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REVIEW

How to explore and explain autonomic changes in multiple sclerosis

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Abstract Autonomic dysfunction (AD) in people with MS (pwMS) is a frequent finding. This narrative review will present an overview of central neural mechanisms involved in the control of cardiovascular and thermoregulatory systems, and methods of autonomic nervous system testing will be discussed thereafter. Since the need for standardization of autonomic nervous system (ANS) testing, we will focus on the standard battery of tests (blood pressure and heart rate response to Valsalva maneuver and head-up tilt, and heart rate response to deep breathing test plus one of the tests for sudomotor function), which can detect ANS pathology in the majority of pwMS. The review will briefly discuss the other types of AD in pwMS and the use of appropriate tests. While performing ANS testing in pwMS one has to consider the multiple sclerosis phenotypes, disease duration, and its activity, the degree of clinical disability of patients included in the study, and the disease-modifying therapies taken, as these factors may have a great influence on the results of ANS testing. In other words, detailed patient characteristics presentation and patient stratification are beneficial when reporting results of ANS testing in pwMS.

Introduction

Multiple sclerosis (MS) is an immune-mediated inflammatory disorder of the central nervous system. It affects more than 2.8 million people worldwide and represents the leading cause of non-traumatic neurologic disability in young adults [1,2]. By its definition MS is characterized by dissemination in space and time, meaning that various parts of the central nervous system may be affected during the course of the disease. This includes structures important for the regulation of the autonomic nervous system (ANS), such as the periventricular nuclei in the brainstem as well as long afferent and efferent tracts in the medulla [3], but also multiple supratentorial areas [4]. Autonomic dysfunction (AD) is frequently found in people with multiple sclerosis (pwMS), according to some studies in more than 80% of pwMS [5]. Symptoms of AD seem to occur even in the prodromal stages of MS [6]. AD increases with disease duration and accumulation of

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neurological disability and is more pronounced in the progressive than in the relapsing stage of the disease [5]. The knowledge of ANS (dys)function in pwMS is not only important because of its direct impact on the quality of life of these patients, but also because of the possible ANS involvement in the pathophysiology of MS [7,8], as well as interaction with some of the MS specific therapies [9,10].

Although the ANS exerts effects on a number of organs and body systems, the autonomic control of the cardiovascular, followed by thermoregulatory systems are the ones that are the most practical to assess. Consequently, the cardiovascular branch of the ANS function is the most studied one in people with MS (pwMS). The review will present a brief overview of central neural mechanisms involved in the control of cardiovascular and thermoregulatory systems, and methods of autonomic nervous system testing will be discussed thereafter. Since the need for standardization of ANS testing has been expressed by relevant societies [11–14] this review will focus on a standardized set of tests. Differences in AD occurrence in various stages of the disease will be presented with each of the diagnostic methods discussed. We will end the review with a very brief overview of other, non-cardiovascular/sudomotor ANS disturbances and the use of appropriate tests in pwMS.

Central neural control of the cardiovascular system

The end goal of the ANS, with regards to the control of the cardiovascular system function, is to ensure adequate perfusion to meet the metabolic demand of different organs and tissues in a variety of conditions and situations a human body can be exposed to. This is achieved by two general mechanisms: 1) reflex regulation or feedback control, which is related to the stimulation of different peripheral receptors, and 2) feedforward regulation, the so-called "central command", conveyed by the descending inputs from higher brain centers [15].

Regarding reflex or feedback regulation, one of the most important and most studied cardiovascular reflexes is the baroreceptor reflex. In short, baroreceptors are stretch receptors sensory neurons located in the walls of the carotid sinus and aortic arch, and their signals are conveyed via glossopharyngeal and vagal nerves afferents, respectively, terminating in the nucleus tractus solitarius (NTS) in the dorsomedial medulla [15]. From NTS, some neurons project directly to preganglionic parasympathetic neurons of the nucleus ambiguous (NA), mediating the fast cardioinhibitory effect via vagal nerve efferents, while other neurons project to GABAergic interneurons of the caudal ventrolateral medulla (CVLM) which in turn inhibit sympathetic premotor neurons of the rostral ventrolateral medulla (RVLM) [15]. RVLM neurons send catecholaminergic projections to the preganglionic sympathetic neurons in the intermediolateral nucleus of the thoracolumbar spinal cord, and the effects on blood vessels and the heart are mediated through postganglionic sympathetic fibers. Both baroreceptor sensory neurons and RVLM neurons are tonically active [15]. This feedback loop is tied to the changes in the arterial pressure in such a way that the elevation in the blood pressure results in an increase in the firing rate of baroreceptors leading to parasympathetic cardiovagal activity (cardioinhibitory effect) and a decrease in the tonic activity of RVLM neurons (i.e., lowering of sympathetic cardiovascular activity), and vice versa. Apart from baroreceptors, reflex regulation is also conveyed through the activity of chemoreceptors, nasopharyngeal, vestibular, and skeletal muscle receptors as well as skin nociceptors [15], but this is outside the scope of this review.

On the other hand, feedforward control does not require any inputs from peripheral receptors [15]. These types of cardiovascular responses are associated with defensive behavior (i.e., fight-or-flight), psychological stress or physical activity and are mediated by highly complex and coordinated mechanisms involving different forebrain and midbrain areas such as the cortex (e.g., medial prefrontal, insular, auditory, olfactory, visual), thalamus and hypothalamus, amygdala, and the periaqueductal gray matter [15]. Some of these areas interact with the baroreflex central neural network and modulate the baroreflex response, but some pathways bypass these structures and exert cardiovascular effects independently from the baroreflex [15].

In other words, central structures involved in autonomic control are termed central autonomic system, which can be divided into a cortical autonomic network or CAN (involved in the feedforward control), and a subcortical autonomic network or SAN (involved in the reflex regulation) [16]. CAN involve all the cerebral lobes with pathways overlapping within the insular cortex, whereas SAN involves the hypothalamus, periaqueductal gray matter (PAG), locus coeruleus, and the beforementioned areas/nuclei of the medulla. [16] The structures and pathways of the central autonomic system are discussed in detail elsewhere [15,16].

