Non-invasive Characterization of Cardiovascular Autonomic Dysfunction in Multiple Sclerosis

Fatemeh Shirbani (*M. Eng*)

A thesis of the Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Macquarie University, submitted in partial fulfilment of the requirements for the degree of Masters of Research.

Supervisor:

Professor Alberto P Avolio

Co-supervisor:

Doctor Edward Barin

May 2016

MACQUARIE University
SYDNEY·AUSTRALIA

Acknowledgement

I would like to thank the people who have contributed to this study. Firstly, I would like to thank my main supervisor **Prof. Alberto Avolio** for his insightful discussion, guidance, motivation and precious help in thesis revisions. I am also deeply grateful to my co-supervisor **Dr. Edward Barin** for providing me with the invaluable advices, the opportunity and the direction of this thesis that without his support and encouragement this study could not be accomplished.

I would like to express my deepest gratitude to **Dr. Mark Butlin** for his wise comments, expert revisions and help in statistical analyses and providing the control group data.

I also would like to thank the MS research group at Royal North Shore Hospital, especially Dr. John Parratt and Dr. Yi-Ching Lee for the opportunity of performing this research in the MS clinic, their medical perspective and providing the clinical information of MS patients.

Finally, I want to express my warmest thanks to my husband for his love, support and helps.

Declaration of originality

I hereby declare that the work presented in this thesis has not been submitted for a higher degree to any other university or institution. To the best of my knowledge this submission contains no material previously published or written by another person, except where due reference is stated otherwise. Any contribution made to the research by others is explicitly acknowledged.

Fatemeh Shirbani

Department of Biomedical Sciences
Faculty of Medicine and Health Sciences
Macquarie University

3rd May 2016

This thesis describes research work conducted across the **10-month period of July 2015 to April 2016,** in partial fulfilment of the conditions of the Masters of Research at Macquarie University.

Declaration of contributions

Blood pressure and heart rate data for the control subjects presented in this thesis were collected by and in collaboration with Dr Mark Butlin, Ms Aarathi Venunadan, Ms Peta Hathway and Mr Ayman AlQahtani. All processing and analysis of these data, as well as the collection, processing and analysis of all data in the Multiple Sclerosis subjects, was conducted by Fatemeh Shirbani.

Ethics approval

All procedures presented in this thesis were conducted under appropriate ethics approval. Study of Multiple Sclerosis patients was approved by the Northern Sydney Local Health District (Ethics Committee reference number: HREC/12/HAWKE/397) with acceptance of this external ethics approval by the Macquarie University Human Ethics Committee (Reference number 5201600002). The study of control (healthy normal) participants was approved by the Macquarie University Human Ethics Committee (reference number 5201300055). Written informed consent was obtained from all Multiple Sclerosis and healthy normal participants.

Publications

Conference Presentations

Shirbani, F.; Barin, E.; Lee, Y.; Ng, K.; Butlin, M.; Avolio, A. P. & Parratt, J. Baroreceptor sensitivity is related to treatment delay in ambulant multiple sclerosis subjects: a non-invasive autonomic function analysis. *High Blood Pressure Council of Australia*, Melb 5-7 Dec,**2015.**

Barin, E.; **Shirbani, F.**; Lee, Y.; Ng, K.; Butlin, M.; Avolio, A. & Parratt, J. Baroreceptor Sensitivity by the Sequence Technique is Retained in Early-Treated but Not Late-Treated Multiple Sclerosis: A Non-Invasive Autonomic Function Analysis. *ECTRIMS Meeting,* **2015**.

Barin, E.; **Shirbani, F.**; Lee, Y. C.; Fontes, A.; Ng, K.; Butlin, M.; Avolio, A. P. & Parratt, J. Non-Invasive Autonomic Function Analysis shows that Disease Duration in Multiple Sclerosis correlates with Spectral Variability of Systolic Blood Pressure, but not Heart Rate or Baroreceptor Function, independently of Age.*ECTRIMS Meeting*, **2016**.

Abstract

Multiple Sclerosis (MS) is associated with autonomic nervous system damaged. Reported cardiovascular autonomic dysfunction (CAD) prevalence in MS varies between studies. As CAD lowers quality of life and may contribute to sudden death in MS, early CAD detection may assist in treatment and in risk identification. A comprehensive suite of cardiovascular autonomic tests was applied to 53 MS patients and results associated with clinical markers of MS severity. CAD was identified through analysis of continuous electrocardiogram and non-invasive finger blood pressure recording during 5-minutes supine rest, short-term deep breathing, Valsalva manoeuvre, orthostatic challenge and isometric exercise. There was greater prevalence of sympathetic (58%) than parasympathetic impairment (34%). Total brain and spine lesions was correlated with dampened sympathetic response in Valsalva manoeuvre and orthostatic challenge. Age corrected score for sympathetic control showed deterioration with longer disease duration and/or treatment delay>10 years. Comparison of a subset of MS patients (n=23) with age and gender-matched controls showed diminished baroreceptor reflex in MS and impaired sympathetic function using frequency domain systolic blood pressure variability analysis, techniques novel to MS investigations. Findings presented in this thesis demonstrate high prevalence of CAD in MS that can be evaluated using a combination of standard and more novel analysis techniques.

Table of Contents

Chapter 1

Introduction and literature review

Over the last 3 decades there has been widespread interest in the study of autonomic dysfunction in Multiple Scleroses (MS) patients. However, while the abnormalities of bladder, bowel and sexual dysfunctions have been well documented, the cardiac autonomic dysfunction is often overlooked. Although impact of cardiac autonomic dysfunction on quality of life is substantial, the contradictory results have left clinical researchers sceptical about the frequency of cardiovascular autonomic dysfunctions in MS, as well as its relation to progress and severity of disease. Therefore, this thesis explores a comprehensive study of non-invasive cardiovascular autonomic test applied to MS patients. In Chapter 1, the relevant physiology of cardiac autonomic nervous system and MS as well as an overview of previous studies are described. Chapter 2 contains a thorough review of cardiac autonomic nervous system testing. Chapter 3 contains methods of this study and the applied statistics. Chapter 4 reports the results which are discussed in Chapter 5, including conclusions made from the findings of this study and suggested future work.

1.1 Autonomic nervous system

The nervous system is categorized into two major parts comprising the central nervous system (CNS) and the peripheral nervous system (PNS). While the CNS consists mainly of the brain and spinal cord, the PNS contains nerves and branches connected to the CNS, and communicates with the rest of body. The PNS itself is divided into two groups of afferent division that transmits sensory information from body to the CNS, and efferent division that transmits signals from the CNS to the body. The efferent division has three parts that mediate voluntary movements (somatic), control gastrointestinal system (enteric) or regulate involuntary body functions (autonomic). Among those, the autonomic nervous system is an involuntary control system that acts unconsciously, but can work in conjunction with the somatic system and is regulated mainly by the hypothalamus in the brain. The ANS regulates body functions such as heart rate, vasomotor activity, cardiac function, digestion, respiratory rate, pupillary response, urination, sexual arousal and reflexes such as coughing, sneezing, swallowing and vomiting[1,2].

The ANS divides into two divisions of sympathetic and parasympathetic nervous systems. The sympathetic nervous system is also sometimes named “flight or fight” system and is responsible for quick responses and stressful situations. The sympathetic nerves start from middle of the spinal cord and usually stimulate organs. For instance, it increases heart rate and blood pressure when necessary. On the other hand the parasympathetic nervous system, designated as a “rest and digest” or “feed and breed” system, functions as a dampening system and usually slows down bodily processes, such as reducing heart rate and blood pressure when necessary. It is associated with conserving energy and restoring tissues for ordinary functions. The parasympathetic nerves usually start from the brainstem or bottom of spinal cord[1,2]. The schematic illustration of sympathetic and parasympathetic nerves and their target organs is shown in Figure 1.1. However, there are certainly some exceptions such as

stimulation of digestion and urination by the parasympathetic nervous system and slowing down by the sympathetic nervous system. Most organs contain nerves from both the sympathetic and parasympathetic systems[1–3].

1.2 Autonomic dysfunction (AD)

When the autonomic nervous system (ANS) does not function properly, it is called autonomic dysfunction (AD) or dysautonomia. The term AD refers to either the failure of the sympathetic or parasympathetic nervous system, or excessive or overactive ANS activity[4]. It often involves organ failure, or the failure of the nerves. Problems with the ANS can range from mild to life threatening and affect only one part (local) or the entire ANS (generalized). Some conditions are temporary and can be reversed, while others are chronic and progressive. AD also may be classified into primary and secondary AD. The primary type of dysautonomia is either when it results from a chronic disease of nervous system degeneration, or without known common pathology[1]. Multiple system atrophy, orthostatic hypotension, pure dysautonomia and Parkinson's disease are all types of primary AD. On the other hand secondary dysautonomia may occur due to injury of the ANS from a disorder such as diabetes, alcoholism or multiple sclerosis[1,4].

