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## **Immune and autonomic nervous system interactions in multiple sclerosis: clinical implications**

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## **Abstract**

Multiple sclerosis is characterized by a wide spectrum of clinical manifestations, among which dysfunction of the autonomic nervous system represents an important cause of multiple sclerosis related disability. The aim of this review is to give an overview of autonomic dysfunction in people with multiple sclerosis, to discuss the interactions between immune and autonomic nervous systems and consequences of these interactions on various aspects of multiple sclerosis.

Autonomic dysfunction in people with multiple sclerosis can be demonstrated clinically and on molecular level. Clinically it can be demonstrated by measuring autonomic symptoms with Composite Autonomic Symptom Score (COMPASS-31) and neurophysiologically, with different autonomic nervous system tests. Both symptomatic and objectively determined autonomic dysfunction can be related to increased risk of multiple sclerosis disease activity. Further supporting these clinical observations are molecular changes on immune cells. Changes in the sympathetic autonomic system, like different expression of dopaminergic and adrenergic receptor on immune cells, or modulation of the cholinergic anti-inflammatory pathway over different subunits of the nicotinic acetylcholine receptor in peripheral immune system, may mediate different effects on multiple sclerosis disease activity.

**Key words:** Multiple sclerosis, autonomic nervous system, cardiovascular autonomic reflexes, sudomotor function

## **Introduction**

Inflammation and neurodegeneration are common underlying processes in multiple sclerosis (MS), triggered by a pathological activation of the immune system. (1) MS is characterized by a wide spectrum of clinical manifestations, among which dysfunction of the autonomic nervous system (ANS) represents an important cause of MS related disability. The reason for this is a variety of clinical manifestations which are consequences of end organ dysfunction that ANS innervates. (2) Recently it has been suggested that pathological interactions between the immune and the autonomic systems may fail to trigger anti-inflammatory mechanisms, which are essential to prevent repeated inflammatory attacks, a key pathogenic feature of MS. (3) Based on limited available data, it can be speculated that both sympathetic and parasympathetic ANS function and/or dysfunction have the influence on inflammatory-anti-inflammatory and neurodegenerative pathways in MS.

The aim of this review is to give an overview of ANS dysfunction in people with MS (pwMS), to discuss the interactions between immune and ANS systems and consequences of these interactions on various aspects of MS.

### **Autonomic nervous system abnormalities in multiple sclerosis**

In general, ANS research can be divided into research regarding patient reported symptoms (usually using different questionnaires) and assessment of the ANS function/dysfunction in the laboratory. One of the most used questionnaire for the investigation of ANS symptoms is the Autonomic Symptom Profile which comprises of 169 questions and assesses 11 domains of autonomic function. (4) Due to several problems with this instrument, a shortened version,

the Composite Autonomic Symptom Score (COMPASS-31) questionnaire was developed (31 questions in 6 autonomic domains: orthostatic intolerance, vasomotor, secretomotor, gastrointestinal, bladder, pupillomotor), which provides an autonomic symptom score from 0 to 100. (5) As COMPASS-31 was found to be suitable for widespread use in autonomic research and clinical practice, it has been validated in several languages and we have recently validated the Croatian version of COMPASS-31 for use in pwMS. (6) We have found significant correlation between expanded disability status scale (EDSS) and the COMPASS-31 total score, as well as significant differences between MS phenotypes (clinically isolated syndrome (CIS), relapsing remitting MS (RRMS), secondary progressive MS (SPMS) and primary progressive MS (PPMS)) in the COMPASS-31 total score, with the lowest score for people with CIS (pwCIS) and highest for people with PPMS (pwPPMS). Another Portuguese study showed alterations in at least one autonomic symptom domain of the COMPASS-31 in 97.1% participants, with gastrointestinal and pupillomotor domains being the most frequently affected. (7) Finally, a study by Cortez and colleagues performed on a small group of people with relapsing remitting MS (pwRRMS) found no significant relationship between COMPASS-31 and EDSS or disease duration, but found significant correlations with the quality of life. (8) The validity of the COMPASS-31 is further supported with a recent study showing that people with laboratory confirmed ANS dysfunction score higher on certain domains of the scale. (9) Taken into account all these studies, we can conclude that COMPASS-31 is an important tool to detect ANS symptoms in pwMS and that it can detect ANS symptoms in different MS phenotypes, including earliest stages of MS, as well as patients with low level of disability.

Assessment of the ANS function in the laboratory is another aspect of ANS research, and in recent years there has been an upsurge in laboratory ANS investigations in pwMS (Table 1). In general, the most extensively investigated part of the ANS is the cardiovascular autonomic

system due to its availability for testing. Studies that had been using cardiovascular autonomic testing have shown that laboratory confirmed parasympathetic nervous system dysfunction exists in 0-55% and sympathetic nervous system dysfunction in 0-61% of pwMS (Table 1). The main problem with the laboratory examination of ANS function is that different tests are used in different laboratories, making comparison between studies very difficult. A nice example of the latter is a study by Acevedo et al. showing parasympathetic nervous system dysfunction in 43% of pwMS when using heart rate response to Valsalva maneuver and 30% when using respiratory sinus arrhythmia. (10) Another factor that can explain observed differences in frequency of autonomic dysfunction (AD) is patient population with different characteristics (age, disease duration, level of disability, MS phenotype) enrolled in different studies. An example of result variability by MS phenotype is study by de Seze et al., that showed sympathetic nervous system dysfunction in 0% of pwRRMS, 20% pwSPMS and 32% pwPPMS. (11) The only way to overcome these problems is to use standardized battery of ANS tests corrected for age and sex in a strictly defined population of pwMS. The initial attempts to overcome this problem in pwMS used a method which scored each autonomic test with 0 points if the result of the test was normal, 1 point if the result was borderline, and 2 points for abnormal results. (12) However, this method again could use many different tests and thus make the comparison between studies difficult. Another approach is the development of the Composite Autonomic Scoring Scale (CASS). CASS is a 10-point scoring scale that uses standardized battery of ANS tests including heart rate response to deep breathing and Valsalva maneuver as measures of parasympathetic nervous system function, blood pressure response to Valsalva maneuver and tilt-table test as measures of sympathetic nervous system function and the quantitative sudomotor axon reflex test (QSART) as measures of sudomotor function. (13) The maximum CASS is 10, with 4 points for adrenergic and 3 points each for

