (Habek et al., 2016). Furthermore, as MS progresses, more of the parasympathetic damage becomes evident, with a fifth of the pwPMS having parasympathetic dysfunction in the current study.

The HRV analysis part of the study has shown that pwPMS have significantly lower SDNN, LF, and HF during both the supine and tilt-up phases, compared to pwRRMS. This represents an overall decrease of HRV in pwPMS, with a decrease of cardiovagal activity, as well as combined sympathetic and parasympathetic cardiac activity. These results are in concordance with CASS results in pwPMS, which also show a higher degree of both adrenergic and cardiovagal dysfunction, compared to pwRRMS. Furthermore, 20% of pwPMS featured a positive cardiovagal index compared to 2.5% of pwRRMS. This finding was also evident on HRV analysis with lower values of HF, an index of cardiovagal activity. These results differentiate pwPMS from patients with CIS as well, since patients with CIS were found to have sympathovagal imbalance, mainly due to diminished sympathetic output; further emphasizing the development of parasympathetic injury in pwPMS (Habek et al., 2016). On the other hand, pwPMS had significantly lower LF/HF during tilt-up, which indicates lower adrenergic activity during orthostatic provocation, thus revealing blunted sympathetic reactivity.

One of the clinical consequences of these observations is limited exercise capacity in pwMS, due to the blunted heart rate and blood pressure response to exercise (Senaratne et al., 1984). As the current study has demonstrated, a significant number of patients with MS have disturbed cardiac autonomic reflexes, and disruption of these reflexes may cause an inadequate cardiac autonomic control during endurance exercise (Hansen et al., 2013). The importance of physical activity has been reinforced by results of a recent study, showing that high-intensity and resistance training actually improves quality of life in patients with MS (Zaenker et al., 2017). Therefore, limited exercise capacity in individuals with MS, due to disturbed cardiovascular autonomic reflexes, can severely affect patients' abilities to properly perform physical rehabilitation – an essential aspect of MS treatment.

The second important finding observed was that sweating dysfunction was present in 35.0% of pwRRMS and 73.3% of pwPMS. These results are of importance as they demonstrate that sweating dysfunction is common in MS, particularly in advanced disease. There have only been a few previous studies assessing sudomotor function in MS (Davis et al., 2005; Habek et al., 2016; Saari et al., 2009). Patients with MS

seem to have a lower sweating response when compared to healthy controls; without a disease specific pattern (Saari et al. 2009). Similar to our findings, thermoregulatory hypohydrosis was associated with increased neurologic disability (Saari et al., 2009). Although QSART has been traditionally used as a marker of peripheral cholinergic postganglionic nerve affection, its values can be abnormal even with preganglionic lesions, as demonstrated in a previous study (Davis et al., 2005). On the other hand, QSART may reflect peripheral nerve affection in MS. Namely, the notion of MS as a pure central nervous system disorder has recently come into question. Jende et al. (2017) have demonstrated peripheral nerve lesion involvement in pwMS using high resolution MRI. These findings may provide a new pathophysiological concept of MS, which has yet to be established.

Another aspect of this observation is the clinical manifestation which thermoregulatory abnormalities may cause. Detecting sweating dysfunction in individuals with MS is of importance, as inadequate thermoregulation can cause reoccurrence of previous symptoms – the Uhthoff's phenomenon. Uhthoff's phenomenon represents transient worsening of symptoms with increased body temperature, due to a disturbance of nerve conduction. It is thought to result from heat induced closure of voltage-gated sodium channels, leading to inadequate action potential depolarization (Frohman et al. 2013). Therefore, inadequate body temperature regulation can lead to transient neurologic worsening in pwMS. Also, the inability to cool down properly can lead to poor exercise tolerance, which can limit physical activity (Huang et al., 2014).

Finally, the multiple regression model showed that type of MS (RRMS or PMS) corrected for age, sex and disease duration was a statistically significant predictor for the presence of AD measured with the total CASS. This finding is interesting in the light of recent ideas that both sympathetic and parasympathetic ANS function and/or dysfunction have an influence on inflammatory and neurodegenerative pathways in MS (Racosta and Kimpinski, 2016). Whether these observed changes are drivers of the inflammation and/or neurodegeneration, or they are just a consequence of MS itself, remains to be elucidated.