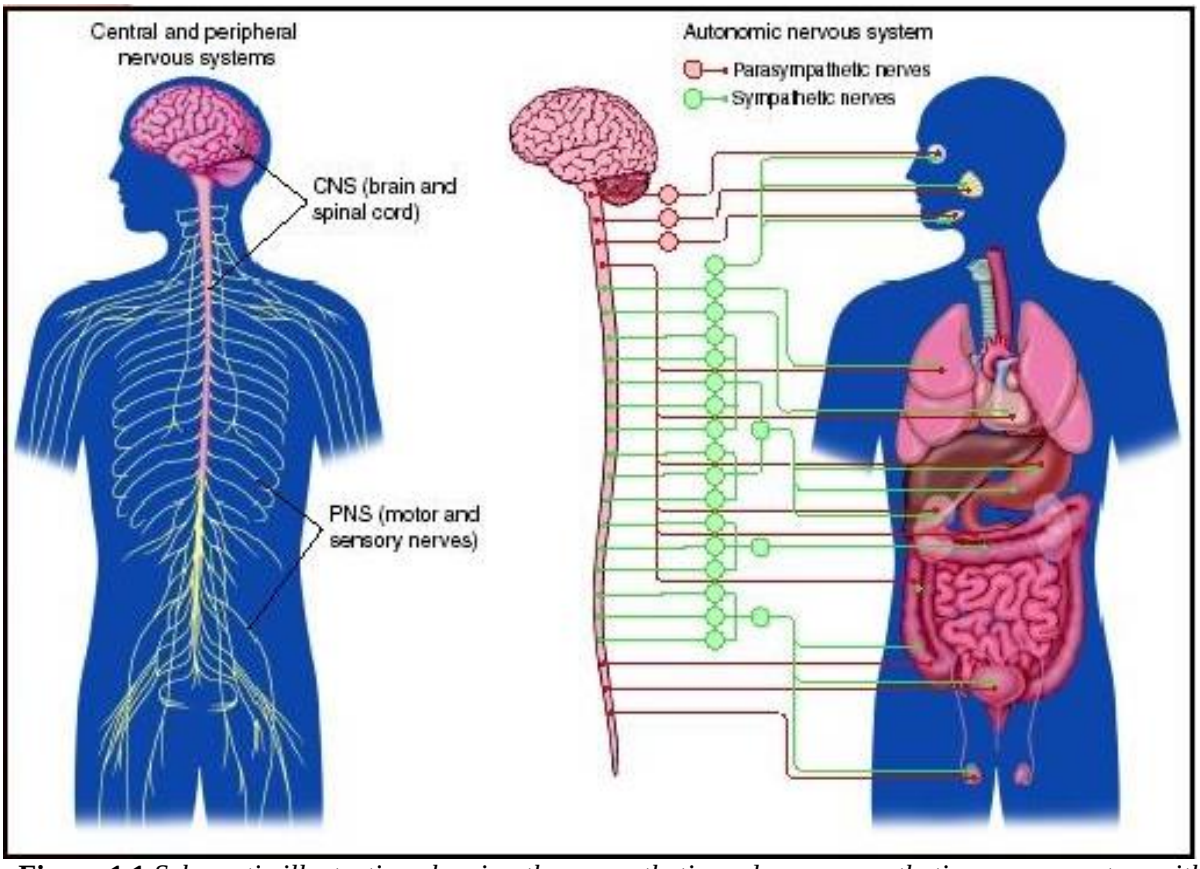


Figure 1.1 *Schematic illustration showing the sympathetic and parasympathetic nervous system with their target organs*[5].

1.3 Symptoms of autonomic dysfunction

Most forms of autonomic failure start with mild symptoms at their onset and are concealed for years because of autonomic or other compensatory mechanisms. The general symptoms of AD may include the following problems and their affects may vary from mild to severe:

a) Orthostatic hypotension (orthostatic intolerance) is a consequence of blood pressure drop at standing. This symptom is usually accompanied by Postural Orthostatic Tachycardia Syndrome (POTS) that is associated with excessive tachycardia and heart palpitation and may result in fainting or light headedness.

b) Exercise intolerance is a term used for inability to alter heart rate with exercise.

c) Sweating abnormalities that could be excessive or insufficient sweat.

d) Slow digestion leading to problems of losing appetite, bloating, diarrhoea or constipation, and difficulty swallowing.

e) Urinary problems include difficulty starting urination, and incomplete emptying of the bladder.

f) Sexual problems in men, identified by difficulty with ejaculation and/or maintaining an erection. In women, it is associated with vaginal dryness and/or difficulty with orgasm.

g) Vision problems include blurry vision, or inability of the pupils to react to changes in light appropriately[1,6].

1.4 Assessment of autonomic dysfunction

The major reasons to assess autonomic function in an individual with a suspected condition are:

a) To identify whether autonomic function is normal or abnormal.

b) To determine the degree of dysfunction and the site of the lesion.

c) To classify the type of AD whether it is of primary or secondary disorders as the prognosis and treatment will depend on the diagnostic category.

d) To investigate the underlying pathophysiological processes (in order to develop novel treatment), and effect of stimuli in daily life on autonomic responses to ensure comprehensive management for AD[1].

In order to assess the autonomic nervous system, usually a set of simple, non-invasive tests are designed to provide reproducible and sensitive information relevant to known physiological functions. The autonomic nervous system is usually tested by evaluating a

reflex arc that involves a stimulus, a receptor, an afferent nerve, central processing, an efferent nerve and an end-organ response. When a standard stimulus is applied, and the normal end-organ function is demonstrated, then by considering all confounding variables and innervations, the autonomic nervous system can be tested. Since in one simple test many organs are dually innervated (such as balance system of sympathetic and parasympathetic pathways), the autonomic nervous system needs to be evaluated recognizing that the result may reflect a decrease in one pathway or an increase in another[7].

1.5 Treatment of autonomic dysfunction

There is no treatment for the primary type of AD, but for the secondary type usually treatment involves curing related symptoms and the underlying condition. However, the damage that had already being affected is difficult to cure. For a symptom such as orthostatic hypotension, for instance, the patients are told to change their lifestyle including taking enough fluid in the daily diet, wearing compression stockings, and elevating the head position in bed. Medications can also be advised. In addition, if the underlying conditions such as alcoholism, diabetes or Parkinson's could be treated, it helps to decrease the AD problem or prevent its progress. For the other major problems caused by AD, physical therapy, walking aides, feeding tubes or other methods are advised[7–9].

1.6 Cardiovascular autonomic nervous system

The cardiovascular autonomic nervous system (CANS), as one of the most important parts of the autonomic system, has a major role in homeostasis, which is largely achieved by adjusting blood flow to different vascular beds in proportion to the level of their metabolic activities. To maintain arterial pressure within relatively fine limits, cardiac output and arterial vascular resistance in different conditions are controlled according to the organs' feedback; such as

arterial pressure and blood gas level[1–3].For instance, in digestion or exercise time, the blood flow to the gastrointestinal tract or to skeletal muscles increase relatively[1].

The cardiovascular control centre (CCC) located in the medulla oblongata (in brainstem), is responsible for autonomic control of heart rate, blood pressure and breathing. The control operation is performed by means of sympathetic and parasympathetic pathways. Sympathetic neurons that influence cardiac activity are localized in the upper thoracic segment of the spinal cord (T1-T4) and the parasympathetic neurons are located in the ventrolateral medulla[10]. The map of cardiac sympathetic and parasympathetic neurons is shown in Figure 1.2.

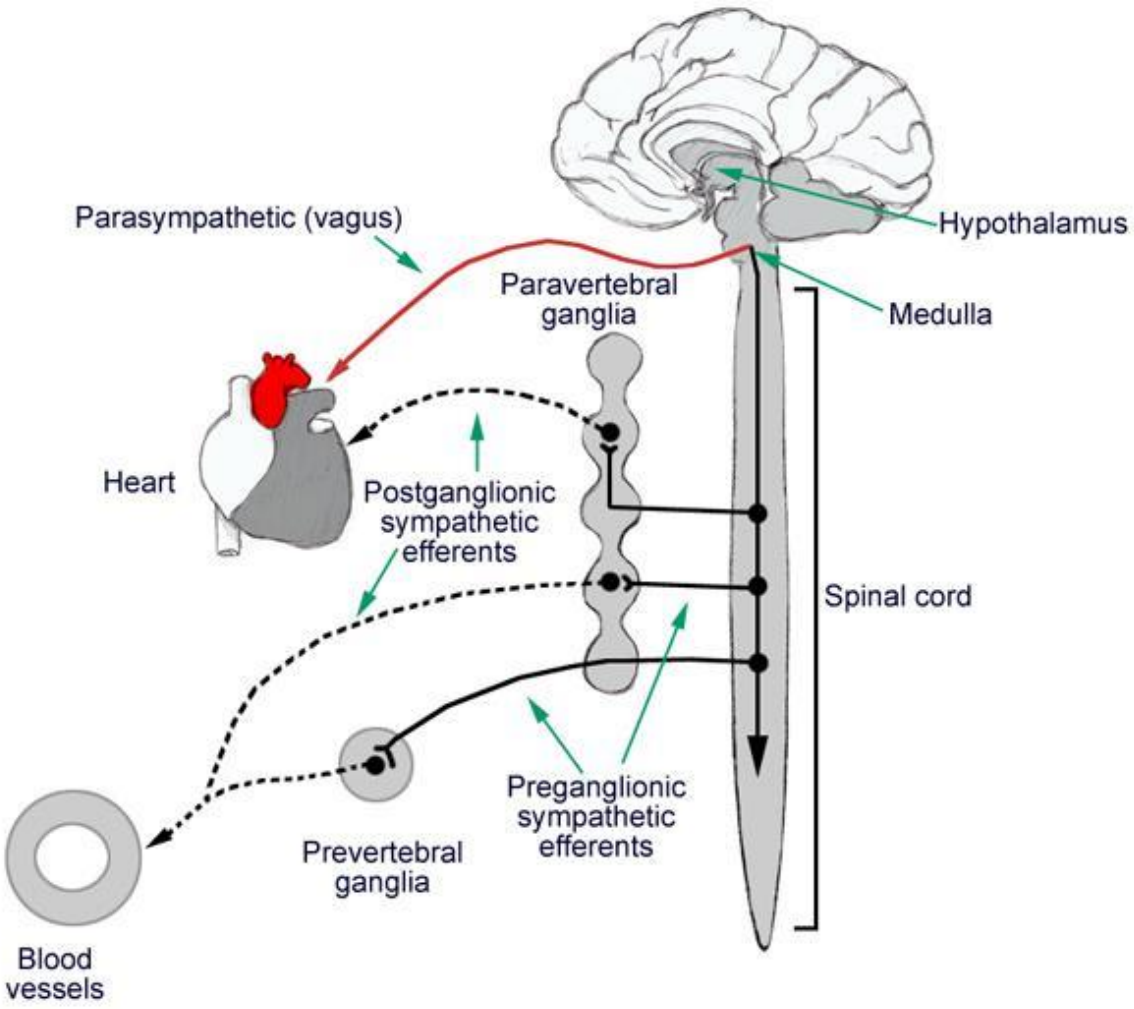


Figure 1.2 *Cardiac sympathetic and parasympathetic systemsmap*[11]

1.7 Control of heart rate and blood pressure

Depending on the body's demand, heart rate is mainly controlled by either direct control or neural innervations (neural control). In direct control of heart rate, when a certain change in body activity or body condition occurs, the change is detected by related receptors, and the Sino Atrial node (SA node) is directly stimulated to increase or decrease heart rate.

Hormonal control and thermal control are two kinds of direct control of heart rate. One example of hormonal control is in situations of fear or stress that the hormone adrenaline is secreted by the adrenal glands, which are above the kidneys, and released into the blood stream. This causes changes in the conductance of pacemaker cells in the SA node, accelerates the action potential occurrence, and consequently heart rate increases. The opposite of the above condition happens by releasing acetylcholine to decelerate the action potential in the SA node, and so decrease heart rate. Furthermore, by doing exercise, muscle movements raise body heat and heart temperature. This augmentation in temperature is sensed by thermoreceptors and then heart rate increases. The reverse process also occurs in case of reduction in body temperature[1,12,13].