Since MS can affect any part of the central nervous system, demyelinating lesions of the beforementioned structures are the potential cause of AD in pwMS (Fig. 1). The most extreme examples of ANS dysfunction in pwMS are the neurogenic stunned myocardium and the neurogenic pulmonary edema, both of which are thought to be caused by sympathetic overactivity [17]. Luckily, these cases are very rare, but according to published data, demyelinating lesions in the medulla are the most common finding in these patients [17].

Evaluation of cardiovascular autonomic function

The exploration of cardiovascular autonomic function in pwMS started in the 1970-ies [18], but most of the studies have been performed in the last 20 years, which is most likely related to the development of a device for noninvasive continuous blood pressure monitoring [19]. The use of various methods and testing protocols in different laboratories has been recognized as one of the main reasons for the lack of comparability between different studies assessing the ANS function in pwMS [20,21]. In the last 10 years, different neurological societies published several consensus statements and recommendations with a tendency to standardize the ANS testing protocols [11–14]. This review will focus mainly on these recommended methods, but some

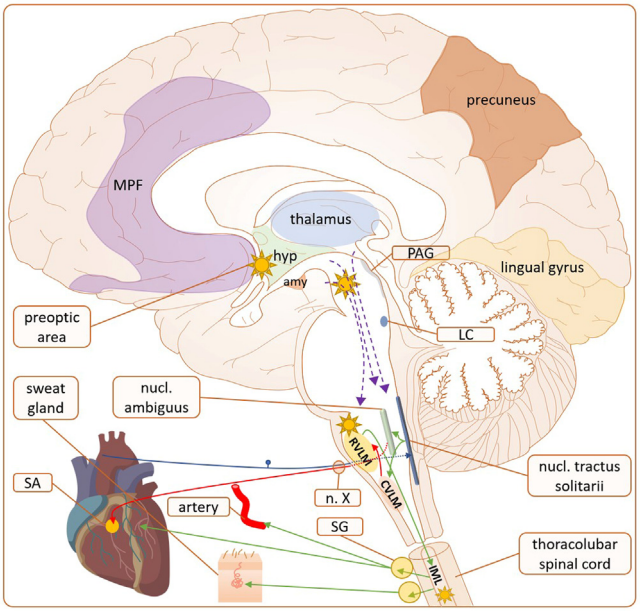

Fig. 1 Schematic presentation of structures involved in the baroreflex response as well as some of the supra- and infratentorial structures associated with the control of the autonomic cardiovascular response. The baroreflex response starts with baroreceptors of the aortic arch and carotid sinus which sense hemodynamic changes, the information is carried via glossopharyngeal and vagal nerve afferent to brainstem centers, which modulate vagal and efferent sympathetic activity accordingly. The blue arrow represents sensory afferent, green arrows represent excitatory, and red inhibitory relations, while purple arrows represent direct and indirect projections of higher structures towards the baroreflex network. Sun shapes are placed in some of the areas of the brain and spinal cord which are known to be involved in the thermoregulatory sweat response. PAG — periaqueductal gray matter, LC — locus coeruleus, n.X — vagal nerve, SA — sinoatrial node, RVLM — rostral ventrolateral medulla, CVLM — caudal ventrolateral medulla, IML — intermediolateral column of the spinal cord, SG — paravertebral sympathetic ganglion, amy — amygdale, hyp — hypothalamus, MPF — medial prefrontal cortex.

additional methods will be discussed as well. Another important consideration when performing ANS testing in pwMS is to consider the MS phenotype, disease duration, and its activity, and the degree of clinical disability of patients included in the study [5,7,22], and the disease-modifying therapies taken [9], as these factors may have a great influence on the results of ANS testing. In other words, detailed patient characteristics presentation and patient stratification are beneficial when reporting results of ANS testing in pwMS.

Sympathetic cardiovascular function tests

Head-up tilt table test (HUTT)

One of the most frequently used tests in ANS testing laboratories is the head-up tilt table test (HUTT), where ideally blood pressure (BP) and heart rate (HR) are continuously measured in a beat-by-beat manner. The details of performing HUTT can be found in the recently published recommendations for tilt table testing [23]. The test starts with at least a 10-minute supine resting position during which stable baseline BP and HR values are acquired. Afterward, the examinee is tilted to a certain degree (optimally to 70°), and BP and HR data are recorded for a variable period ranging from 5 to 60 min, depending on the differential diagnosis and the response measured [24]. As is the case with almost all ANS testing procedures, it is by measuring an indirect response in ANS activity - in this case changes in BP and HR with respect to baseline values — to a specific stimulus that we assess the functional integrity of the ANS. In the case of HUTT, this stimulus is gravitational redistribution of the blood towards the lower body, which lowers baroreceptors activity leading to disinhibition (i.e., increased activity) of sympathetic nerves producing a vasoconstrictive and positive chrono- and inotropic cardiac effects to counteract this blood redistribution. A normal BP and HR response to HUTT would be a transient drop in systolic BP (sBP) up to 20 mmHg and in diastolic BP (dBP) up to 10 mmHg, coupled with about 10 beats per minute (bpm) increase in heart rate within the first 20 s after tilting with a later tendency of BP to reach baseline values [25]. Apart from the normal, there are three other types of responses that can be recorded during HUTT - orthostatic hypotension, postural tachycardia syndrome, and syncope. When combined, these responses can be found in over 60% of pwRRMS tested with HUTT [22]. Also, abnormal HUTT responses are more frequent already at the stage of the clinically isolated syndrome (CIS) when compared to healthy controls [26].

Orthostatic hypotension

Orthostatic hypotension (OH) is defined as a sustained reduction of sBP of $>$20 mmHg and/or dBP reduction of $>$10 mmHg within the first 3 min of active standing or HUTT [12]. However, it should be noted that not all patients who fulfill these criteria have an underlying AD. Other potential causes, such as different medications, hypovolemia, cardiac pump failure, venous pooling, etc., should be carefully assessed and in such instances, the term non-neurogenic OH is used [27]. On the other hand, the neurogenic OH (nOH) is a result of an inadequate sympathetic nervous system (SNS) response (i.e., vasoconstriction and heart rate increase) as a consequence of the damage to the ANS, to the explained gravitational redistribution of blood which may or may not lead to OH characteristic symptoms. Apart from detailed patient history, blood pressure response to the Valsalva maneuver [14] and a ΔHR/ΔsBP ratio at 3 min of tilt $<$ 0.5 beats/min per mmHg [28] have been proven beneficial when discerning neurogenic from non-neurogenic causes of OH.