The limitation of this study may be selection bias, as patients were recruited in a tertiary medical center and a referral center for diseases of the ANS. However, the patients were not part of a preselected study group and were consecutively included, thus being representative of a population of individuals with MS.

In conclusion, results of this study demonstrate that more than half of pwRRMS experience AD, with the frequency rising to 90% in pwPMS. There is a significant difference in autonomic function in pwRRMS and pwPMS, with pwPMS having a higher burden of AD. Furthermore, a substantial number of tested patients had sudomotor dysfunction - significantly more often in the pwPMS, a part of ANS testing frequently neglected. Moreover, HRV analysis has shown to be overall lower in pwPMS compared to pwRRMS, with a blunted sympathetic reactivity. These results suggest that autonomic affection is an intricate part of MS activity. It is known that ANS has an important role in the regulation of the immunological system via adrenergic and cholinergic receptors on the immune cells (Kohm and Sanders 2001). Further research is

needed to asses a possible causative association between immunological derangement present in MS and ANS function. Given the effect that AD can have on physical activity and thermoregulation, specific care should be taken to address any autonomic symptoms and signs in MS patients – especially in the progressive stage.

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Tables

Table 1. Patients' characteristics, disease duration and EDSS.

	pwRRMS	**pwPMS**	**p-value**
Total number	40	30	
Female/Male	33/7	17/13	0.031[a]
Mean age	35.45±9.15	48.03±10.58	<0.001*[b]
Mean disease duration (days)	1980.60±2246.50	4774.23±2283	<0.001*[c]
Median EDSS (range)	1.25 (0 to 3.5)	6.5 (3.5 to 8.0)	<0.001*[c]

pwRRMS- patients with relapsing-remitting multiple sclerosis. pwPMS- patients with progressive multiple sclerosis. EDSS-Expanded Disability Status Scale, * - statistically significant, [a] χ^2 test, [b] t-test, [c] Mann-Whitney test, Bonferroni corrected p-value = 0.0125

Table 2. Autonomic testing results for relapsing remitting and progressive multiple sclerosis patients.

Autonomic dysfunction	pwRRMS			pwPMS			p-value
	N	Median	Range	N	Median	Range	
Adrenergic index	39	0	0 to 3	23	1	0 to 3	0.029
Cardiovagal index	40	0	0 to 1	25	0	0 to 2	0.018
Sudomotor index	40	0	0 to 2	30	1	0 to 3	<0.001*
CASS	39	1	0 to 5	23	3	0 to 6	<0.001*

pwRRMS- patients with relapsing-remitting multiple sclerosis. pwPMS- patients with progressive multiple sclerosis. CASS-Composite Autonomic Scoring Scale, * - statistically significant, Mann-Whitney test, Bonferroni corrected p-value = 0.0125

Table 3. Comparison of frequency of different types of QSART responses between relapsing-remitting and progressive multiple sclerosis patients.

	QSART result	pwRRMS N	pwPMS N	p value
	Normal	37	22	
	Hypohydrosis	0	5	
Forearm	Hyperhydrosis	2	0	0.001
	Pers. sweating	0	1	
	Anhydrosis	0	2	
	Normal	31	12	
	Hypohydrosis	5	9	
Proximal leg	Hyperhydrosis	1	1	0.004
	Pers. sweating	3	3	
	Anhydrosis	0	5	
	Normal	30	13	
	Hypohydrosis	2	4	
Distal leg	Hyperhydrosis	4	2	0.004
	Pers. sweating	4	4	
	Anhydrosis	0	7	
	Normal	34	16	
	Hypohydrosis	4	6	
Foot	Hyperhydrosis	0	0	0.003
	Pers. sweating	1	0	
	Anhydrosis	1	8	

pwRRMS- patients with relapsing-remitting multiple sclerosis. pwPMS- patients with progressive multiple sclerosis. QSART- quantitative sudomotor axon reflex test. Pers.-persistent. Dist.-distal, χ^2 test, Bonferroni corrected p-value = 0.0125