On the other hand, the control methods by chemoreceptors, proprioceptors and "baroreceptors" are named as neural innervations for controlling heart rate. The chemoreceptors located in the aorta and central chemoreceptor centre (with direct connection to CCC), are able to sense the state of chemical balance in blood. In exercise for example, rise in the level of CO_2 and fall in the level of O_2 is followed by reduction in blood pH level. This is detected by chemoreceptors and followed by increase in heart rate. Also, tension in muscles is detected by proprioceptors, fibres in muscles with the ability of sensing muscle contraction.

The feedback signal is then sent to CCC to increase SA activation and accelerate heart rate, or to decrease heart rate if tension is removed[1,13,14].

1.7.1 Baroreceptor and baroreflex sensitivity

The arterial baroreflex system is one of the important neural mechanisms for blood pressure control by means of baroreceptors. They play a major role in preventing short term wide fluctuations of arterial blood pressure by providing a continuous stream of information about BP changes to the CNS[15,16]. Baroreceptors are mechanoreceptors located mainly in the walls of aortic arch and internal carotid artery; they are sensitive to stretch and are able to detect blood pressure changes and thus initiate baroreflex. When there is an increase in blood pressure, it is followed by increase in diameter of arteries (stretch). This leads to deformation of baroreceptors and so detection of changes in blood pressure. The reflex signal (action potential) is then sent and the CCC, commands increase in parasympathetic stimulation and decrease in sympathetic stimulation of the heart. The command is transferred via parasympathetic fibres (vagus nerve) to release acetylcholine at the SA node. The permeability of the pacemaker cells to K^+ ion then rises resulting in less frequent occurrence of action potential occurrence and in slower heart rate. Therefore, reduced sympathetic stimulation results in lower cardiac output and parasympathetic stimulation leads to lower peripheral resistance and blood pressure[16,17].

In case of detection of a fall in blood pressure, the correction signal from CCC is sent via the sympathetic nerve commanding sympathetic activation and parasympathetic inhibition. In this case, norepinephrine in the SA node is released to increase permeability to Ca^{2+} and Na^+ ions. The flow of positive ions inside increase pacemaker cells potential and heart rate and cardiac output increases as a result (sympathetic activation). Also, parasympathetic inhibition is followed by increase in systemic vascular resistance (SVR) and blood pressure[15–17]. The feedback loop of baroreceptors is illustrated in Figure 1.3.

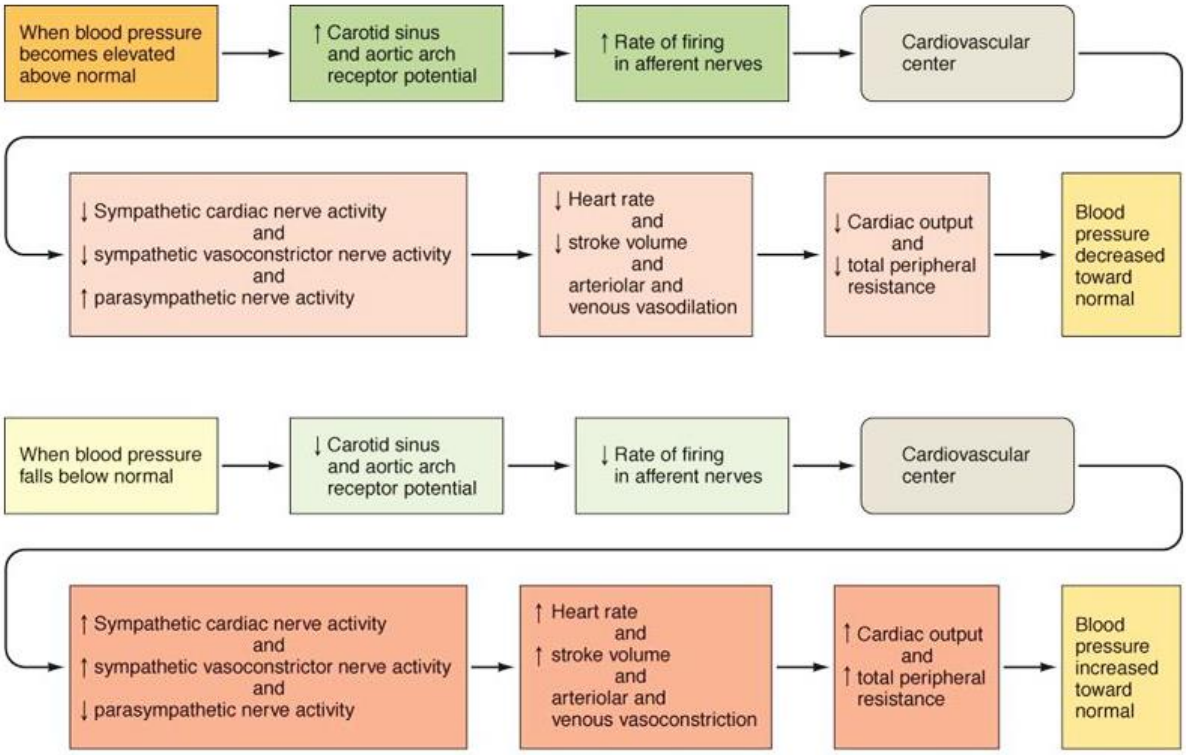


Figure 1.3 *Baroreflex loop* [18]

1.8 Cardiac autonomic dysfunction (CAD)

Cardiac autonomic dysfunction (CAD) is any abnormality in autonomic control of heart rate and blood pressure. This type of dysautonomia is usually accompanied by the main clinical symptoms of fatigue, pathological responses to orthostatic challenge such as syncope, palpitation, dizziness, nausea, general weakness, hot flashes and sweating[19].

1.9 Assessment of autonomic nervous system

CANS assessment usually involves a set of reflex hemodynamic tests and monitoring Heart Rate (HR) and Blood Pressure (BP) changes as response. The common tests include:

a) Short term deep breathing: deep breathing for 1 minute with the rate of 6 breath/minute.
b) Orthostatic challenge: active or passive standing from supine position.
c) Valsalva manoeuvre: short term increase in the thoracic pressure by blowing out through a blocked mouthpiece.
d) Sustained hand grip: squeezing a hand grip for 3-5 minutes for 30% of maximum strength.

Apart from the reflex tests above, spontaneous changes of HR and BP in 5-minute supine position are also calculated in both frequency and time domains. Tests for CANS assessments are explained in detail in Chapter 2.

1.10 Nervous system disorders

Different known and unknown factors can cause nervous system disorders. They can be categorised into vascular disorders, such as stroke; infections, such as meningitis; structural disorders, such as brain or spinal cord injury, brain or spinal cord tumours; functional disorders, such as headache, epilepsy, and dizziness; degeneration, such as Parkinson disease, multiple sclerosis, amyotrophic lateral sclerosis (ALS), and Alzheimer disease.

1.11 Multiple Sclerosis disease

Multiple sclerosis (MS) is a chronic autoimmune, inflammatory neurological disease of the CNS[20].The word "sclerae" means scars or better known as plaques or lesions. In MS disease, the loss of oligodendrocytes, the cells responsible for creating and maintaining myelin sheaths in the CNS, results in thinning or complete loss of myelin, and as the disease advances, there is breakdown of the axons of neurons [20,21].

Myelin as a fatty white layer around the axons has a critical role in providing support, nutrition and insulation for the neurons and it enables signal transmission through the nervous system[20,21]. A neuron is depicted in Figure 1.4. When a neuron receives a stimulus, the positive ions flow inside the neuron via voltage gated ion channels in dendrites. Then the highly concentrated positive ions repel and move along the axon till reaching equilibrium (electrotonic conduction). This method of signal transmission is fast, but as illustrated in Figure 1.5, it is dissipating in time and space. Thus, the Nodes of Ranvier as unmyelinated parts of axons with the activatable ion channels, in between myelinated parts, have the role of boosting the signal (by action potential) and transmit it through the next myelinated part[3,22].

However, the voltage of the attenuated signal should be still higher than a threshold to trigger channels in the Nodes of Ranvier. When the myelin which has high electrical resistance is lost or becomes thinner, the signal is dissipated and the neuron can no longer effectively conduct electrical signals (Figure. 1.6)[3,21,22].

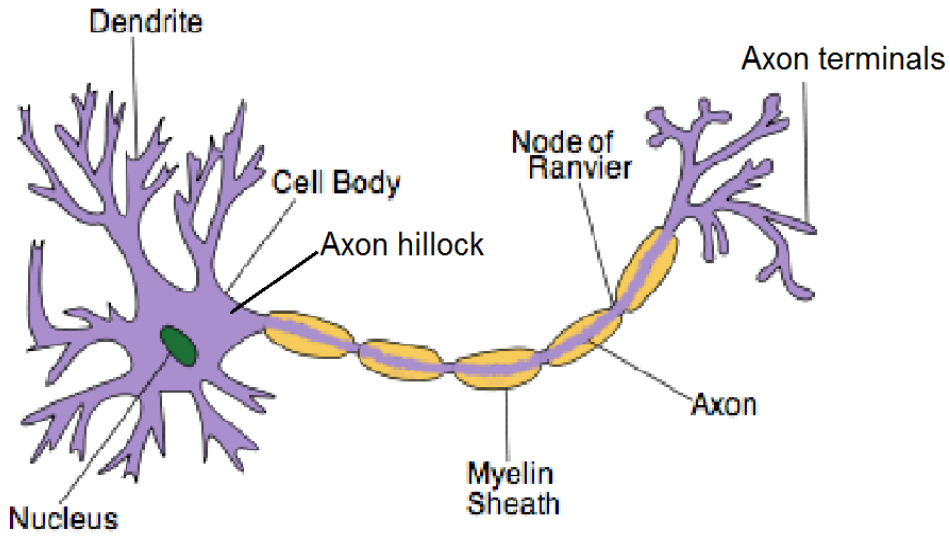


Figure 1.4 *Neuron schematic*[22].