sudomotor and cardiovagal failure. Although CASS was initially developed on patients with multiple system atrophy, Parkinson's disease and autonomic neuropathy, it has been successfully used in pwMS as well. We have used CASS in a large, well defined cohort of people with clinically isolated syndrome (pwCIS) and found that it can detect autonomic dysfunction in a large proportion of patients, namely parasympathetic dysfunction in 5 %, sympathetic in 42.6 % and sudomotor in 32.7 % of participants. (14) In a subsequent study we used CASS in a cohort of pwRRMS and progressive MS (PMS), and found that type of multiple sclerosis (RRMS or PMS) corrected for age, sex and disease duration, was a statistically significant predictor of CASS value. (15) Furthermore, both disease duration and EDSS positively correlated with total CASS. These studies indicate that CASS is a valuable tool for evaluation of ANS dysfunction in pwMS and using this test enables comparison of the results across different studies.

Interestingly, in structural disorders of the ANS, like MS, there is a great discrepancy between patient reported symptoms of the ANS and laboratory ANS findings. Studies have 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) in up to one third of cases can be completely asymptomatic during the head-up tilt table test. (16) Similarly, one study has shown no significant association between the presence of symptoms of AD and laboratory confirmed autonomic damage. (17)

The third aspect of the CASS is sudomotor testing. Traditional neurophysiologic measurements of sudomotor function include thermoregulatory sweat testing, QSART, silicone impressions and sympathetic skin response (SSR). (18) Sweating dysfunction is common in MS, particularly in advanced course of disease. The most frequently used test for evaluation of sudomotor function in pwMS is SSR, and it has shown sudomotor abnormalities in 18-94% pwMS (Table 1). However, this methodology is only a surrogate measure of

sudomotor function, it shows a high variability within and between subjects and may not be evident in many subjects older than 50 years. (18) On the other hand, QSART is used to evaluate postganglionic sympathetic cholinergic sudomotor function by measuring the axon-reflex mediated sweat response and because of this it has rarely been studied in pwMS. However, with increased duration of the preganglionic lesion the response on QSART may become abnormal as well. This has been shown in a study investigating cholinergic sweating responses with pilocarpine iontophoresis in pwMS, which showed diminished peripheral sweating responses as a consequence of impairments in central autonomic control of sudomotor function. (19) This indicates that QSART may be used for detection of sudomotor dysfunction in MS as well. In line with this, we have shown QSART abnormalities in 33% of pwCIS, 35.0% of pwRRMS and 73.3% of pwPMS. (14,15) Sudomotor index correlated with both disease duration and EDSS in all patients and pwPMS had significantly worse QSART results on all tested areas compared to pwRRMS. (15) Whether QSART abnormalities can be used as markers of disease progression remains to be elucidated.

In figure 1, four main questions which arise from these studies are depicted: 1) Can AD be related to demyelinating lesions on the MRI?, 2) *What happens with AD with the progression of MS?*, 3) How is AD related to MS disease course? and 4) Is AD related to MS comorbidities?

#### *Can AD be related to demyelinating lesions on the MRI?*

As the MRI is most widely used test in the diagnosis and follow-up of pwMS, several studies tried to correlate AD with the presence of demyelinating lesions in different brain and/or spinal cord regions. Only few studies have found a correlation between ANS dysfunction and brainstem lesions on the MRI, two of which found correlation between sympathetic



cardiovascular dysfunction and brainstem lesions. (14,17,20) On the other hand, one study has shown that ANS dysfunction correlates with a spinal cord atrophy, suggesting that autonomic dysfunction is secondary to axonal loss, rather than to demyelination. (11) Although these results are not robust, as both, brainstem and spinal cord are areas of the central nervous system responsible for autonomic dysfunction, they are not surprising. Studies on larger well defined cohorts of pwMS using newer MRI techniques might give more evidence supporting this association.

#### *What happens with AD with the progression of MS?*

Only three studies so far investigated longitudinal evolution of cardiovascular autonomic dysfunction in pwMS. (21,22,23) The first study addressing this question enrolled people with advanced RRMS and secondary progressive MS (SPMS) with a disease duration from 2-32 years. Authors only looked at parasympathetic cardiovascular autonomic function over a one-year period and found progression in two tests measuring parasympathetic function (maximum change in heart rate after standing up and the Max/Min ratio after standing up). (21) In a study that used similar tests but included people with active RRMS with a disease duration ranging from 2-13 years and had a follow-up of two years, authors found a progression of the parasympathetic autonomic dysfunction. (22) In the third and final study that longitudinally investigated cardiovascular autonomic function in pwMS, authors enrolled people with active and stable MS with an average disease duration of 5.5 and 9.3 years, respectively, and used tests of parasympathetic and adrenergic sympathetic function. (23) During two-year follow-up, only test results of the parasympathetic function worsened, while there was no change in results of the adrenergic sympathetic function tests. While there is a

clear lack of studies with longitudinal evaluation of adrenergic sympathetic function, some hypotheses can be generated from cross-sectional studies which evaluated both, adrenergic sympathetic and parasympathetic branches, in different MS phenotypes (CIS, RRMS and SPMS) which are clearly related to time. It has been shown that in pwCIS parasympathetic dysfunction is present in 5% and sympathetic in 43% (14), in pwRRMS parasympathetic dysfunction is present in 2% and sympathetic in 36%, while in pwPMS parasympathetic dysfunction is present in 20% and sympathetic in 61% (15). If we put these studies into a context of longitudinal studies, there seems to exist a distinct pattern of dysautonomia which depends on different phases of the disease.