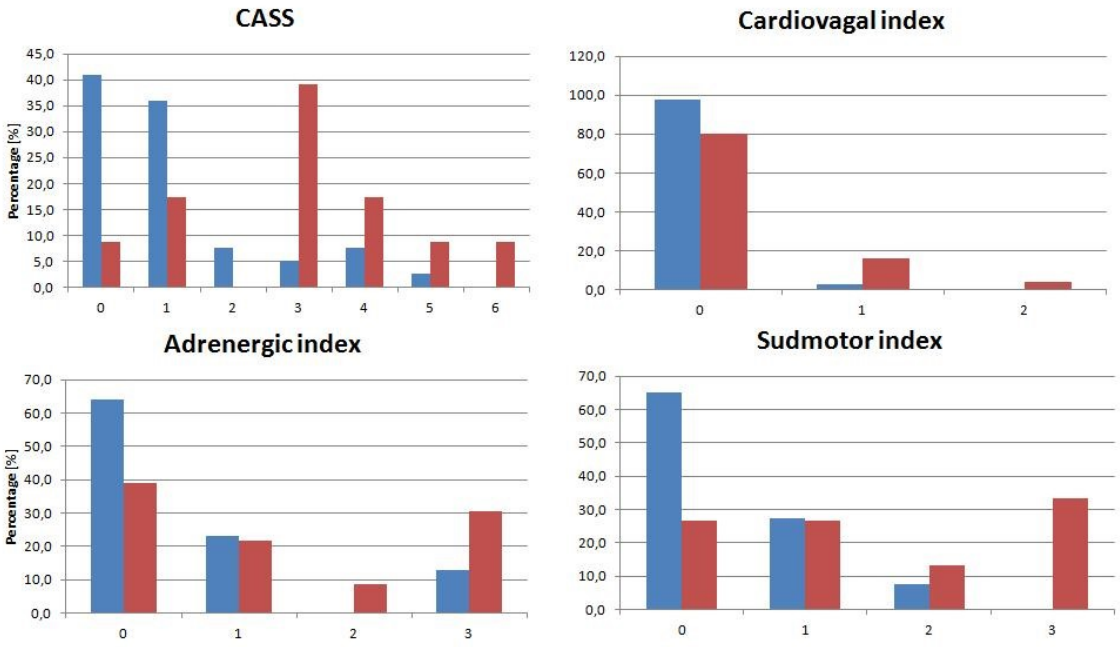
Table 4. Comparison of HRV analysis data for patients with relapsing remitting multiple sclerosis (RRMS) and progressive multiple sclerosis patients (PMS)

	pwRRMS					pwPMS				
	Mean	Median	St. Dev	Min	Max	Mean	Median	St. Dev	Min	Max
LF supine	759.43	436.00*	1432.48	68.00	8935.00	198.11*	129.50	208.66	11.00	888.00
HF supine	892.70	397.00*	1316.41	79.00	5981.00	230.79*	154.00	281.84	6.00	1382.00
LF/HF supine	1.18*	0.93	1.14	0.13	6.77	1.29*	1.25	0.85	0.24	4.24
HFnu supine	53.09*	51.70	17.27	12.90	87.70	48.56*	44.40	15.29	19.00	80.50
SDNN supine	37.67*	32.25	21.52	15.40	121.00	20.53*	17.90	9.70	6.30	52.40
LF upright	521.28*	393.50	400.13	62.00	1583.00	161.43	73.00*	216.15	4.00	928.00
HF upright	199.95	74.50*	330.18	15.00	1750.00	87.32	33.00*	135.91	1.00	666.00
LF/HF upright	5.65*	4.52	4.71	0.36	17.87	3.18	2.59	2.63	0.33	11.43
HFnu upright	23.41*	18.10	15.76	5.30	73.40	34.37	27.90	20.39	8.00	75.20
SDNN upright	27.33*	23.35	13.33	11.80	84.80	16.39	13.05	8.81	3.40	40.10

pwRRMS- patients with relapsing-remitting multiple sclerosis. pwPMS- patients with progressive multiple sclerosis. LF-low frequency. HF-high frequency. HF.nu-High frequency normalized units. SDNN- standard deviation of all normal RR intervals, mean* parametric distribution, median* non-parametric distribution

Figures

Figure 1. Frequency of autonomic, parasympathetic, sympathetic and sudomotor dysfunction in relapsing-remitting and progressive multiple sclerosis patients with CASS, cardiovagal index, adrenergic index and sudomotor index, respectively. CASS-composite autonomic scoring scale. Blue – relapsing-remitting multiple sclerosis. Red – progressive multiple sclerosis.