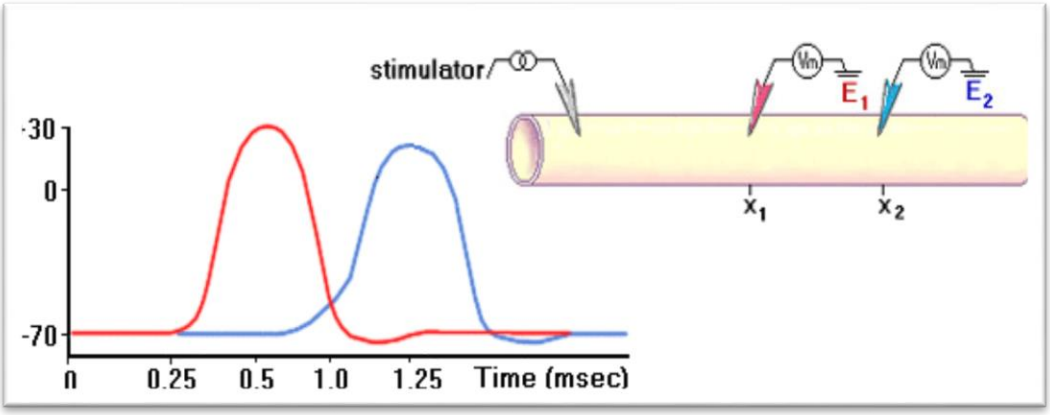


Figure1.5 *Conduction of action potential in a myelinated axon*[23].

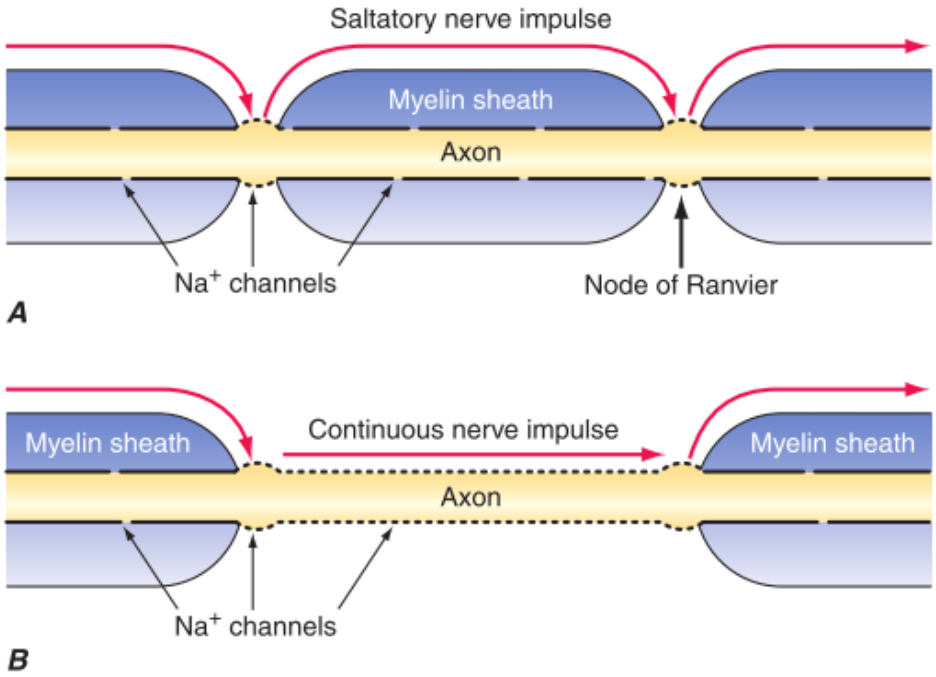


Figure1.6 *Signal transmission in myelinated and demyelinated axon* [24].

In multiple sclerosis, a repair process, called remyelination, takes place in early phases of the disease, but the oligodendrocytes are unable to completely rebuild the cell's myelin sheath[21].

Apart from demyelination, inflammation is another sign of MS. The inflammatory process is caused by T cells which area type of lymphocyte for the body's defences. The T cells gain entry into the brain via disruptions in the blood–brain barrier (BBB) and recognize myelin as a foreign agent and attacks it. Inflammation causes tissue damage, named lesions. These lesions most commonly affect the white matter in the optic nerve, brain stem, basal ganglia, and spinal cord, or white matter tracts close to the lateral ventricles[25]. The peripheral nervous system is rarely involved. These lesions are hardened by scar tissue as a result of repeated attacks, named plaques. When these plaques are built up around the damaged axons, the ability of parts of the nervous system to communicate is disrupted, resulting in a wide range of symptoms[21].

1.11.1 Epidemiology of Multiple Sclerosis

Multiple Sclerosis (MS), as the most common demyelinating disease of the central nervous system (CNS), is the primary cause of neurological disability in young adults (20-45 years old)[26]. In 2010, the number of people with MS was about 2.5 million globally. About twice as many women are affected as men with the age distribution of 7 females(6 patients in20-30, 1 patient in 40 years old) and3 males (1 patient in 20-30, 2 patients in 35-45 years old) out of 10 patients[20]. Also MS is distinctly more common in white and Hispanic people compared to Asians and African people. In Figure.1.7 the distribution of MS around the world is illustrated[27].

1.11.2 Causes and risk factors of Multiple Sclerosis

Although the underlying mechanism of MS is thought to be either destruction by the immune system or failure of the myelin-producer cells, the exact cause of MS is not clear but a number of risk factors are shown to contribute to MS such as viral (e.g. Epstein - Barr virus), autoimmune diseases (thyroid, Type 1 diabetes, inflammatory bowel disease), genetic /family history, race, low level of vitamin D, smoking and cold climate[26].

Figure 1.7 *Distribution of Multiple Sclerosis disease*[28].

1.11.3 Types and severity of Multiple Sclerosis

Neurologists describe 4 distinct categories of MS based on the course of disease:

1. Relapsing Remitting MS (RRMS) is the most common type, as about 85% of MS patients start from RRMS in their late 20s. It is described by a number of attacks (relapses or exacerbations) of symptoms followed by periods of remission (recovery), when symptoms recover or disappear[26].

2. Secondary Progressive MS (SPMS) is a type of MS in which the disease course continues to worsen with or without periods of remission or levelling off of symptom severity (plateaus). This type may develop in many patients with relapsing–remitting disease that is illustrated in Figure 1.8. However, treatment with disease-modifying agents helps to delay such progression.
3. Primary Progressive MS (PPMS) affects approximately only 10% of MS patients and is more resistant to the MS drugs. In this type symptoms continue to worsen gradually and there is no spontaneous recovery from the initial phase of the disease [26]. There are no relapses or remissions, but there may be occasional plateaus. It can also be a second stage of relapsing remitting type.
4. Progressive Relapsing MS (PRMS) is a rare form of MS, affecting fewer than 5% of patients. It is progressive from the start, with intermittent flare-ups of worsening symptoms and there are no periods of remission[20].All four types of disease are displayed in Figure 1.9.

Also MS may be categorised based on the localization of lesions or topography of lesions such as dwMS (deep white matter MS), gMS (generalized MS), bcMS (brain stem/cerebellar MS) and sMS (spinal predominant MS)[29].

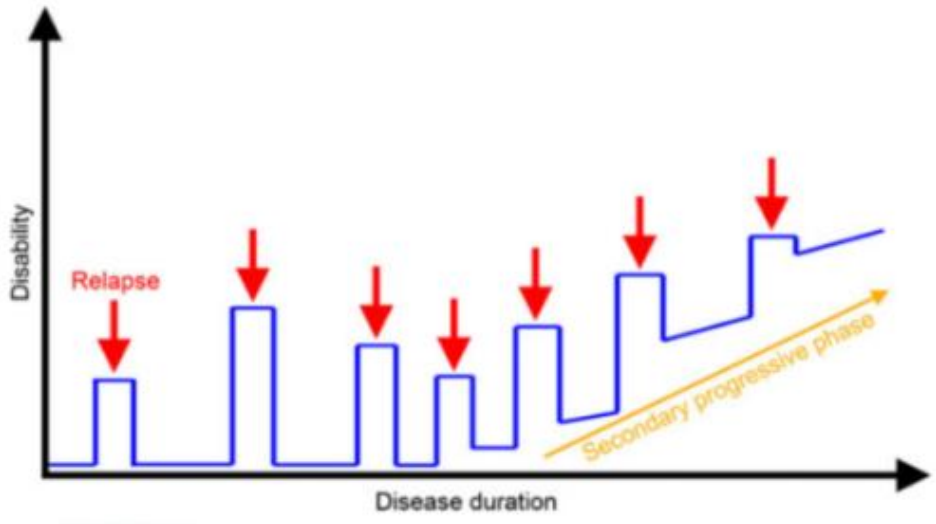


Figure1.8 *Progression of disease type from RRMS to SPMS*[26].

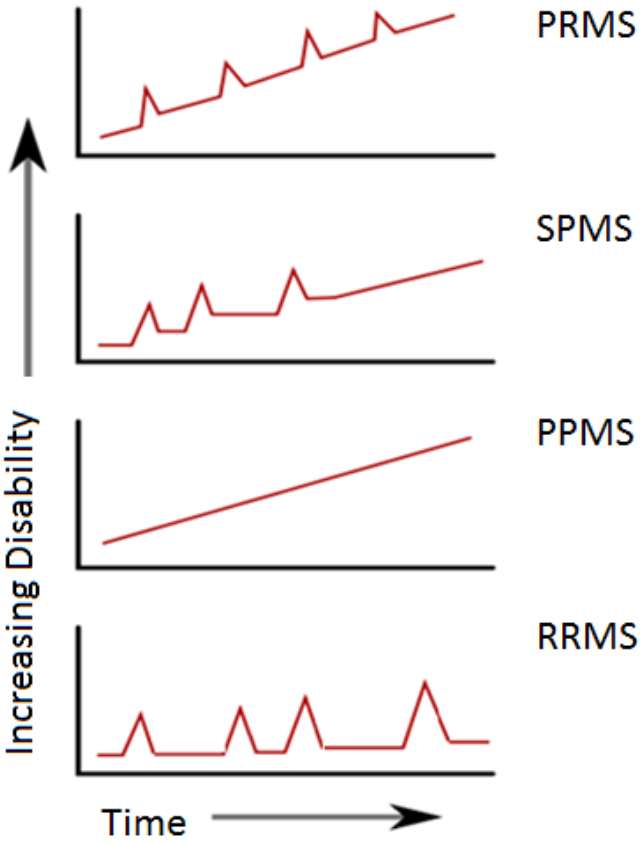


Figure1.9 *Four types of MS based on the course of disease* [30].

1.11.4 Symptoms of Multiple Sclerosis

Depending on the location and severity of lesions, a person with MS can have almost any neurological symptoms or signs, with autonomic, visual, motor, and sensory problems. In most patients, the disease is characterized initially by episodes of reversible neurological deficits, which is often followed by progressive neurological deterioration over time. Usually the first symptoms are recovered quickly, thus the initial symptoms are usually overlooked. The condition begins in 85% of cases as a clinically isolated syndrome over a number of days with 45% having motor or sensory problems, 20% having optic neuritis, and 10% having symptoms related to brainstem dysfunction, while the remaining 25% have more than one of the previous difficulties[20].