The reported OH frequency as HUTT finding in pwMS is variable, ranging from 10% [22] to over 37% [29]. Our study has shown that there is no significant difference in the incidence of OH when comparing RRMS and PMS (primary and secondary) phenotypes [5], while others report significantly higher OH prevalence in PMS phenotypes compared to RRMS [30]. Others report a higher frequency of OH in pwMS associated with a higher degree of disability assessed by the Expanded Disability Status Scale (EDSS) [29]. Also, OH seems to be more frequent in people with RRMS during remission

when compared to people with RRMS during active relapse [22]. Regarding the association of OH with specific lesions within the CNS, new-onset severe OH associated with a lesion in the dorsal medulla potentially involving the NTS has been reported [31], while Saari et al. [32] reported a correlation of decreased BP responses to HUTT and total midbrain and parietal lesion volume.

Postural tachycardia syndrome (POTS)

Postural tachycardia syndrome (POTS) is defined as the presence of sustained HR increase of ≥30 bpm or HR over 120 bpm within 10 min of HUTT (Fig. 2) or active standing in absence of OH associated with ≥ 6 months of the frequent and chronic presence of typical symptoms [12,33]. Other potential causes of orthostatic tachycardia, such as deconditioning, dehydration, anemia, hyperthyroidism, or medications that impair autonomic regulation should be excluded [34]. POTS is a multifactorial condition, and several, often overlapping forms of POTS have been identified over the last two decades – hypovolemic, neuropathic, hyperadrenergic, associated with norepinephrine transporter deficiency, autoimmune, mast cell activation syndrome [33]. Hyperadrenergic POTS is associated with elevated levels of plasma norepinephrine during orthostasis. While this hyperadrenergic state is most commonly secondary to partial dysautonomia (i.e., autonomic neuropathy) or hypovolemia, it has been shown that a subset of these patients has an increased sympathetic nervous system activity [35]. In a case report of a patient with the first MS relapse who was also diagnosed with POTS, we hypothesized that the onset of POTS is related to a thalamic lesion. On the follow-up HUTT, after this patient received intravenous methylprednisolone, the criteria for POTS were not fulfilled [36]. One study indicates that POTS is more prevalent in pwMS when compared to people referred to HUTT because of orthostatic intolerance symptoms and without known neurological illnesses [37]. Interestingly, there are reports of patients who developed POTS several years before establishing the diagnosis of MS [38], and a case of a patient with POTS and radiologically (RIS) isolated syndrome with positive cerebrospinal fluid (CSF) oligoclonal bands have recently been reported [39]. Furthermore, a study that evaluated the predictive value of ANS findings in 84 patients diagnosed with clinically isolated syndrome (CIS) found that POTS is an independent predictor of conversion to clinically definite MS (i.e., a predictor of clinical or MRI disease activity) during 6-month follow-up with an odds ratio of over 12 [40]. Moreover, POTS has been found to be significantly more common in RRMS patients who are in relapse compared to those in remission [22]. In the context of MS, POTS may be a result of lesions involving the central autonomic network leading to sympathetic overdrive. Although to our knowledge there are no studies specifically investigating MRI findings in pwMS with POTS, two brain morphometric studies - one involving POTS patients, and the other pwMS - share some similarities. Umeda et al. [41] report lower gray matter volume in the left insula, right cingulate gyrus, right middle frontal gyrus, and lower white matter volume in the right pre- and post-central gyrus, paracentral lobule, and superior frontal gyrus in POTS patients, while Winder et al. [4] report higher sympathetic cardiovascular activity in pwMS associated with demyelinating lesions in the left insular and hippocampal regions, as well as right frontal inferior opercular region and right posterior parietal white matter. The previously mentioned association of POTS and MS activity may be related to the immunomodulatory effects of increased sympathetic activity [42]. Okamoto et al. found that POTS patients have increased levels of proinflammatory interleukin-6 (IL-6) which correlated to an index of vascular sympathetic activity [43]. On the other hand, it has been shown that pwMS have elevated plasma and CSF levels of IL-6 [44,45], and a significant correlation of CSF IL-6 detectability and disease activity has been reported in pwRRMS [45].

Syncope

Syncope is defined as a transient loss of consciousness and postural tone due to cerebral hypoperfusion with rapid and spontaneous recovery. Although there are multiple potential causes of syncope (e.g., neurally mediated, cardiogenic, hypovolemic, orthostatic hypotension, etc.) they all result in global cerebral hypoperfusion. The most common type of syncope is neurally mediated or reflex syncope, but the pathophysiological mechanism is still incompletely understood. Although some laboratories perform up to 60 min tilt-up when assessing a patient with a history of syncope, our work has shown that the duration of testing can be greatly reduced by employing a painful stimulus after a 10 min tilt-up period, while increasing the sensitivity for detecting a

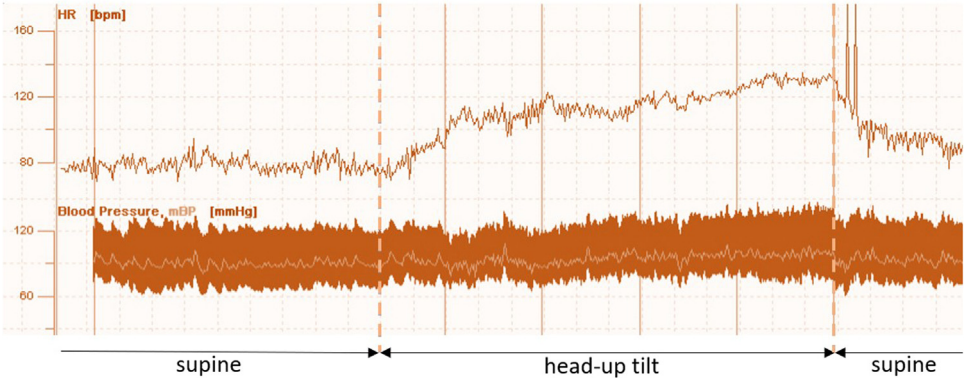

Fig. 2 A typical recording of heart rate (upper part) and blood pressure (lower part) response in a patient with postural tachycardia syndrome. Note the gradual increase in heart rate and stable blood pressure response to head-up tilt.

syncopal response. Our previous studies found that the prevalence of syncope in pwRRMS is between 17 and 27% [22,46], which is in line with the prevalence of syncope in the general population. [47] (Cheshire, 2017) Interestingly, syncope can be provoked significantly more often in RRMS patients who are in remission compared to those in relapse [22]. Also, our previous work indicates that the preserved brainstem function is necessary for the development of syncope, which is in line with the hypotheses that syncope is a useful "reflex" which has been preserved throughout the course of human evolution [46].