#### *How is AD related to MS disease course?*

There are several line of evidence that interactions between the immune and the autonomic systems may alter the disease course of MS. (3) However, data on how these abnormalities influence evolution of MS over time are sparse. Only two studies investigated the role of ANS abnormalities in pwCIS. In a first study, authors investigate whether ANS dysfunction presenting as postural orthostatic tachycardia syndrome (POTS) can predict conversion to MS in pwCIS over 6-months follow-up. POTS was identified as a significant predictor of early conversion to MS with an odds ratio of 2.34. (24) The second study aimed to evaluate the potential role of ANS abnormalities on disease activity (relapses and new MRI lesions) and disease progression in 121 pwCIS over a mean duration of follow-up of 2.9 years. (25) The results have shown that symptoms of AD measured with COMPASS-31 (COMPASS-31 > 7.32) increase the risk of next relapse by 2.7 folds in pwCIS. These results are of particular interest because a recent study has observed pwMS had significantly higher risk of presenting up to

10 years prior to a first demyelinating event with gastric, intestinal, urinary and anorectal disturbances, anxiety, depression, insomnia, fatigue, headache and various types of pain. (26) These autonomic symptoms that precede MS for up to 10 years are called MS prodrome and the more symptoms are present so is the risk of MS greater. If we put the results of the former study into the context with the autonomic MS prodrome, we may speculate that ANS is an important predictor of disease activity, even before the first demyelinating event. If these results would be confirmed in a second independent study, they would be of great importance for early detection of pwMS at risk for higher disease activity.

#### *Is AD related to MS comorbidities?*

As discussed previously, one of the most frequent ANS abnormalities in MS are abnormalities of the cardiovascular autonomic system. These abnormalities might be related to epidemiological studies showing that pwMS may have an increased risk of ischemic heart disease and congestive heart failure when compared with the general population (26), and that pwMS have a markedly increased risk of myocardial infarction in the first year after the MS diagnosis. (28) A recently published retrospective study found that pwMS have adrenergic hyperactivity expressed as an increase in  $\alpha$  adrenergic baroreflex sensitivity ( $\alpha$ -BRSa) compared with healthy controls (HC). (29) In the same study authors have also observed a positive correlation between  $\alpha$ -BRSa and systolic BP in the tilted position. These results are interesting knowing that that adrenergic hyperactivity, which is a hallmark of arterial hypertension, (30) may contribute to the increased risk of ischemic heart disease and congestive heart failure in pwMS. Further studies are needed in order to confirm these preliminary results.

## **ANS-immune system interactions and their role in MS**

Interactions between ANS and immune system exist on several levels and are out of scope of this review article. Several recent articles review experimental evidence that the ANS has a crucial role in the communication between the nervous system and the immune system. (53,54)

Few studies investigated changes in the interaction between ANS and immune system in pwMS. In vitro studies have shown that interferon beta leads to reduction of intracellular and increased extracellular levels of epinephrine, norepinephrine and dopamine. (55) This effect is the result of induction of catecholamine release from the cells to the medium and increased production of all three catecholamines. These results are interesting if we put them into a clinical context, with a study that showed reduction of the likelihood for a relapse with increasing levels of serum epinephrine. (25)

In both RRMS and PPMS, there is an increase in the beta2 adrenergic receptor density on mononuclear cells in peripheral blood which is associated with clinical and radiological disease activity. (56,57) Furthermore, responsiveness of beta2 adrenergic receptors to isoproterenol was shown to be absent in untreated patients and restored after interferon beta treatment. (58) On the other hand, gene expression studies showed that the expression of mRNA for beta adrenergic receptors is reduced in mononuclear cells of peripheral blood from untreated patients with relapsing MS. (59) In untreated pwMS there is a reduction of expression and activity of D1-like dopaminergic receptors and  $\beta$ 2-adrenergic receptors on circulating peripheral blood mononuclear cells and on CD4+ T effector lymphocytes and overexpression of D1-like dopaminergic receptors on CD4 + CD25<sup>high</sup> T regulatory lymphocytes. (60) The

opposite is seen in pwMS treated with interferon beta. (59,60) Furthermore, dopaminergic receptor D3 and  $\alpha$ 2A-adrenergic receptor mRNAs in peripheral blood mononuclear cells, and dopaminergic receptor D5 mRNA in T regulatory cells may be associated with the risk of conversion to MS in pwCIS within 12 months of clinical presentation. (61)