The specific symptoms are determined by the locations of the lesions within the nervous system, thus many of them are not unique to MS and can happen in other neurological diseases. These symptoms include: (i) vision problem (gaze difficulty, double or blurred vision); (ii) weak, stiff or painful spasm of muscles; (iii) loss of sensitivity or changes in sensation such as tremor, tingling or numbness; (iv) dizziness, (v) bladder control problem or bowel difficulties, (vi) gate or balance problem, (vii) slurred speech or swallowing problem, (viii)later symptoms (mental or physical fatigue, depression or euphoria, inability to multitask or concentrate effectively, difficulty making decisions and planning)[26,30].

Relapses are usually not predictable, occurring without warning. Exacerbations rarely occur more frequently than twice per year. Some relapses, however, are preceded by common triggers and they occur more frequently during spring and summer. Similarly, viral infections such as the common cold, influenza, or gastroenteritis increase their risk. Stress may also trigger an attack. Symptoms may develop in several days or in some people takes longer, may be recovered quickly or last for months[20,26,30].

1.11.5 Diagnosis of Multiple Sclerosis

Usually no single test is used for definite diagnosis of MS, especially when the patient is seeking diagnosis after experiencing the initial symptom[24]. Tests include:

a) Blood test is usually required for identification of infectious or inflammatory diseases.
b) Magnetic resonance imaging (MRI) of the brain and spinal cord is the best initial test and most accurate one. MRI scans of brain or spinal cord images are taken before and after injection of a contrast agent (such as Gadolinium). In lesions or plaques with active inflammation of MS, blood brain barrier (BBB) disruption exists and thus contrast agent would leak into these lesions. So by comparing to injection free MRI scans, areas of demyelination may be distinguished. An example of comparison between 2 brain MRI images of a healthy individual and MS subject with brain lesions is displayed in Figure 1.10.

c) Evoked potential test is done by sending electrical signals and measure the accuracy and speed of the nervous system in responding to the stimulation. Since the nervous system in MS may responds less actively to stimulation of the optic nerve and sensory nerves due to demyelination of such pathways, the brain responses can be examined using visual- and sensory-evoked potentials.

d) Lumbar puncture or spinal tap is another test to obtain samples of cerebral spinal fluid (CSF). The CSF is tested to find oligoclonal bands of IgG (Immunoglobulin G) antibodies on electrophorese which is found in CSF of 75-85% of MS patients.

However, the international panel on the diagnosis of MS is based on evidence of (1) at least two separate lesions (plaques or scars) in the white matter of the CNS (perform MRI and spinal tap); (2) at least two different episodes in the disease course that the damage areas developed at least one month apart; (3) chronic inflammation of the CNS, as determined by analysis of the CSF. The presence of one or more of these criteria allows a general diagnosis of MS, which may be refined according to the subsequent course of the disease[20].

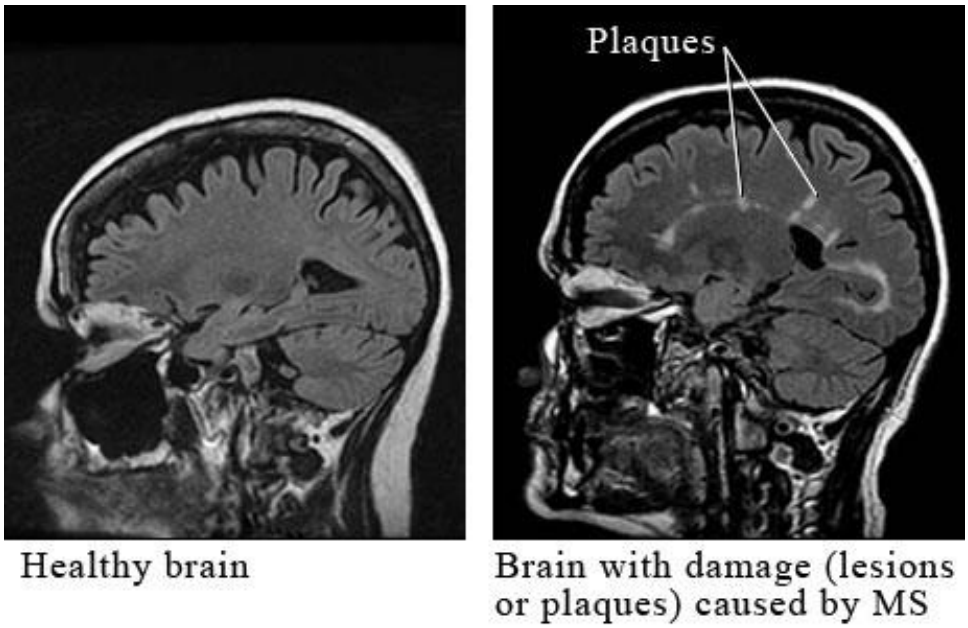


Figure 1.10 *MRI scans of Healthy Brain and MS brain with lesions*[31]

1.11.6 Treatment of Multiple Sclerosis

There is no curative, FDA-approved therapy known for MS. However, a number of medications can be used to treat the disease symptomatically. Generally, MS drugs try to treat symptoms, treat attacks, and reduce the number of attacks, slow disease progression and improve function after an attack. However, research show that over 50% of people with MS may use complementary and alternative medicine such as yoga, vitamin D, herbal medicine, diet change, oxygen therapy and relaxation. The evidence for the effectiveness for such treatments in most cases is weak or absent. MS medications are listed in Table 1.1 [20].

Table1.1 *Drugs categories for treating MS*[20]

Table 1 FDA-Approved Disease-Modifying Agents for the Treatment of Multiple Sclerosis		
Drug	**Brand (Manufacturer)**	**Dosing Frequency**
Interferon beta-1a	Avonex (Biogen Idec)	Once weekly
Interferon beta-1a	Rebif (Pfizer)	Three times weekly
Interferon beta-1b	Betaseron (Bayer)	Every other day
Interferon beta-1b	Extavia (Novartis)	Every other day
Glatiramer acetate	Copaxone (Teva)	Once daily
Mitoxantrone	Novantrone (EMD Serono)	Short infusion (about 5 to 15 minutes) every 3 months
Natalizumab	Tysabri (Biogen Idec)	1-hour infusion every 4 weeks
Fingolimod	Gilenya (Novartis)	Once daily

1.11.7 Prognosis of Multiple Sclerosis

The main measure of disability in MS patients is the Kurtzke Expanded Disability Status Scale (EDSS). The EDSS quantifies disability in eight Functional Systems (FS) and allows neurologists to assign a Functional System Score (FSS) in each of these. The FS include pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, cerebral and other. The EDSS scale which can be from 0 (normal neurological exam) to 10 (death due to MS) with

steps of 0.5 score, allows worldwide standardisation of neurostatus scoring. EDSS steps 1.0 to 4.5 refer to people with MS who are fully ambulatory. EDSS steps 5.0 to 9.5 are defined by the impairment to ambulation. The expected future course of the disease depends on the subtype of the disease, the individual's sex, age, initial symptoms and the degree of disability.[32].

Another informative score is Multiple Sclerosis Severity Score (MSSS) simply mathematically calculated from EDSS, and shows the progress of disease disability based on disease duration. MSSS corrects EDSS for duration of disease by comparing an individual's disability with the distribution of scores in subjects having equivalent disease duration. The reference database is from research done on 9892 MS patients[33].

1.12 Autonomic dysfunction in Multiple Sclerosis

One of the neurological problems in MS patients is autonomic dysfunction (AD). In fact, since the pathophysiology of MS is characterized by dissemination in space, as well as in time, the autonomic nervous system is inevitably damaged in the course of the disease. Also the proportion of affected patients is reported to increase with disease duration[34]. AD can be represented in different types. The most common type of AD in MS patients is fatigue which is reported in up to 90% of MS patients. However, it is arguable whether this symptom is linked to sympathetic vasomotor dysfunction with normal cardiovagal activity or impairment of sympathovagal balance with decreased parasympathetic function and a normal to low sympathetic activity [35,36].

Another common AD in MS is bladder dysfunction infecting approximately 75% of patients. The symptoms of this dysautonomia include urinary urgency and increased frequency, and emptying problems. Those symptoms represent the first manifestation of MS in the 5–9% of patients[8].Bowel dysfunction is also common with more than 70% presence in MS patients.

Bowel discontinuous, diarrhoea and constipation are the general symptoms of this type of AD. From those, constipation is a result of both decreased mobility and disease exacerbation. Also medications taken may alter bowel movement and result is constipation[34,37,38].Sexual dysfunction also affects 50-80% of MS patients. This type of AD often happens to be overlooked or underestimated as a symptom and therefore denied the possibility of treatment. The most common symptoms are erectile dysfunction in men and diminished lubrication and reduced ability to experience an orgasm in women[8,34].

Sleeping related difficulties are present in more than 50% of MS patients. This type of AD which can vary from moderate to severe sleep problems, may lead to increased fatigue, respiratory dysfunction, aggravation and deteriorate patients' perception of well-being. The most common symptoms include difficulty initiating and maintaining sleep and frequent awakenings because of leg muscle spasm, pain, and breathing disturbance. Treatment of sleep disorder is by short-term use of medications with education on sleep hygiene, relaxation techniques and behavioural therapy.

MS disease is sometimes accompanied with impairment of sweat gland function. This type of dysautonomia which is named sudumotor dysfunction is an under-investigated area in MS. Heat is known to worsen MS symptoms; this may be due to the impairments in autonomic control of sudomotor function and a consequence of abnormal sympathetic activity. Since the symptoms of dysfunction in thermoregulation can be long-lasting, avoiding of prolonged exposure to heat is strictly recommended to all MS patients[8,34].

1.12.1 CAD in Multiple Sclerosis

Cardiovascular autonomic dysfunction (CAD) in MS is one of the important types of AD in MS but has been studied less than the other types of dysfunctions. A meta-analysis surveyed 3125 studies of cardiac dysfunction in MS, published from 1984 to 2013[39]. Reviewing of 16

eligible studies from those with 611 patients, reported varying range of 0-76% prevalence of CAD in MS with the sample size ranges of 10-65 patients. Thus, while the abnormalities of bladder, bowel and sexual dysfunction have been well documented, the frequency of CAD in MS is not well known[34,39,40].

One reason of CAD prevalence uncertainty in MS is the definition of abnormality based on the minimum number of abnormal tests in CANS assessment. If at least two pathological cardiac autonomic tests are considered as abnormal, the prevalence is as low as 19%, however with the definition of at least one abnormal test, the prevalence of CAD in MS is 42%. The symptoms of CAD in MS also include orthostatic intolerance, POTS and vasovagal syncope. Cardiac arrhythmias also may appear as a consequence of CANS dysfunction caused by MS[39].