Blood pressure response to Valsalva maneuver

The Valsalva maneuver has been utilized in ANS testing now for over half a century [48,49]. In order to analyze the response to the Valsalva maneuver, a beat-by-beat recording of BP and HR values is a prerequisite. In the autonomic laboratory setting Valsalva maneuver is done by a person blowing against resistance for 15 s [50], ideally with the pressure produced by blowing measuring 40 mmHg corresponding to the intrathoracic pressure [51]. Four distinct phases of the BP response to the Valsalva maneuver have been distinguished (see Fig. 3a). While the BP changes recorded during phases I and III are related to the mechanical effect of sudden increase and decrease (respectively) of intrathoracic pressure corresponding to the start and the cessation of the Valsalva maneuver respectively, BP changes during phases II and IV reflect the SNS activity counteracting these sudden changes of intrathoracic pressure and its effects on the cardiovascular system [50]. Fig. 3a presents a normal BP response to the Valsalva maneuver. Late phase II is dependent on vascular α-adrenergic innervation, and phase IV depends mostly on cardiac β-adrenergic innervation [52]. Mean BP (mBP) dropping over 20 mmHg during early phase II, mBP not reaching baseline values in late phase II, absence of mBP overshoot in phase IV, sBP recovery time (PRT) to baseline value in phase IV longer than 4 s, and pulse pressure drop (PPD) lower than 50% is considered as abnormal responses and a sign of sympathetic dysfunction [53,51]. Our study indicates that a third of patients diagnosed with CIS (most of whom would fulfill the later published revised 2017 McDonald criteria for RRMS) [54] exhibit abnormal BP responses to Valsalva maneuver, and the most common abnormalities detected are the diminished PPD (≈28%) and mBP not reaching baseline values in late phase II (≈9%) [55]. Others report no differences in BP response to Valsalva maneuver between early RRMS patients and healthy controls, but the PPD is not taken into the account and the sample size is considerably smaller [56]. PwPMS may exhibit more pronounced abnormalities of BP responses such as the absence late phase II, absence of phase IV or prolonged PRT [5]. Although to our knowledge there are no published studies investigating correlation of MS lesions and alterations of BP response to Valsalva maneuver, a functional MRI study nicely depicts the importance of the previously discussed central autonomic network structures such as insular cortex, medial and dorsal medulla, dorsal pons, and midbrain in cardiovascular response to the Valsalva maneuver [57].

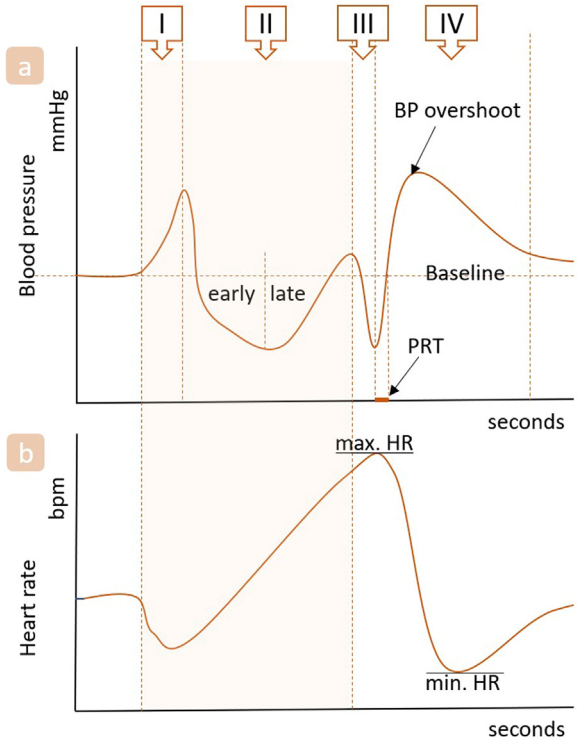

Fig. 3 Schematic representation of normal a) four-phased blood pressure response to Valsalva maneuver (the phases are indicated by roman numerals) and b) heart rate response to Valsalva maneuver. Light orange color indicates the period during which the patient performs the Valsalva maneuver (i.e., blowing against resistance). PRT – pressure recovery time.

Parasympathetic cardiovascular function

Heart rate response to Valsalva maneuver

In addition to sympathetic function assessment through the analysis of the blood pressure response, the Valsalva maneuver provides insight into parasympathetic function through the analysis of heart rate response. Valsalva ratio (VR) is calculated by dividing the maximum HR induced by the Valsalva maneuver by the minimum HR during the phase IV BP overshoot (see Fig. 3b). The VR is dependent mostly on parasympathetic cardiac activity but is also affected by peripheral and cardiac sympathetic activity [52]. Nevertheless, VR is regarded as a test of the vagal component of the arterial baroreflex [11]. Since a large normative database is available, VR results are compared to age and sex-adjusted referent values [11]. In our previous work, we found that the VR is decreased/abnormal in only about 1% of CIS patients [55]. In a follow-up study we found no significant changes in VR in CIS patients over a two-year period [58]. Flachenecker et al. report significantly higher VR in active RRMS patients compared to those with stable disease [7]. This and another study [59] report no significant changes in VR over a two-year period in pwRRMS. The VR seems to be normal in patients with secondary progressive MS as well [9]. De Seze et al. report that primary progressive MS patients have the smallest VR values (although not abnormal when compared to referent values), while RRMS and SPMS patients exhibited practically the same responses [30].