A second branch of the ANS, the parasympathetic nervous system has a major role in alerting the central nervous system about the presence of inflammation via inflammatory cytokines. (62) These afferent signals are transmitted by the vagal nerve and trigger an anti-inflammatory response, termed “cholinergic anti-inflammatory pathway”, which is postulated to suppress inflammatory and immune responses by integrating signaling in the immune and nervous systems. (3,63) This efferent part of the cholinergic anti-inflammatory pathway ends in the alpha 7 subunit of nicotinic acetylcholine receptors, which are expressed in immune cells (T cells, B cells, monocytes and endothelial cells), leading to the release of anti-inflammatory cytokines. (63,64)

Several studies on animal models of MS argue about the relevance of cholinergic anti-inflammatory pathway in MS pathogenesis. In mice with autoimmune experimental acute encephalomyelitis, deficient on subunit alpha 7 nicotinic acetylcholine receptors, subtle changes in the expression of pro-inflammatory and anti-inflammatory cytokines were observed with a higher expression of interleukin 10, interleukin 1 factor 9, and inhibin alpha. (65) More pronounced changes were observed in another study, which demonstrated that subunit alpha 7 nicotinic acetylcholine receptors play an important role in the reduction of inflammatory response conferred by nicotine in autoimmune experimental acute encephalomyelitis. (66) However, recent data demonstrate that several nicotinic acetylcholine receptor subtypes are involved, albeit differently, in the cholinergic anti-inflammatory pathway, indicating that each nicotinic acetylcholine receptor subtype may

modulate unique cellular immune functions. (67) Specifically, it has been suggested that disease exacerbation (or even induction) is being mediated at least in part via alpha 9 nicotinic acetylcholine receptor in peripheral immune cells, but also protective roles of central nervous system alpha 7 nicotinic acetylcholine receptor have also been suggested. (68) Discovery of acetylcholine producing T cells capable to regulate inflammation has proved importance of neural circuit as rheostat mechanism in immunomodulation. (69)

As nicotine, which acts via nicotinic acetylcholine receptors, is one of the principal components of cigarette smoke, it is interesting that substantial body of evidence supports the causal involvement of smoking in the development and progression of MS. (70) Studies on the effect of nicotine on immune system have been contradictory, some finding pro- and some anti-inflammatory effects. (71) Studies on animal models of MS, experimental autoimmune encephalomyelitis (EAE), found that nicotine improves EAE symptoms, the clinical scores and demyelination status, in contrast to cigarette smoke condensate which caused a worsening course of the disease. (72) Even more interestingly, a therapeutic intervention in EAE combining mesenchymal stem cells and nicotine led to a significant reduction in the cumulative disease disability better than the treatment with either therapy alone. (73) Furthermore, the combination treatment caused a significant decline in the production of the pro-inflammatory interleukin-17, tumor necrosis factor- $\alpha$ , and interferon- $\gamma$  cytokines and simultaneously caused a meaningful increase in the production of the anti-inflammatory interleukin-10.

All these data give a molecular background to previously mentioned clinical and neurophysiological data supporting the role of ANS dysfunction in MS. However, correlation studies taking into account clinical, neurophysiological and molecular aspects are missing and would help us in better understanding of immune-ANS interaction disturbances in MS.

## **Conclusion**

Results of the studies published so far have shown a distinctive pattern of AD in pwMS. While the disease activity, which is more prevalent in pwCIS and early RRMS, is associated with sympathetic nervous system dysfunction, parasympathetic nervous system dysfunction becomes more evident with the progression of the disease, with highest percentages of involvement seen in advanced progressive MS. On a molecular level, changes in expression of different receptors responsible for the communication between ANS and immune systems may modulate inflammatory response and thus have an influence on MS disease activity or progression. Whether these observed ANS changes are drivers of the inflammation and/or neurodegeneration, or they are just a consequence of MS lesions in the central nervous system remains to be elucidated.

## References

1. Wu GF, Alvarez E. The immuno-pathophysiology of multiple sclerosis. *Neurol Clin*. 2011;29:257–278.
2. Adamec I, Habek M. Autonomic dysfunction in multiple sclerosis. *Clin Neurol Neurosurg* 2013;115 Suppl 1:S73-8.
3. Racosta JM, Kimpinski K. Autonomic dysfunction, immune regulation, and multiple sclerosis. *Clin Auton Res* 2016;26:23-31.
4. Suarez GA, Opfer-Gehrking TL, Offord KP, Atkinson EJ, O'Brien PC, Low PA. The Autonomic Symptom Profile: a new instrument to assess autonomic symptoms. *Neurology* 1999;52:523-528.
5. Sletten DM, Suarez GA, Low PA, Mandrekar J, Singer W. COMPASS 31: a refined and abbreviated Composite Autonomic Symptom Score. *Mayo Clin Proc* 2012;87:1196-201.
6. Drulović J, Gavrilović A, Crnošija L, Kisić-Tepavčević D, Krbot Skorić M, Ivanović J, Adamec I, Dujmović I, Junaković A, Marić G, Martinović V, Pekmezović T, Habek M. Validation and cross-cultural adaptation of the COMPASS-31 in Croatian and Serbian patients with multiple sclerosis. *Croat Med J* 2017;58:342-348.
7. Vieira B, Costa A, Videira G, Sá MJ, Abreu P. Prevalence of autonomic dysfunction in patients with multiple sclerosis. *Acta Med Port* 2015;28:51-5.
8. Cortez MM, Nagi Reddy SK, Goodman B, Carter JL, Wingerchuk DM. Autonomic symptom burden is associated with MS-related fatigue and quality of life. *Mult Scler Relat Disord* 2015;4:258-63.
9. Ruška B, Pavičić T, Pavlović I, Junaković A, Adamec I, Crnošija L, Krbot Skorić M, Habek M. Performance of the COMPASS-31 questionnaire with regard to autonomic nervous system testing results and medication use: a prospective study in a real-life setting. *Neurol Sci* 2018;39:2079-2084.
10. Acevedo AR, Nava C, Arriada N, Violante A, Corona T. Cardiovascular dysfunction in multiple sclerosis. *Acta Neurol Scand* 2000;101:85-8.
11. de Seze J, Stojkovic T, Gauvrit JY, Devos D, Ayachi M, Cassim F, Saint Michel T, Pruvo JP, Guieu JD, Vermersch P. Autonomic dysfunction in multiple sclerosis: cervical spinal cord atrophy correlates. *J Neurol* 2001;248:297-303.
12. Flachenecker P, Wolf A, Krauser M, Hartung HP, Reiners K. Cardiovascular autonomic dysfunction in multiple sclerosis: correlation with orthostatic intolerance. *J Neurol* 1999;246:578-86.
13. Low PA. Composite autonomic scoring scale for laboratory quantification of generalized autonomic failure. *Mayo Clin Proc* 1993;68:748–752.
14. Habek M, Crnošija L, Lovrić M, Junaković A, Krbot Skorić M, Adamec I. Sympathetic cardiovascular and sudomotor functions are frequently affected in early multiple sclerosis. *Clin Auton Res* 2016;26:385-393.
15. Adamec I, Crnošija L, Junaković A, Krbot Skorić M, Habek M. Progressive multiple sclerosis patients have a higher burden of autonomic dysfunction compared to relapsing remitting phenotype. *Clin Neurophysiol* 2018;129:1588-1594.
16. Arbogast SD, Alsheklee A, Hussain Z, McNeeley K, Chelimsky TC. Hypotension unawareness in profound orthostatic hypotension. *Am J Med* 2009;122:574-80.