Another reason could be unclear definition of CAD in MS. Although POTS, orthostatic intolerance and fatigue are linked to CAD, because this type of dysfunction is mostly asymptomatic, CAD could be hidden for years if not assessed very well[41].

Finally, studies include a wide spectrum of MS patients (in terms of different severity and progress scores, type of disease, and location of lesions). Therefore, cardiovascular autonomic function is not assumed to be consistent.

1.12.2 Overview of previous CANS studies for Multiple Sclerosis

In the studies done for assessment of CANS in MS, the main clinical symptoms and complaints are found to be fatigue, orthostatic intolerance (palpitation, nausea, syncope, dizziness), weakness, hot flashes and sweating[19]. However, test results were not consistent in different studies. For example, abnormal blood pressure in standing test found with varying range of 0-25% compared to controls[8,42–44]. Abnormal heart rate changes in standing was present from 15% to 28% of subjects[40,43]. Deep breathing test also showed abnormalities in

range of 17%-36% of subjects[38,44,45]. The highest range of abnormalities was found for hand grip test with almost consistent range of 40%-43% of subjects[8,38]. On the other hand, Valsalva test showed hardly any significant abnormality in comparison with controls, however, it was reported to be significantly different in active and inactive MS disease[37].Other parameters such as supine Heart Rate Variability (HRV) in time domain and supine and standing HRV and Systolic Blood Pressure (SBP) variability in frequency domain were calculated in both MS and control groups. Nevertheless, opposite results were reported in previous studies as significantly different or similar results in comparison with controls[36,44,45]. Furthermore, baroreceptor sensitivity calculation with neck suction stimulation and frequency technique differed significantly between MS and control, while sequence technique and backward tilt table test for BRS showed varying significant or no significant differences[40,46,47]. However, CAD in MS is usually identified by the number of abnormal results in patients.

Other studies attempted to link cardiac autonomic dysregulation with the location of lesions. Some simply explained each type of autonomic dysfunction by presence of lesions in regions responsible for autonomic regulation, such as nuclei in the periventricular region of fourth ventricle in the brainstem as well as medullar lesions[34,39]. For instance, brainstem lesions are identified as the feasible morphological substrates of CAD. In contrary other groups showed association of total midbrain lesion volume and total parietal lesion volume with CAD[39].

However, the majority of studies surveyed the trend of sympathovagal abnormalities for MS patients. Previous studies agreed on the fact that both sympathetic and parasympathetic parts, albeit not always simultaneously, were compromised in MS[34,39]. It has been suggested that parasympathetic dysfunction correlates with disease progression (restless worsening) and EDSS score, and its impairment could be the consequence of MS[39]. Sympathetic dysfunction, on the other hand is shown to be associated with clinical activity of MS and may even have a pathogenetic role in the development of MS[19,34,37,39].

For assessment of predominantly parasympathetic function the most common tests include heart rate responses to the Valsalva manoeuvre, deep breathing, orthostatic challenge and cutaneous axon reflex test. On the other hand, blood pressure alterations in orthostatic challenge and sustained hand grip estimate predominantly sympathetic function. Other assessment of CANS include heart rate variability in both time and frequency domain, baroreflex estimation and muscle sympathetic nerve activity that are suggested to provide evaluation of more subtle deterioration of cardiovascular autonomic function[35,40,46,48–50].

1.12.3 Importance of CANS assessment

Since CANS evaluation studies do not always lead to the consistent results (in terms of rate of CAD and abnormal variables), and because of the critical importance of CANS assessment in MS, there is still need for further research in this field. Some of the main important reasons for CANS study include the following:

a) CAD caused by the lesions in cardio-respiratory control centre may be the reason for sudden death in MS patients. A study done on 50 unexpected deaths in MS, found 9 cases with only MS-related cause of death (other causes were suicide, accidents). Among those, although the mechanism of death was uncertain, demyelisation lesions of neural structures that control cardiovascular and/or respiratory functions (hypothalamus and brainstem) were reported to be the reason for death[51]. Also, the cause of sudden death for another case with RRMS was clearly linked to the sudden onset of cardiac arrhythmias attributed to active MS lesions in medulla[52]. In addition, active inflammation of the medulla in progressive MS accounted for sudden death in a patient, due to respiratory failure as a consequence of active inflammation in the medulla[53].
b) Cardiac dysfunction is usually asymptomatic and hidden at the first stages. Therefore, if it is not thoroughly assessed, CAD is not detected in MS until there is significant

impairment of the autonomic system. The symptoms of CAD usually become evident when the disease progresses, thus it will add to the patient's disability level and treatment complications.

c) Early detection of CAD, on the other hand, has the benefit of identifying the risk of cardiovascular events for a patient, and in performing multimodal and individualized treatment strategies.

d) The investigation on the nature of sympathovagal imbalance is of great importance in terms of treatment decisions. For instance, for impaired parasympathetic function in MS patients, reduced fat intake and mild to moderate intensity aerobic exercise can enhance parasympathetic function. Also, adequate hydration, short bursts of high intensity aerobic and head-up tilt sleeping position are suggested for patients for decreased sympathetic function[19,54]. Medication also varies in relation to the symptoms of CAD.

e) CANS investigation in MS can provide useful benchmark information about MS disease and treatment planning. For instance, identification of CAD was shown to be correlated with land-mark pathophysiological processes of MS (e.g. underlying inflammation and neurodegeneration)[40].

1.13 Justification and purpose of this thesis

Motivation of study: The impact of CAD on quality of life is substantial, but unfortunately, often overlooked. For instance, the Kurtzke expanded disability status scale (EDSS) only contains bowel and bladder dysfunction[32]. The reason might be that the CAD is not very well clearly described in MS due to either inconsistent results in different studies, or partial investigation of CANS assessment.

Aim of study: The study presented in this thesis aimed to use a comprehensive set of non-invasive cardiac autonomic tests, and investigate any distinctions between MS and control variables, and any relations between multiple sclerosis CANS test variables and clinical variables. The clinical variables include MS disease severity scores (EDSS, MSSS), disease duration, treatment delay, type of disease based on progress (RRMS, PRMS, SPMS, PPMS), disease classification based on localization of lesions (dwMS, gMS, bcMS, sMS) and finally number of lesions. The comprehensive CANS test in this study includes 4 common reflex tests of deep breathing, orthostatic challenge, Valsalva manoeuvre and isometric exercise. In addition, detailed analysis of spontaneous changes of heart rate and blood pressure for multiple sclerosis patients are performed.

Focus of study: The focus of this research is on calculation and interpretation of heart rate variability in both time and frequency domains, systolic blood pressure variability in both time and frequency domains and baroreceptor sensitivity with sequence technique and frequency technique. Performing short-term analysis of HRV and BRS (5 minute) might be advantageous especially for two groups of MS patients: (i) Patients in early stages of MS with no symptoms of CAD that reflex test does not show any abnormalities (early CAD diagnosis), and (ii) MS patients with special conditions or higher severity scores where it may not be possible to perform some of the reflex tests such as orthostatic challenge (due to immobility), sustained hand grip (due to muscle weakness) and Valsalva Manoeuvre (in case of pregnancy). This is beneficial, especially if simple and potentially widely applicable techniques are discovered and tailored to individual patients.

Novelty of study: The novelty of this study is in it being comprehensive. The suite of tests proposed in this project has not been done before in such a comprehensive manner and in a large group of MS subjects. Specifically, baroreceptor sensitivity with both frequency and sequence techniques (two criteria) and from spontaneous variation in BP and HR has never

been tested for MS subjects to this extent. This is of a great importance since baroreceptor sensitivity of MS subjects may provide valuable information regarding the interaction of sympathetic and parasympathetic activity in assessing the overall autonomic function related to extent and severity of the disease.

Chapter 2

Review of cardiovascular autonomic testing

For assessment of cardiovascular autonomic function, several direct/invasive or non-invasive methods exist. The methods that more directly assess autonomic function include the analysis of catecholamine and catecholamine metabolite levels (extremely high catecholamine level in blood can be attributed to high sympathetic activity caused by stimulation or damage of brainstem), cardiovascular autonomic imaging, and sympathetic microneurography (directly measurement of muscle sympathetic nerve activity from sympathetic nerve fibers in a peripheral nerve)[55]. Although these tests provide valuable information on the understanding of pathophysiological changes of CANS, they are less common due to complications, difficulty for participants and operators, invasiveness and unsuitability for longitudinal evaluations. Accordingly, a series of non-invasive CANS tests is usually preferred both in clinical and research platforms. In these methods, CANS is evaluated via a reflex arc, involves a stimulus received by a receptor and an afferent nerve, processed by CNS, the response sent back through an efferent nerve and made apparent by an end-organ reflex[7]. However, in most reflexes both sympathetic and parasympathetic pathways have innervations. Synapses and neurotransmitters are the other factors involved in each arch reflex. Another difficulty of

CANS assessment with reflex tests, relates to the anatomic location of CANS that prevent their direct physiological testing. For these reasons, stimulus should be standardized, and normal response should be determined scientifically. Furthermore, a group of clinical tests should be performed and CANS should be assessed by interpretation of all results together[7,55–57].

In this section, the conditions needed to participate in CANS test in order to obtain universally approved and reproducible results are explained. Then, collected data is described, and finally, the most common tests for CANS assessment are explained in details. In addition, the expected output and interpretation of out of range results are demonstrated.

2.1 Subject Condition

To obtain reliable results from CANS experiments, it is vital to eliminate any other factors that might affect autonomic responses. Therefore, since it is known that excessive eating, drinking coffee or alcohol, smoking, medicines and excessive exercise may affect the cardiovascular autonomic nervous system, the subjects should not take any of those for about 8 hours prior to test. Also, the subjects with any other diseases affecting autonomic function should be excluded from the study, including cardiac disease, alcohol dependency, collagen disease, diabetes mellitus, renal failure, liver failure, and peripheral neuropathys[1,55].

2.2 Required Data

Continuous and non-invasive measurement and recording of beat-to-beat HR and BP is required for this test. The ECG (electrocardiogram) electrodes are also needed for extracting morphological information of the ECG. Signal. The data should be recorded for offline analysis.

2.3 Control Subjects

Assessment of the cardiovascular autonomic nervous system has become an essential tool in both clinical autonomic laboratories and the research setting. For interrelating the results of autonomic testing, the results should be compared to universally applied “normal control” values [1]. However, there are some stimuli that affect the ranges of responses such as specific laboratory condition (room temperature), testing equipment, the protocol and the quality of testing by staff. Therefore, it is suggested that each study, have their own control subjects.