CASS
Percentage [%]
45,0
40,0
35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
0
1
2
3
4
5
6
Cardiovagal index
120,0
100,0
80,0
60,0
40,0
20,0
0,0
0
1
2
Adrenergic index
Percentage [%]
70,0
60,0
50,0
40,0
30,0
20,0
10,0
0,0
0
1
2
3
Sudmotor index
70,0
60,0
50,0
40,0
30,0
20,0
10,0
0,0
0
1
2
3

Supplementary Table 1: Multiple regression analysis, performed for HRV variables, * presents statistically significant predictors, p value=0.05

	Unstandardized Coefficients		Standardized Coefficients		
HRV variable	B	Std. Error	Beta	t	Sig.
LF_supine					
(Constant)	1366.800	788.536		1.733	0.088
Age	-19.235	14.314	-0.197	-1.344	0.184
Sex	184.743	324.266	0.072	0.570	0.571
Disease duration	-0.030	0.062	-0.067	-0.475	0.636
MStype	-204.099	364.322	-0.089	-0.560	0.577
HF_supine					
(Constant)	1892.511	694.028		2.727	0.008
Age	-22.276	12.599	-0.241	-1.768	0.082
Sex	131.123	285.402	0.054	0.459	0.648
Disease duration*	0.151	0.055	0.362	2.759	0.008
Mstype*	-748.772	320.657	-0.346	-2.335	0.023
LF/HF_supine					
(Constant)	0.722	0.726		0.994	0.324
Age	0.011	0.013	0.125	0.834	0.407
Sex	0.019	0.299	0.008	0.062	0.951
Disease duration	-9.886E-05	0.000	-0.248	-1.724	0.090
MStype	0.232	0.336	0.112	0.692	0.492
HF.nu_supine					
(Constant)	66.631	11.206		5.946	0.000
Age	-0.080	0.203	-0.056	-0.392	0.697
Sex	-2.762	4.608	-0.074	-0.599	0.551
Disease duration*	0.002	0.001	0.383	2.784	0.007
Mstype*	-10.552	5.177	-0.317	-2.038	0.046
SDNN_supine					
(Constant)	66.662	12.228		5.452	0.000
Age*	-0.486	0.222	-0.290	-2.191	0.032
Sex	0.044	5.028	0.001	0.009	0.993
Disease duration	0.001	0.001	0.163	1.278	0.206
MStype*	-14.273	5.649	-0.363	-2.526	0.014
LF_upright					
(Constant)	1471.268	224.533		6.553	0.000
Age*	-8.756	4.076	-0.268	-2.148	0.036
Sex*	-191.897	92.334	-0.225	-2.078	0.042
Disease duration	-0.005	0.018	-0.033	-0.273	0.786

MStype*	-279.780	103.739	-0.366	-2.697	0.009
HF_upright					
(Constant)	580.455	185.004		3.138	0.003
Age	0.037	3.358	0.002	0.011	0.991
Sex	-123.635	76.078	-0.202	-1.625	0.109
Disease duration	0.026	0.015	0.245	1.779	0.080
MStype*	-207.625	85.476	-0.378	-2.429	0.018
LF/HF_upright					
(Constant)	13.945	2.596		5.372	0.000
Age*	-0.131	0.047	-0.367	-2.782	0.007
Sex	-1.471	1.068	-0.158	-1.378	0.173
Disease duration	0.000	0.000	-0.196	-1.547	0.127
MStype	-0.333	1.199	-0.040	-0.278	0.782
HF.nu_upright					
(Constant)	-5.042	11.364		-0.444	0.659
Age*	0.554	0.206	0.348	2.688	0.009
Sex	3.060	4.673	0.074	0.655	0.515
Disease duration*	00.002	0.001	0.335	2.691	0.009
MStype	-1.570	5.250	-0.042	-0.299	0.766
SDNN_upright					
(Constant)	59.114	7.851		7.529	0.000
Age	-0.186	0.143	-0.168	-1.302	0.198
Sex*	-7.961	3.229	-0.276	-2.466	0.016
Disease duration	0.001	0.001	0.104	0.837	0.406
Mstype*	-11.707	3.628	-0.453	-3.227	0.002