Deep breathing test

Variation of HR associated with respiration is also known as respiratory sinus arrhythmia (RSA). Since both afferent and efferent limbs underlying the RSA are conveyed by the vagal nerve it has been analyzed in various ways to gain insight into cardiac parasympathetic function. Heart rate response to deep breathing or the deep breathing test (DBT) is the most widely used method since it is easy to perform and analyze, it is highly reproducible, and sensitive in detecting ANS pathologies [11]. The test is performed with the patient in a supine position, instructed to continuously inhale and exhale, with each phase lasting 5 s, while continuously recording heart rate. RSA amplitude is then calculated as the difference between the HR at the end of inspiration and HR at the end of expiration (see Fig. 4). Typically, six cycles are averaged to a final RSA amplitude value, which is compared to age-adjusted referent values [51].

In our study, abnormal DBT was found in about 4% of patients with CIS [55], and a significant decline in RSA amplitude after a two-year follow-up has been recorded in this CIS patient cohort [58]. A study showed that pwPMS have a significantly lower RSA amplitude compared to pwRRMS [5]. When comparing the DBT and Valsalva ratio findings, it seems that DBT is a more sensitive method in detecting pathologies, as well as the progression of cardiovagal dysfunction in the MS population. This is in line with the studies on diabetic neuropathy [60,61].

Evaluation of sudomotor autonomic function

The sudomotor function is one of two major thermoregulatory mechanisms, the other being peripheral vasodilatation [62]. Studies indicate that the thermoregulatory sweating response is controlled by brain temperature and is secondarily modulated by mean skin temperature [63]. The topmost hierarchical structure controlling the thermoregulatory responses is most likely located in the preoptic area of the anterior hypothalamus [64] (Cheshire, 2020), which contains thermosensitive neurons [62]. An fMRI study indicates rostral lateral midbrain and rostral lateral medulla involvement in thermal sweating response (see Fig. 1) [65]. Efferent thermoregulatory pathways descend further to the intermediolateral column (IML) of the thoracolumbar spinal cord. Preganglionic sympathetic neurons project from the IML of the spinal cord and synapse onto the postganglionic sympathetic neurons in the paravertebral sympathetic chain ganglia from which unmyelinated C-fibers travel along arteries to innervate the eccrine skin glands [64]. The primary neurotransmitter of the postganglionic cholinergic sudomotor nerves is acetylcholine which binds to muscarinic receptors on eccrine sweat glands. In the context of MS lesions of the abovementioned central structures may be related to sudomotor dysfunction. On the other hand, there is also evidence of somewhat controversial peripheral nervous system involvement in pwMS [66].

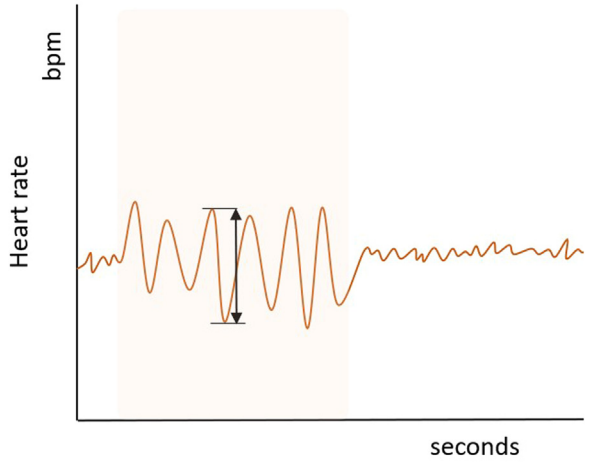

Fig. 4 Heart rate response to deep breathing test. Light orange color indicates the period of guided deep breathing (5-second inhale, 5-second exhale). The arrow indicates the respiratory sinus arrhythmia amplitude (RSA) of one breathing cycle. At least six cycles are averaged to the final RSA result.

Sudomotor function has rarely been studied in pwMS. Most of the published studies utilized the quantitative sudomotor axon reflex test, the thermoregulatory sweat test, or the sympathetic skin response (SSR) and these methods will be discussed hereafter.

Sympathetic skin response

In short, the physiological basis of SSR is the existence of a polysynaptic reflex elicited by an arousal stimulation ultimately leading to the activation of the sweat glands mediated by the sympathetic activity [67]. In the SSR the transient reflex changes in electrodermal potential related to the production of sweat are most commonly measured with active electrodes placed over palms or soles and referent electrodes placed on the dorsum of the respective body part [68]. The most common stimulus is the electrical stimulation of the median nerve, but SSR can be recorded in response to mental stress, cough, inspiratory gasp, loud noise, and various other somatic or psychic stress stimuli [68]. The presence of the response indicates the activation of eccrine glands and thus indirectly the activity of the sympathetic nerves. Quantifiable parameters of the SSR are the latency and the amplitude of the response, although the amplitude is highly variable and is usually not considered as a reliable index of abnormality [69]. The test is easily performed on most standard EMG equipment, but the results are highly variable and the SSR is generally considered to have limited sensitivity and specificity [64]. This variability of the reported results is at least partly related to a considerable number of factors that can influence the measured response (e.g., skin and ambient temperature, stimulus strength and type, habituation of the response with repeated stimuli, mental and/or emotional state, etc.) [67]. On the other hand, Margaritella et al. published a meta-analysis of case-control studies of SSR in pwMS and their results suggest that, despite a considerable heterogeneity of findings, the SSR may be useful in this patient population [70]. The pooled analysis showed that 34% of pwMS had absent responses on at least one of the tested sites in comparison to none in the healthy controls group. Also, the proportion of pwMS with absence of response was moderately correlated to the disease duration, and weakly correlated with the EDSS [70]. Moreover, pwMS had significantly longer

latencies of the SSR both on the upper and lower extremities. The authors suggest latency threshold values for the lower extremities only, as the latency threshold for upper extremities did not reach a significant diagnostic odds ratio [70]. Saari et al. demonstrated a correlation between the SSR abnormalities and the total brain lesion volume, lesion volume in the pons, right temporal lobe and left cerebellar lobe, and the presence of thoracic spinal cord lesions in pwMS [32]. Interestingly, cervical spinal cord lesions were not associated with the SSR findings [32]. Another study nicely demonstrates the presence or absence of the SSR on upper or lower extremities in relation to the spinal cord lesion level [71]. The absence of SSR in the legs has been correlated with the presence of leg motor weakness, neurogenic bladder, and the spinothalamic tract involvement in pwMS [72]. Also, a significant correlation of the SSR and cardiovascular autonomic abnormalities has been reported [73]. The abnormal SSR can be detected in up to 43% of pwMS without clinical signs of AD [74]. and the abnormalities are more frequently found in the progressive forms of MS [30].