2.4 Cardiovascular Autonomic Nervous System testing

2.4.1 Supine-Rest Test

2.4.1.1 Data Collection and Preparation

Evaluation of CANS usually only includes reflex tests. However, studies suggested that spontaneous changes of HR and BP either in time or frequency domain, or studying the correlation of their synchronic changes, have the potential to provide important information on the autonomic cardiovascular system. In fact, HRV is the variation in length of consecutive RR intervals determined by dynamic interaction between the spontaneous cardiac impulses of SA node. The SA node itself, receives several different inputs including sympathetic and parasympathetic nervous system and humoral factors. The factors that affect these inputs include baroreflex, thermoregulation, hormones, sleep-wake cycle, meals, physical activity, breathing and stress[58]. Therefore, short term (around 5 minutes) recording of beat-to-beat BP and HR is suggested.

Various parameters are derived from normal to normal (NN) RR tachogram. The term "NN" means that all of the beats are normal and ectopic beats are excluded. The necessity of removing non sinus (ectopic) beats is because of two reasons. First, ectopic beats are not

generated by the mechanism that is responsible for the variability in the RR intervals. Second, ectopic beats often occur substantially earlier or later than when it is expected, and a prolonged pause is followed or preceded by them. Therefore, they deviate measurements and add high frequency components to variations and should be removed[59]. For a typical RR tachogram, the relative deviations of RR intervals from the mean value usually do not exceed 20–30%[60]. One method to automatically find ectopic beats is to check if the new RR interval deviates by more than 10% from the median of the last nine NN intervals[61]. About 5 minutes normal to normal RR-tachogram is needed for spectral analysis. The measurements can be calculated of this test explained in the following order:

2.4.1.2 Morphological Values of ECG and Average Values of HR and BP

ECG morphological normal values are described and illustrated in Table 2.1 and Figure 2.1. The morphological parameters of ECG (including PQ, QRS, QT and RR interval) are usually calculated automatically by ECG monitors and averaged from 5 non-consecutive cardiac beats[57].

Interpretation: For the reason that QT is HR (frequency) dependent, the corrected format (QT_c) based on Bazett's formula (Table 2.1) is used to adjust QT based on RR interval. The QT_c parameter evaluates the function of the left afferent sympathetic cardiac fiber. This test is rarely abnormal. However, prolonged QT_c is attributed to cardiac autonomic nervous dysfunction and studies show it is a predictor of cardiac arrhythmias and sudden death [7,57,62,63].

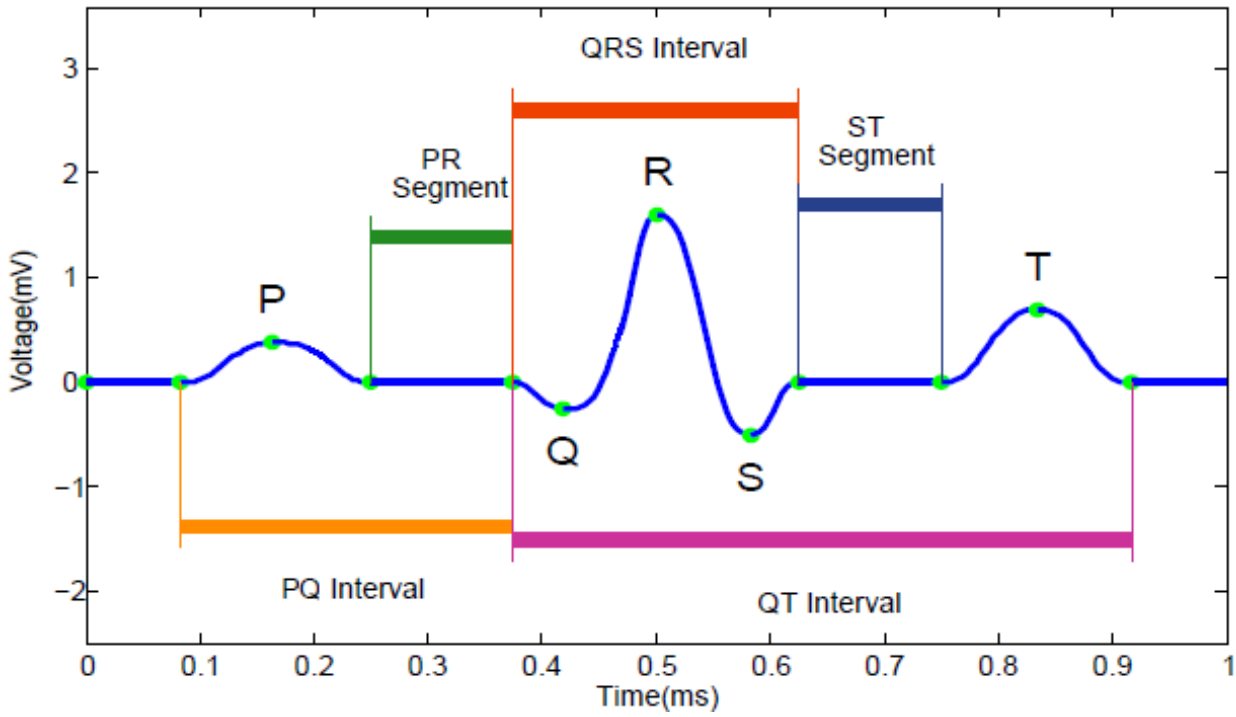


Figure 2.1*Main morphological features contained within ECG* [64]

Table 2.1 *ECG morphological variables and normal ranges*[65,66]

Parameter	Normal ranges
PQ interval	120ms<PQ <200ms
QRS interval	60ms<QRS<120ms
QT_c (by Bazett's correction formula) = $QT / \sqrt{RRinterval(\sec)}$	**350<QT_c<460 for women** **<450 for men**

2.4.1.3 Average HR and BP Values

Average values of HR, SBP, DBP and MBP are usually calculated from 1-2 minute normal to normal beat-to-beat information. Normal values are displayed in Table 2.2.

Interpretation: The basic parameter of average HR is calculated to identify tachycardia and bradycardia, and the average values of BP are measured to identify high BP and low BP. These abnormalities, affect the other autonomic variables. For instance, BRS (baroreceptor

sensitivity) is impaired in hypertensive patients [16,67], or LF of HRV tends to be higher in the presence of hypertension [68].

Table 2.2*Normal values for HR and BP* [69]

Parameters	**Ranges**			
Heart rate (beat/min)	Bradycardia		Normal HR	Tachycardia
	HR<60		60-100	HR>100
Systolic blood pressure (SBP) mmHg	Low BP	Normal BP	Pre-Hypertension	Hypertension
	<90	90-120	120-139	>140
Diastolic blood pressure (DBP) mmHg	<60	60-80	80-89	>90

2.4.1.4 Heart Rate Variability (HRV) in time domain

The parameters of short-term HRV in time domain proposed by the task force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology (the task force on HRV), are described in Table 2.3[68]. Normal range values that are based on a review study of 44 publications; each including more than 30 healthy adult participants is also displayed. Data length was 5 minutes of NN RR-interval[68,70].

Interpretation: Time-domain indexes provide comprehensive information about distribution around the mean. However, time domain indexes offer no information about the variability of the signal with respect to the length of time [1,68]. Among these factors, rMSSD and PNN50 mainly reflect the fastest components of heart rate variability, which are largely due to parasympathetic control. Studies suggest that a low SDNN value is predictor of high mortality in cardiovascular diseases. However, it is dependent on the length of data. The rMSSD value is an indicator of parasympathetic activity and low values are attributed to poor parasympathetic mediation of HRV. The value of PNN50 also is an indicator of parasympathetic activity [1,68,71].

Table 2.3*Time domain variables of short-term RR interval*[68,70,72]

(N is the number of NN RR intervals)

Parameters	**Description**	**Formula**	**Mean ± std** **Range**
Mean RR	Average of R-R interval beats	$\overline{RR}$	926±90 785-1160
SDNN	Standard Deviation of all Normal to Normal R-R intervals	$\sqrt{\frac{\sum_{j=1}^{N}(RR_j - \overline{RR})^2}{N-1}}$	50±51 32-93
rMSSD	square root of the Mean of Squared Successive Differences in R-R intervals	$\sqrt{\frac{\sum_{j=1}^{N-1}(RR_{j+1} - RR_j)^2}{N-1}}$	42±42 19-75
pNN50	percentage of NN R-R intervals that are at least 50 msec different from the previous interval	$\frac{numberof(RR_{j+1} - RR_j) > 50ms}{N-1} * 100$	8.9±7.2 [49]

2.4.1.5 HRV and BP variability in Frequency Domain

Calculation of RR interval variability in the frequency domain provides more detailed information about CANS. Numerous studies on humans show that the regular oscillations with frequency greater than 0.025 Hz (i.e. relatively fast oscillation with period shorter than 30s) appear to reflect sympathetic and parasympathetic modulation of the heart and vascular tone in different bands[1,55,60].

The identified features of this spectrum are a low frequency (LF) component centered around 0.1 Hz (band: 0.04-0.15 Hz), a high frequency (HF) component with a peak found in a frequency range greater than 0.15 Hz (band: 0.15-0.5Hz) and a very low frequency (VLF) band between 0.0033 and 0.04 Hz[49,55,68]. Integration of the power spectrum density in each

frequency band provides the so-called parameters of LF, HF, VLF and total power (TP) which is the total area under the power spectrum density (PSD) curve (TP=VLF+LF+HF)[73].

The most popular methods of HRV analysis in the frequency domain are based on fast Fourier transform (FFT) and is the method suggested by the taskforce for HRV as well [68]. For this, the following method measures HRV and also SBPV (Systolic Blood Pressure Variability). Investigation of SBPV is not conventional in CANS tests. However, it is necessary to be calculated for baroreceptor sensitivity evaluation by the frequency technique.

Fast Fourier Transform by the Welch method: The conventional method for frequency analysis of the RR tachogram proposed by the task force on HRV, is based on the discrete fast Fourier transform (DFFT). DFFT is applicable to uniformly-spaced samples of a continuous function, and decompose the signal into its frequency spectrum components[68]. However, RR interval is sampled at the timing of the cardiac cycle and inherently irregularly spaced in time. Thus, interpolation (for example, linear or cubic spline) is necessary or some modifications and should be done to apply FFT on unevenly sampled time series [74]. In Figure 2.2, ECG tracing for 12 seconds, RR tachogram and its linear and cubic spline interpolation are displayed.