17. Vita G, Fazio MC, Milone S, Blandino A, Salvi L, Messina C. Cardiovascular autonomic dysfunction in multiple sclerosis is likely related to brainstem lesions. *J Neurol Sci* 1993;120:82-6.
18. Illigens BM, Gibbons CH. Sweat testing to evaluate autonomic function. *Clin Auton Res* 2009;19:79-87.
19. Davis SL, Wilson TE, Vener JM, Crandall CG, Petajan JH, White AT. Pilocarpine-induced sweat gland function in individuals with multiple sclerosis. *J Appl Physiol* (1985) 2005;98:1740-4.
20. Saari A, Tolonen U, Pääkkö E, Suominen K, Pyhtinen J, Sotaniemi K, Myllylä V. Cardiovascular autonomic dysfunction correlates with brain MRI lesion load in MS. *Clin Neurophysiol* 2004;115:1473-8.
21. Nasser K, TenVoorde BJ, Adèr HJ, Uitdehaag BM, Polman CH. Longitudinal follow-up of cardiovascular reflex tests in multiple sclerosis. *J Neurol Sci* 1998;155:50-4.
22. Nasser K, Uitdehaag BM, van Walderveen MA, Ader HJ, Polman CH. Cardiovascular autonomic function in patients with relapsing remitting multiple sclerosis: a new surrogate marker of disease evolution? *Eur J Neurol* 1999;6:29-33.
23. Flachenecker P, Reiners K, Krauser M, Wolf A, Toyka KV. Autonomic dysfunction in multiple sclerosis is related to disease activity and progression of disability. *Mult Scler* 2001;7:327-34.
24. Habek M, Krbot Skorić M, Crnošija L, Gabelić T, Barun B, Adamec I. Postural Orthostatic Tachycardia Predicts Early Conversion to Multiple Sclerosis after Clinically Isolated Syndrome. *Eur Neurol* 2017;77:253-257.
25. Krbot Skorić M, Crnošija L, Gabelić T, Barun B, Adamec I, Junaković A, Pavičić T, Ruška B, Habek M. Autonomic symptom burden can predict disease activity in early multiple sclerosis. *Mult Scler Relat Disord* 2019;28:250-255.
26. Disanto G, Zecca C, MacLachlan S, Sacco R, Handunnetthi L, Meier UC, Simpson A, McDonald L, Rossi A, Benkert P, Kuhle J, Ramagopalan SV, Gobbi C. Prodromal symptoms of multiple sclerosis in primary care. *Ann Neurol* 2018;83:1162-1173.
27. Marrie RA, Reider N, Cohen J, Stuve O, Trojano M, Cutter G, Reingold S, Sorensen PS. A systematic review of the incidence and prevalence of cardiac, cerebrovascular, and peripheral vascular disease in multiple sclerosis. *Mult Scler* 2015;21:318-331.
28. Christiansen CF, Christensen S, Farkas DK, Miret M, Sørensen HT, Pedersen L. Risk of arterial cardiovascular diseases in patients with multiple sclerosis: a population-based cohort study. *Neuroepidemiology* 2010;35:267-74.
29. Habek M, Mutak T, Nevajdić B, Pucić D, Crnošija L, Krbot Skorić M. Adrenergic hyperactivity: a missing link between multiple sclerosis and cardiovascular comorbidities? *Acta Neurol Belg* 2018 Nov 23. doi: 10.1007/s13760-018-1051-4.
30. Grassi G, Seravalle G, Brambilla G, Pini C, Alimento M, Facchetti R, Spaziani D, Cuspidi C, Mancia G. Marked sympathetic activation and baroreflex dysfunction in true resistant hypertension. *Int J Cardiol* 2014;177:1020-5.
31. Noronha MJ, Vas CJ, Aziz H. Autonomic dysfunction (sweating responses) in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 1968;31:19-22.
32. Mutani R, Clemente S, Lamberti A, Monaco F. Assessment of autonomic disturbances in multiple sclerosis by measurement of heart rate responses to deep breathing and to standing. *Ital J Neurol Sci* 1982;3:111-4.