Thermoregulatory sweat test (TST)

The TST is performed by applying an indicator powder to as much of the skin surface of the patient and elevating the patient's core temperature to about 1 °C in a controlled environment. The indicator powder changes color in contact with the sweat which allows for the identification of the areas of anhidrosis [64]. The main advantages of this test are that the whole anterior body is tested, and it detects abnormalities (i.e., anhidrosis) regardless of the anatomical location of the pathology (i.e., central or peripheral, pre- or postganglionic) [11]. On the other hand, the test is technically demanding, results are qualitative (although the percentage of surface anhidrosis can be calculated), and the procedure may be time-consuming and uncomfortable to the patient leading to reluctance to have repeated testing [62].(Buchmann et al., 2019) Different quantifiable versions of TST have been utilized as well. For example, the passive heating-induced sweating response has been measured by using an evaporimeter [75] and capacitance hygrometry [76]. In those cases, the response is measured at small skin surfaces on different body sites thus greatly reducing the spatial resolution compared to the previously described qualitative/indicator powder version.

The TST with indicator powder quinizarin was utilized in MS studies by Noronha et al. [77], Vas [78], and Cartlidge [79]. Noronha et al. found that 42% of examined pwMS had abnormal TST, and the incidence of abnormal responses was much higher in pwMS with more severe and advanced disease [77]. Vas investigated thermoregulatory sweating response in males with MS with and without impotency, and it was found that subjects with normal sexual function had normal TST results, in the partially impotent subjects anhidrosis was detected in lower limbs, while totally impotent participants did not perspire below the waist [78]. Concurrent results were reported by Cartlidge [79]. In a more recent study Saari et al. [75] found that evaporimeter measured sweating response to heat exposure was significantly lower in pwMS (21 RRMS and 8 SPMS patients; EDSS range 0−9) compared to healthy controls at the forehead, legs, and feet level. The response was lower in the SPMS group compared to RRMS patients. They also found a negative correlation between sweating response and the EDSS, and total lesion volume in the whole brain correlated with decreased sweating in the foot and hand, while no correlation was found for spinal cord lesions [75].

Quantitative sudomotor axon reflex test (QSART)

The QSART is the most widely used test of sudomotor function [62]. This is a test of postganglionic sudomotor function which uses iontophoresis of 10% acetylcholine (or another cholinergic agonist) solution as a stimulus of the efferent sudomotor nerve endings, and a change of the relative humidity is measured over time with a special sweat capsule placed on four typical body areas - forearm, proximal and distal leg, and dorsum of the foot. Measured values (μL of sweat) are compared to age and location-adjusted normative values [51]. The advantage of this test is that the results are quantifiable, they have temporal resolution and relatively low variability [62]. The limitations are the high cost of equipment, technical demands, and the very low spatial resolution since it tests only four small-sized areas, and the iontophoresis can be uncomfortable for the patient [62].

Davis et al. conducted the first QSART study in MS patients using pilocarpine (a cholinergic agonist) iontophoresis of the right forearm [80]. It was found that MS patients and matched controls had no differences in the number of activated sweat glands per square centimeter, while MS patients had significantly lower sweating rates and sweat gland output compared to controls. Additionally, a proportion of MS patients undertook a 15-week exercise protocol, and the QSART was repeated with no significant changes compared to pre-exercise results. The authors hypothesize that although they tested postganglionic (i.e., peripheral) sudomotor function the results suggest central etiology of sudomotor dysfunction leading to insufficient sympathetic input to sweat glands and their subsequent adaptations such as reduced cholinergic sensitivity and/or atrophy of sweat glands. This is in line with the findings of diminished pilocarpine-induced postganglionic (i.e., peripheral) sweat response in patients with cervical spinal cord injury regardless of their physical activity status [81]. Our work demonstrated that acetylcholine-induced QSART response is abnormal in over 30% of CIS patients, and abnormalities were detected in both the upper and lower extremities [55]. Abnormal QSART results did not correlate with the presence of brainstem or cervical spinal cord lesions, nor with the total number of T2 lesions [55]. Over 15% of these patients experienced worsening of sudomotor function over a two-year follow-up period, while about 13% of the sudomotor function improved [58]. Similarly, about 35% of RRMS patients with longer disease duration (≈5.5 years) have abnormal QSART results, mostly mild hypohidrosis of the lower extremities [5]. On the other hand, in pwPMS the QSART abnormalities can be found significantly more often (≈73%) compared to relapsing MS phenotype, the abnormalities reflect more severe sudomotor dysfunction (i.e., hypohidrosis detected at multiple sites, and more severe hypohidrosis or complete anhidrosis) [5].

Composite autonomic scoring scale (CASS)

The CASS is a method of generating a quantitative composite score to describe the severity of the autonomic failure [53]. It uses BP response to the Valsalva maneuver and HR and BP responses to HUTT to address the adrenergic cardiovascular dysfunction (adrenergic score), HR response to the Valsalva maneuver (i.e., Valsalva ratio) and deep breathing test (i.e., RSA amplitude) to evaluate the cardiovagal function (cardiovagal score), and the QSART test to evaluate postganglionic sudomotor function (sudomotor score). While the cardiovagal and sudomotor score range is 0−3, the adrenergic score range is 0−4, the reason being that adrenergic failure has a greater effect on the patient. Total CASS score can therefore range from 0 to 10, with scores from 1 to 3 representing mild, scores 4−6 moderate, and scores of 7−10 severe autonomic failure [53]. The testing protocols and normative values are described in detail by Novak [51]. This method is useful since it quantifies the severity of the autonomic failure and can easily be used to monitor its progression.

In our work we found that about 60% of patients with CIS have CASS score >1, with most of the patients (≈54%) having mild autonomic failure (CASS score 1−3), and about 5% of the patients had a moderate autonomic failure (CASS score 4−6) [55]. About 42% of patients had some degree of adrenergic failure, and ≈32% had a sudomotor failure, while mild cardiovagal failure was present in just 5% of patients [55]. The CASS score and its subscores did not correlate with the total number of brain and spinal cord T2 lesions. On the other hand, a correlation between adrenergic score and brainstem lesions was found, but with no statistically significant correlation with specific brainstem parts [55]. In a two-year follow-up study of these CIS patients, we found that about 39% of pwMS experience worsening of autonomic failure expressed as total CASS score, about 26% had improvements in CASS score, and in about 35% there was no change in CASS score [58]. The changes in CASS scores were related to changes in adrenergic and sudomotor scores, while cardiovagal scores did not change during this follow-up period [58]. When comparing the CASS score in pwRRMS and pwPMS we found that progressive MS patients had more pronounced autonomic failure reflected in higher CASS scores, and this difference is mostly related to higher sudomotor scores [58]. The total CASS score and sudomotor score positively correlated with disease duration and the EDSS [58].