33. Senaratne MP, Carroll D, Warren KG, Kappagoda T. Evidence for cardiovascular autonomic nerve dysfunction in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 1984;47:947-52.
34. Pentland B, Ewing DJ. Cardiovascular reflexes in multiple sclerosis. *Eur Neurol* 1987;26:46-50.
35. Yokota T, Matsunaga T, Okiyama R, Hirose K, Tanabe H, Furukawa T, Tsukagoshi H. Sympathetic skin response in patients with multiple sclerosis compared with patients with spinal cord transection and normal controls. *Brain* 1991;114:1381-94.
36. Anema JR, Heijnenbroek MW, Faes TJ, Heimans JJ, Lanting P, Polman CH. Cardiovascular autonomic function in multiple sclerosis. *J Neurol Sci* 1991;104:129-34.
37. Thomaidis TN, Zoukos Y, Chaudhuri KR, Mathias CJ. Physiological assessment of aspects of autonomic function in patients with secondary progressive multiple sclerosis. *J Neurol* 1993;240:139-43.
38. Gutrecht JA, Suarez GA, Denny BE. Sympathetic skin response in multiple sclerosis. *J Neurol Sci* 1993;118:88-91.
39. Elie B, Louboutin JP. Sympathetic skin response (SSR) is abnormal in multiple sclerosis. *Muscle Nerve*. 1995 Feb;18(2):185-9.
40. Linden D, Diehl RR, Berlit P. Subclinical autonomic disturbances in multiple sclerosis. *J Neurol* 1995;242:374-8.
41. Caminero AB, Pérez-Jiménez A, Barreiro P, Ferrer T. Sympathetic skin response: correlation with autonomic and somatic involvement in multiple sclerosis. *Electromyogr Clin Neurophysiol* 1995;35:457-62.
42. Linden D, Diehl RR, Kretzschmar A, Berlit P. Autonomic evaluation by means of standard tests and power spectral analysis in multiple sclerosis. *Muscle Nerve* 1997;20:809-14.
43. Merkelbach S, Dillmann U, Kölmel C, Holz I, Müller M. Cardiovascular autonomic dysregulation and fatigue in multiple sclerosis. *Mult Scler* 2001;7:320-6.
44. Gunal DI, Afsar N, Tanridag T, Aktan S. Autonomic dysfunction in multiple sclerosis: correlation with disease-related parameters. *Eur Neurol* 2002;48:1-5.
45. McDougall AJ, McLeod JG. Autonomic nervous system function in multiple sclerosis. *J Neurol Sci*. 2003 Nov 15;215(1-2):79-85.
46. Labuz-Rozsak B, Pierzchala K. Difficulties in the diagnosis of autonomic dysfunction in multiple sclerosis. *Clin Auton Res* 2007;17:375-7.
47. Lorberboym M, Lampl Y, Nikolov G, Sadeh M, Gilad R. I-123 MIBG cardiac scintigraphy and autonomic test evaluation in multiple sclerosis patients. *J Neurol* 2008;255:211-6.
48. Saari A, Tolonen U, Pääkkö E, Suominen K, Pyhtinen J, Sotaniemi KA, Jauhiainen J, Myllylä VV. Sympathetic skin responses in multiple sclerosis. *Acta Neurol Scand* 2008;118:226-31.
49. Hale LA, Nukada H, Du Plessis LJ, Peebles KC. Clinical screening of autonomic dysfunction in multiple sclerosis. *Physiother Res Int* 2009;14:42-55.
50. Aghamollai V, Harirchian MH, Modabbernia A, Ghaffarpour M, Mousavi M, Tafakhori A. Sympathetic skin response (SSR) in multiple sclerosis and clinically isolated syndrome: a case-control study. *Neurophysiol Clin* 2011;41:161-71.
51. Adamec I, Bach I, Barušić AK, Mišmaš A, Habek M. Assessment of prevalence and pathological response to orthostatic provocation in patients with multiple sclerosis. *J Neurol Sci* 2013;324:80-3.