Additional methods for assessing the autonomic cardiovascular function in PWMS

Although the standard battery of autonomic tests (those included in CASS) detect AD in pwMS, it is still unclear whether this approach is the most suitable when assessing it in pwMS, especially with regards to the possible interplay of ANS function and other important MS-related parameters (e.g., disease activity). Autonomic dysfunction can occur not only because of the "loss-of-function" type pathology (e.g., neurogenic orthostatic hypotension), but also in the setting of an imbalance in the activity of the sympathetic and parasympathetic arms of the ANS. In other words, the overactivity of one of the ANS branches should also be considered an AD. When using exclusively test included in the CASS methodology, the only finding that in some cases may suggest that the AD is caused by an overactive ANS is POTS detected by the tilt-up test, while all the other findings focus on the diminished activity of a particular ANS branch in response to different challenges imposed. This is particularly important since it has been shown that MS-related lesions in specific brain regions may shift the cardiovascular sympathetic-parasympathetic balance toward an increased sympathetic tone [4]. The importance of sympathetic overactivity in MS is reflected in a finding that POTS was found to be a statistically significant prognostic factor for MS activity (clinical or neuroradiological) [40]. Furthermore, it has been suggested that the use of newer ANS testing methods, such as heart rate variability (HRV) and blood pressure variability (BPV), may be more useful than conventional ANS tests when assessing the AD in pwMS [82,83]. As studies on BPV in pwMS are very limited, this section will briefly review the heart rate variability.

Analysis of the heart rate variability

The sympathetic and parasympathetic nerves are tonically active at rest. Consequently, the heart rate reflects the net effect of vagal and sympathetic outflow toward the heart. Parasympathetic nerves exert their effect more rapidly (<1 second), while the sympathetic outflow enacts its effect at a much slower pace (>5 s) and the effect is longer lasting [84]. Parasympathetic activity leads to the prolongation of R-R intervals in the ECG (i.e., slower HR), while the opposite effect is exerted by sympathetic activity. These facts are exploited in the analysis of heart rate variability (HRV). HRV analysis can be performed on at least 5-minute-long ECG recordings (short-term variability) or on longer recordings like 24 h ambulatory ECG recordings (long-term variability). Details on the methodologies of HRV analysis have been published in 1996 in a seminal paper by a Task Force supported by the European Society for Cardiology and the North American Society of Pacing and Electrophysiology [85]. Numerous methods of HRV analysis exist, but the most frequently used time-domain and frequency-domain power spectrum analysis parameters will briefly be discussed here. In the frequency-domain analysis high-frequency power (HF) is considered a marker of vagal activity, while low-frequency power (LF) is thought to reflect primarily the sympathetic modulation of the heart rate, LF/HF ratio is thought to mirror sympathovagal balance. The LF and HF can be expressed in normalized units (LFnu, HFnu) which represent the relative value of each power component in proportion to the total power minus the very low-frequency power [85]. Two most commonly used time-domain parameters are the standard deviation of normal-to-normal intervals (SDNN), which is an estimate of total heart rate variability (normal-to-normal (NN) intervals represent all RR intervals between adjacent QRS complexes resulting from sinus node depolarizations), and the square root of the mean of the sum of the squares of differences between adjacent NN intervals which is highly correlated to HF spectrum i.e. vagal activity [85]. Currently, HRV analysis is used primarily by researchers because of still lacking consensus on referent values and standardized protocols [86].

A study comparing time and frequency domain parameters of RRMS patients with a healthy control group found that the MS group had significantly lower LF, HF, SDNN and RMSSD, while no differences were found for LF/HF ratio [87]. When RRMS patients were stratified with regards to disease duration cut-off of 5 years, all the analyzed parameters except the LF/HF were significantly lower in the longer disease duration group implying progressive worsening of the autonomic nervous system [87]. Studer et al. [88] performed frequency-domain HRV analysis in 120 MS patients (84 RRMS, 36 PMS (primary and secondary), and 60 healthy controls. They found no significant differences in LFnu, HFnu, or LF/HF ratio between MS (RRMS and PMS) and the control group. Interestingly it was found that patients with disease duration longer than 5 years exhibit higher sympathetic activity (higher LFnu and LF/HF ratio, lower HFnu), and a positive correlation was found for disease duration and LF/HF ratio [88]. The PMS group had a significantly higher LF/HF ratio compared to the RRMS or control group which indicates a predominance of sympathetic activity at rest in this group. Furthermore, the authors report a significantly lower LF/HF ratio in RRMS patients with active demyelinating lesions on postcontrast MRI compared to those with no evidence of active lesions. The authors concluded that the sympathetic system is overactive in PMS patients and defective in active RRMS patients [88]. Monge-Argilés et al. [89] recorded 24 h ambulatory ECG in MS patients and the control group, and they found that LF/HF ratio is lower in the MS group during the experimental supine position recording, while during the rest of the day, MS patients had significantly higher LF/HF ratio indicating sympathetic predominance. Winder et al. [4] used voxel-based lesion-symptom mapping (VLSM) to determine associations between cardiovascular HRV parameters and cerebral MS-related lesion sites and they found an association between increased LF/HF ratio and lesions in left insular, hippocampal, and right frontal inferior opercular regions. The use of HRV and its association to the disease-related parameters in the MS population has been nicely covered in a recent review by Garis et al. [90].