52. Crnošija L, Adamec I, Lovrić M, Junaković A, Krbot Skorić M, Lušić I, Habek M. Autonomic dysfunction in clinically isolated syndrome suggestive of multiple sclerosis. *Clin Neurophysiol* 2016;127:864-869.
53. Benarroch EE. Autonomic nervous system and neuroimmune interactions: New insights and clinical implications. *Neurology*. 2019;doi: 10.1212/WNL.0000000000006942.
54. Sternberg EM. Neural regulation of innate immunity: a coordinated nonspecific host response to pathogens. *Nat Rev Immunol* 2006;6:318–328.
55. Cosentino M, Zaffaroni M, Ferrari M, Marino F, Bombelli R, Rasini E, Frigo G, Ghezzi A, Comi G, Lecchini S. Interferon-gamma and interferon-beta affect endogenous catecholamines in human peripheral blood mononuclear cells: implications for multiple sclerosis. *J Neuroimmunol* 2005;162:112-21.
56. Karaszewski JW, Reder AT, Anlar B, Arnason GW. Increased high affinity beta-adrenergic receptor densities and cyclic AMP responses of CD8 cells in multiple sclerosis. *J Neuroimmunol* 1993;43:1–7.
57. Zoukos Y, Thomaidis TN, Kidd D, Cuzner ML, Thompson A. Expression of beta 2 adrenoreceptors on peripheral blood mononuclear cells in patients with primary and secondary progressive multiple sclerosis: a longitudinal six month study. *J Neurol Neurosurg Psychiatry* 2003;74:197–202.
58. Giorelli M, Livrea P, Trojano M. Post-receptorial mechanisms underlie functional dysregulation of beta2-adrenergic receptors in lymphocytes from Multiple Sclerosis patients. *J Neuroimmunol* 2004;155:143-9.
59. Zaffaroni M, Marino F, Bombelli R, Rasini E, Monti M, Ferrari M, Ghezzi A, Comi G, Lecchini S, Cosentino M. Therapy with interferon-beta modulates endogenous catecholamines in lymphocytes of patients with multiple sclerosis. *Exp Neurol* 2008;214:315–321.
60. Marino F, Cosentino M. Adrenergic modulation of immune cells: an update. *Amino Acids* 2013;45:55-71.
61. Cosentino M, Zaffaroni M, Legnaro M, Bombelli R, Schembri L, Baroncini D, Bianchi A, Clerici R, Guidotti M, Banfi P, Bono G, Marino F. Dopaminergic receptors and adrenoceptors in circulating lymphocytes as putative biomarkers for the early onset and progression of multiple sclerosis. *J Neuroimmunol* 2016;298:82-9.
62. Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. *J Leukoc Biol* 2007;81:1–5.
63. Tracey KJ. Reflex control of immunity. *Nat Rev Immunol*. 2009;9:418-28.
64. De Jonge WJ, Ulloa L. The alpha7 nicotinic acetylcholine receptor as a pharmacological target for inflammation. *Br J Pharmacol* 2007;151:915–929.
65. Nicolussi EM, Huck S, Lassmann H, Bradl M. The cholinergic anti-inflammatory system limits T cell infiltration into the neurodegenerative CNS, but cannot counteract complex CNS inflammation. *Neurobiol Dis* 2009;35:24–31.
66. Hao J, Simard AR, Turner GH, et al. Attenuation of CNS inflammatory responses by nicotine involves  $\alpha 7$  and non- $\alpha 7$  nicotinic receptors. *Exp Neurol* 2011;227:110–119.
67. Simard AR, Gan Y, St-Pierre S, Kousari A, Patel V, Whiteaker P, Morley BJ, Lukas RJ, Shi FD. Differential modulation of EAE by  $\alpha 9^*$ - and  $\beta 2^*$ -nicotinic acetylcholine receptors. *Immunol Cell Biol* 2013;91:195-200.
68. Liu Q, Whiteaker P, Morley BJ, Shi FD, Lukas RJ. Distinctive Roles for  $\alpha 7^*$ - and  $\alpha 9^*$ - Nicotinic Acetylcholine Receptors in Inflammatory and Autoimmune Responses in the

Murine Experimental Autoimmune Encephalomyelitis Model of Multiple Sclerosis. *Front Cell Neurosci* 2017;11:287.

69. Rosas-Ballina M, Olofsson PS, Ochani M, et al. Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. *Science* 2011;334:98-101.
70. Degelman ML, Herman KM. Smoking and multiple sclerosis: A systematic review and meta-analysis using the Bradford Hill criteria for causation. *Mult Scler Relat Disord* 2017;17:207-216.
71. Gomes JP, Watad A, Shoenfeld Y. Nicotine and autoimmunity: The lotus' flower in tobacco. *Pharmacol Res* 2018;128:101-109.
72. Gao Z, Nissen JC, Ji K, Tsirka SE. The experimental autoimmune encephalomyelitis disease course is modulated by nicotine and other cigarette smoke components. *PLoS One* 2014;9:e107979.
73. Khezri S, Abtahi Froushani SM, Shahmoradi M. Nicotine Augments the Beneficial Effects of Mesenchymal Stem Cell-based Therapy in Rat Model of Multiple Sclerosis. *Immunol Invest* 2018 Feb;47(2):113-124.

**Table 1.** Studies investigating different types of autonomic dysfunction in patients with multiple sclerosis using different autonomic tests.

Reference	Number of participants	MS phenotype	Sympathetic dysfunction	Parasympathetic dysfunction	Sudomotor dysfunction
Noronha et al., 1968 (31)	60	NR	NR	NR	42% <sup>a</sup>
Mutani et al., 1982 (32)	10	NR	NR	40% <sup>b</sup>	NR
Senaratne et al., 1984 (33)	11	NR	NR	55% <sup>b</sup>	NR
	19	NR	16% <sup>d</sup>	21% <sup>c</sup>	NR
Pentland et al., 1987. (34)	50	NR	0% <sup>e</sup>	8% <sup>c</sup> , 30% <sup>b</sup>	NR
Yokota et al., 1991 (35)	28	RRMS	NR	NR	75% <sup>f</sup>
Anema et al., 1991 (36)	34	NR	13% <sup>e</sup> (out of 30 patients)	36% <sup>b</sup>	NR
Thomaides et al., 1993 (37)	10	SPMS	0% <sup>g</sup>	0% <sup>b,c</sup>	NR
Gutrecht et al., 1993 (38)	29	NR	NR	NR	59% <sup>f</sup>
Vita et al., 1993 (17)	40	NR	0% <sup>e</sup>	18% <sup>b</sup>	NR
Elie et al., 1995 (39)	70	RRMS 41, PMS 29	NR	NR	94% <sup>f</sup>
Linden et al., 1995 (40)	30	NR	14% <sup>e</sup> (out of 22 patients)	10% <sup>b</sup>	67% <sup>f</sup>
Camirero et al., 1995 (41)	63	NR	NR	NR	41% <sup>f</sup>
Linden et al., 1997 (42)	20	NR	5% <sup>g</sup>	25% <sup>b</sup>	75% <sup>f</sup>

Nasseri et al., 1999 (22)	20	RRMS	NR	20% <sup>b</sup>	NR
Flachenecker et al., 1999 (12)	40	RRMS, SPMS	8% <sup>e</sup>	3% <sup>c</sup> , 10% <sup>b</sup>	NR
Acevedo et al., 2000 (10)	40	RRMS 30, PMS 10	38% <sup>e</sup>	43% <sup>c</sup> , 30% <sup>b</sup>	NR
de Seze et al., 2001 (11)	25	RRMS	0% <sup>e</sup>	0% <sup>h</sup>	30% <sup>f</sup>
	25	SPMS	20% <sup>e</sup>	12% <sup>h</sup>	48% <sup>f</sup>
	25	PPMS	32% <sup>e</sup>	12% <sup>h</sup>	48% <sup>f</sup>
Merkelbach et al., 2001 (43)	54	RRMS	22% <sup>e</sup>	2% <sup>c</sup> , 22% <sup>b</sup>	NR
	14	SPMS	29% <sup>e</sup>	7% <sup>c</sup> , 29% <sup>b</sup>	NR
	16	PPMS	31% <sup>e</sup>	25% <sup>c</sup> , 31% <sup>b</sup>	NR
Gunal et al., 2002 (44)	22	RRMS	9% <sup>e</sup>	14% <sup>c</sup> , 18% <sup>b</sup>	18% <sup>f</sup>
McDougall et al., 2003 (45)	63	RRMS 39, SPMS 21 and PPMS 3	3% <sup>d</sup> , 3% <sup>g</sup>	0% <sup>c</sup> , 16% <sup>b</sup>	45% <sup>f</sup>
Labuz-Roszak et al., 2007 (46)	24	RRMS 11, SPMS 10 and PPMS 3	19% <sup>e</sup>	4% <sup>c</sup> , 29% <sup>b</sup>	75% <sup>f</sup>
Lorberboym et al., 2008 (47)	10	RRMS 7, SPMS 3	NR	30% <sup>c</sup> , 50% <sup>b</sup>	NR
Saari et al., 2008. (48)	27	RRMS 21, SPMS 6	NR	NR	52% <sup>f</sup>
Hale et al., 2009 (49)	31	RRMS 22, SPMS 5 and PPMS 2 (2 unknown type)	26% <sup>g</sup>	6% <sup>c</sup> , 10% <sup>b</sup>	NR
Aghamollai et al., 2011 (50)	30	CIS 9, RRMS 21	NR	NR	77% <sup>f</sup>

Adamec et al., 2013 (51)	112	RRMS	11% <sup>g</sup>	NR	NR
Crnošija et al, 2016 (52)	24	CIS	38% <sup>d</sup> , 8% <sup>g</sup>	4% <sup>c</sup> , 0% <sup>b</sup>	31% <sup>i</sup> (out of 16 patients)
Habek et al., 2016 (14)	104	CIS	34% <sup>d</sup> , 8% <sup>g</sup>	1% <sup>c</sup> , 4% <sup>b</sup>	33% <sup>i</sup>
Adamec et al., 2018 (15)	40	RRMS	36% <sup>j</sup>	3% <sup>k</sup>	35% <sup>i</sup>
	30	PMS	61% <sup>j</sup>	20% <sup>k</sup>	73% <sup>i</sup>

NR not reported; CIS clinically isolated syndrome; RRMS relapsing remitting multiple sclerosis; SPMS secondary progressive multiple sclerosis; PPMS primary progressive multiple sclerosis; PMS progressive multiple sclerosis; <sup>a</sup> thermoregulatory sweat testing; <sup>b</sup> deep breathing test; <sup>c</sup> Valsalva ratio, <sup>d</sup> systolic BP response to Valsalva manoeuvre, <sup>e</sup> active standing, <sup>f</sup> sympathetic skin response, <sup>g</sup> tilt table, <sup>h</sup> composite measure of 3 parasympathetic tests including deep breathing and Valsava ratio, <sup>i</sup> QSART, <sup>j</sup> adrenergic index, <sup>k</sup> cardiovagal in

## Figures

**Figure 1.** Autonomic dysfunction (AD) in people with multiple sclerosis (pwMS) can be demonstrated clinically and on molecular level. Clinically it can be demonstrated by measuring autonomic symptoms with Composite Autonomic Symptom Score (COMPASS-31) and neurophysiologically with different autonomic nervous system tests. Both symptomatic and objectively determined AD can be related to increased risk of multiple sclerosis (MS) disease activity, and abnormalities on different autonomic nervous system tests may be associated with an increased risk of cardiovascular comorbidities. The cause of AD in pwMS is probably associated with demyelinating lesions in the brainstem and atrophy of the cervical spinal cord, however once present it can cause a vicious circle of new lesions which can again worsen AD. Further supporting these clinical observations are molecular changes on immune cells. Changes in the sympathetic autonomic system like different expression dopaminergic receptor (DR) D3 and  $\alpha$ 2A-adrenergic receptor (AR) mRNAs in peripheral blood mononuclear cells, and dopaminergic receptor D5 mRNA in T regulatory cells may be associated with MS disease activity. Similarly, modulation of the cholinergic anti-inflammatory pathway over different subunits of the nicotinic acetylcholine receptor (nAChR) in peripheral immune may mediate different effects on MS disease activity.