Other autonomic nervous system disturbances and appropriate tests

Bladder dysfunction

Urinary bladder dysfunction has been reported in up to 97% of pwMS [91]. Most of the lesions associated with bladder dysfunction symptoms in pwMS are located in the spinal cord causing disconnection of the pontine micturition center and the parasympathetic sacral micturition center [92], although the suprapontine structures (e.g., cingulate cortex, supplementary motor area) are also likely involved [93]. The related symptoms can be a result of either storage phase dysfunction (e.g., increased frequency, urgency, urgency incontinence, incontinence) or voiding phase dysfunction (e.g., urinary retention, incomplete bladder emptying). The urodynamic studies are utilized to assess bladder dysfunction. In a recent study by Erden et al. [94] who retrospectively analyzed urodynamic test results in 75 MS patients, the authors reported that ≈77% of patients had storage dysfunction, about 81% had voiding dysfunction, while detrusor overactivity and detrusor hypocompliance were identified in ≈74% of patients. A longitudinal study, in which repeated urodynamic tests were performed in pwMS over a 14-year period, found that a significant proportion of patients will develop changes in urodynamic patterns and detrusor compliance suggesting repeated testing may be beneficial in some patients to optimize their clinical management [95].

Bowel dysfunction

In a large MS population survey study 68% of respondents had fecal incontinence and/or constipation, both of which positively correlated with the disease duration and the presence of genitourinary symptoms [96]. The digestive tract is controlled by an intrinsic enteric nervous system which is modulated extrinsically by the parasympathetic nerves (i.e., vagal and sacral (S2−4) nerves) and the sympathetic nerves originating from 5th thoracic through 2nd lumbar spinal cord segments [97]. Bowel dysfunction in the context of MS is most likely associated with lesions of the supraspinal or descending pathways that control the sacral parasympathetic outflow [97]. On the other hand, decreased ambulation may also alter bowel movements. The most used tests to assess the digestive tract ANS function are the gastrointestinal and colonic transit and esophageal and anorectal manometry. Khanna et al. [98] recently reported the results of a retrospective study involving 166 MS patients (111 RRMS, 52 PMS) who underwent measurements of gastrointestinal and colonic transit, and anorectal manometry. The authors reported delayed gastric emptying in 16%, accelerated gastric emptying in 22%, and delayed colonic transit in 7% of the examined patients, while in a proportion of patients with constipation, a higher resting anal sphincter pressure suggested evacuation disorder [98].

Sexual dysfunction

The importance of functional sympathetic and parasympathetic innervation of the genitals for normal sexual function is well known. According to the results of two meta-analysis the prevalence of sexual dysfunction (SD) in men and women with MS is practically identical at about 63% [99,100]. Most common presentations of SD in men with MS are erectile dysfunction (50−70%), ejaculatory and/or orgasmic dysfunction (50%), reduced libido (39%) and anorgasmia (37%), while SD in women with MS most commonly presents as sensory genital dysfunction (61%), difficulty achieving orgasm (24−60%), decreased vaginal lubrication (36%) and reduced libido (40%) [101]. The SD in pwMS can be related to demyelinating lesions [102,103], but other factors related to MS (e.g., fatigue, immobility, spasticity, cognitive impairment, pain, anxiety, depression, psychosocial issues etc.) can also greatly influence the sexual function of these patients [104,105]. When assessing SD in pwMS The Committee 3 of the International Consultation on Sexual Medicine suggests using the Multiple Sclerosis Intimacy and Sexuality Questionnaire (MSISQ-19) and the Multiple Sclerosis−Female (SEAMS-F) questionnaire [106]. In their study, Seçil et al. investigated the genital sympathetic skin (g-SSR) response to assess its potential use as a marker of SD in pwMS [107]. The

authors reported that 50% of MS patients had pathological responses when compared to healthy controls. Moreover, 80% of the patients with severe symptoms of SD had no response on g-SSR [107]. Tests such as penile and vaginal plethysmography, and corpus cavernosum electromyography may be used to assess the sexually related ANS function although the use of these tests in MS population to our knowledge has not yet been documented.

Pupillary motricity disorders

The ANS is directly or indirectly engaged in various functions of the eye (e.g., aqueous humor formation and outflow, ocular blood flow, ocular accommodation etc.), but probably the most studied function is the regulation of the pupil diameter. The pupil diameter is regulated by the parasympathetically innervated sphincter papillae muscle, and sympathetically innervated dilator pupillae. Parasympathetic innervation originates from the preganglionic neurons in the Edinger-Westphal nucleus located in the rostral midbrain, whereas the sympathetic innervation arises from the IML of the C8-Th2 spinal cord segments [108]. Pupillometric studies can discern between afferent and efferent (i.e., regulated by the ANS) pupillary pathways, and can thus provide information about ocular ANS function. Pupillometry has rarely been utilized in pwMS, and various protocols and variables have been used. According to the available data it seems that autonomic pupilar abnormalities are commonly present in pwMS and are more pronounced in primary progressive MS patients [109]. These abnormalities can be detected in up to 15% of RRMS patients with mean disease duration of 6 years [110]. Bitirgen et al. report a significant correlation of alterations in pupillary light reflex responses and neurological disability and retinal axonal loss [111]. De Seze et al. demonstrated a significant correlation between the efferent pupillary pathway defects and the spinal cord atrophy, but the predominance of PMS patients in study sample should be taken into consideration [109]. Of note, pupillometric parameters seem not to be affected by the history of optic neuritis and have not shown correlation to visual evoked potentials findings [109,111,112].

Conclusion

The cause of AD in MS may be related to lesions in specific parts of the central autonomic network (see Fig. 1), and it has been correlated with spinal cord atrophy. Autonomic dysfunction in pwMS is a frequent finding and can even be detected in a large proportion of patients presenting with the first demyelinating event. The current data suggest that AD pattern is variable depending on the phenotype (relapsing-remitting or progressive), disease duration, EDSS, and MS activity (relapses and/or MRI activity). Also, there is evidence that some MS treatments may influence the results of ANS testing. Therefore, we advise that those variables be considered when performing ANS-related studies in pwMS, as well as when analyzing and presenting the data. The standard battery of tests (BP and HR response to Valsalva maneuver and HUTT, and HR response to deep breathing test plus one of the tests for sudomotor function) can detect ANS pathology in a large proportion of MS patients, however other methods such as heart rate variability may provide additional insight into ANS dysfunction in MS, especially in relation to disease activity parameters. The study of autonomic function in the MS population is relevant not only because of the direct influence on the patient's quality of life but also because of the potential autonomic and immunological systems interplay which may be relevant to the pathophysiology and the course of MS.

Declaration of Competing Interest

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