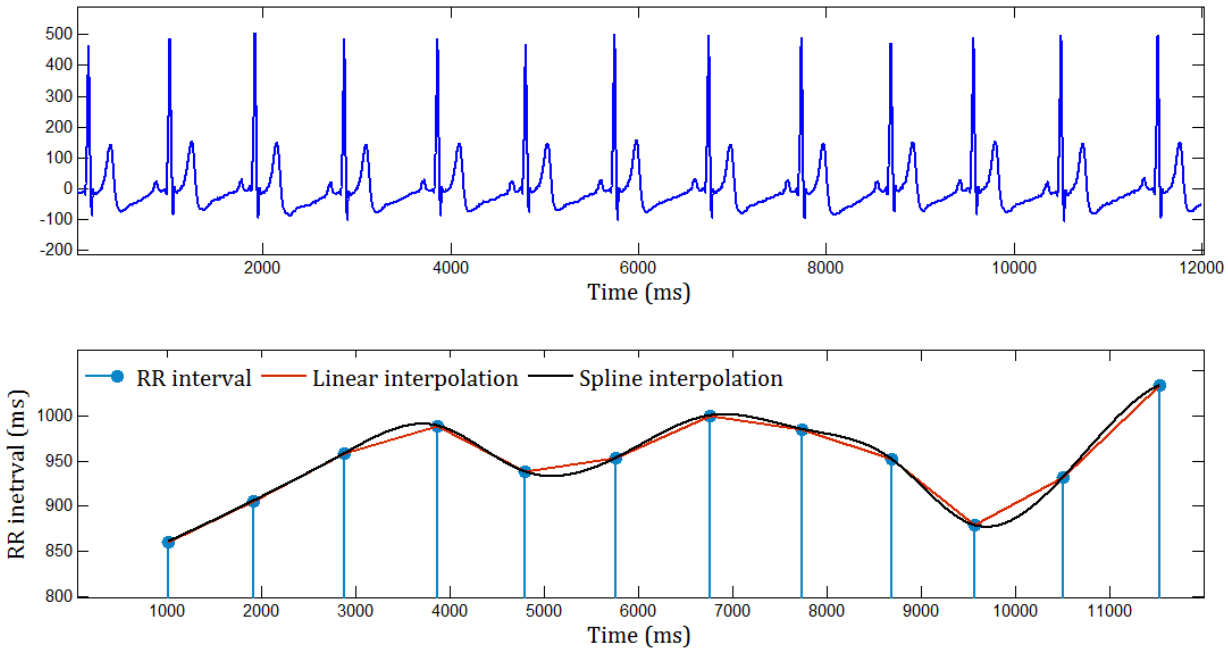


Figure 2.2 *ECG tracing (12 sec) and linear and spline interpolation of RR intervals to produce RR tachogram with even sampling.*

Interpolation by the rate of 1sample/1ms provides evenly sampled data tracing the RR interval with 300,000 data points for a 5 minute ECG recording (5 min x 60 sec x 1000ms =300,000). To obtain the PSD, the Welch periodogram can be calculated using the "pwelch" command in MATLAB. The Welch periodogram is a modified version of DFFT, in which the signal is divided to defined overlapped segments by a defined window. Then DFFT is performed on each window and the output spectrums are averaged in order to reduce spectral leakage commonly associated with non-stationary input (RR interval). The window size, overlapping size, and down sampling rate should be optimized so as to obtain the highest frequency resolution, and more reliable results. In Figure 2.3, the Welch periodogram method is illustrated. Parameter L is the window size input for DFFT with the overlap size of L-D. The input data for MATLAB command of "pwelch" is below:

[Pxx,F] = pwelch(X,WINDOW,NOVERLAP,NFFT,Fs) **Eq. 2.1**

The window type selected was the default window (Hamming window) of the *pwelch* function. The term Pxx is the output frequency spectrum with the size of $\frac{NFFT}{2}+1$, as NFFT is the number of FFT points to calculate PSD (NFFT is equal to L in Figure 2.3). In this study, the term NOVERLAP (size of overlaps in windows), is selected to be the maximum possible overlap (NFFT-1) to obtain more reliable results. So the optimization question is reduced as below:

$$Frequencyresolution of PSD = \frac{PSD frequency range}{number of Pxx} = \frac{input frequency range}{1+\frac{NFFT}{2}}$$, **Eq. 2.2**

$NFFT = \{256,512,1024, \dots\}$, NFFT> number of input data points **Eq. 2.3**

Regardless of the input's frequency range, the maximum detectable frequency in input is 1000 Hz, due to interpolation with the rate 1sample/msec. However, the maximum desired frequency of this study for HRV is 0.5 Hz. Therefore, down sampling can provide lower resolution in the time domain, lower range of detectable frequency in frequency domain and thus higher resolution in frequency domain.

Down sampling rate (DS rate) $= \frac{1000}{input frequency range new\ (>0.5\ Hz)} \Longrightarrow$ DS rate < 2000 **Eq. 2.4**

New number of input data points $= \frac{\text{number of input data points}}{\text{DS rate}} = \frac{300{,}000}{DS rate}$ **Eq. 2.5**

The optimization problem is selection of NFFT and DS rate to obtain maximum frequency resolution. Considering equations 2.2 to 2.5, the final optimized parameters are:

NFFT= 256, DR rate=1000, F_s=$\frac{1000}{Dsrate} = 1Hz$

$$frequencyresolutionofPSD = \frac{\frac{Fs}{2}=0.5}{129} = 0.0039\ Hz \qquad \textbf{Eq. 2.6}$$

By applying the optimized parameters in the Welch periodogram method, the HRV spectrum of a normal RR tachogram is as illustrated in Figure 2.4. Also, an example of HRV PSD in supine position, published by the task force on HRV is shown in Figure 2.5. The ranges of HRV parameters calculated for healthy controls in previous studies are displayed in Table 2.4.

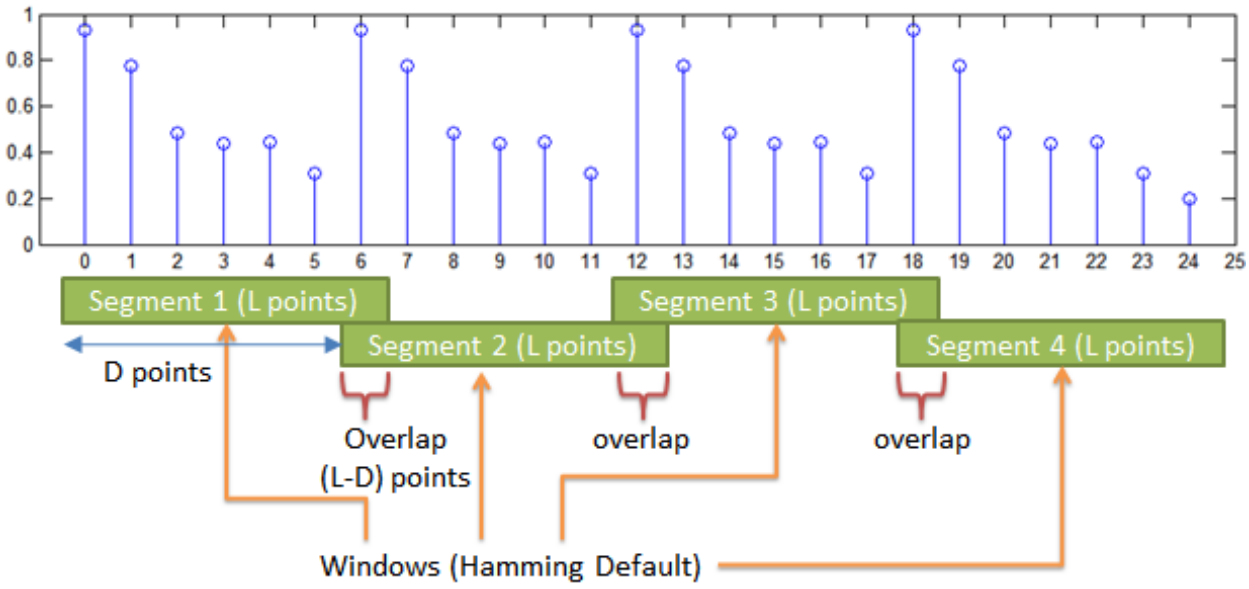


Figure 2.3 *Welch periodogram for overlapped windowing DFF* [75]

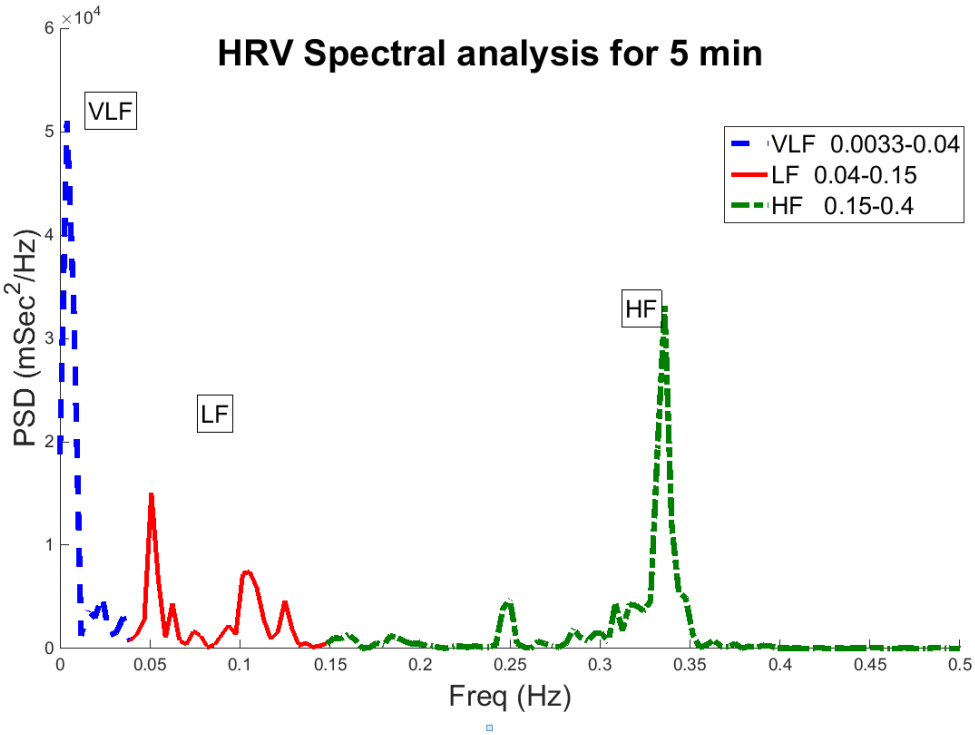


Figure 2.4 *Spectral analysis of HRV in supine position (pwelch)*

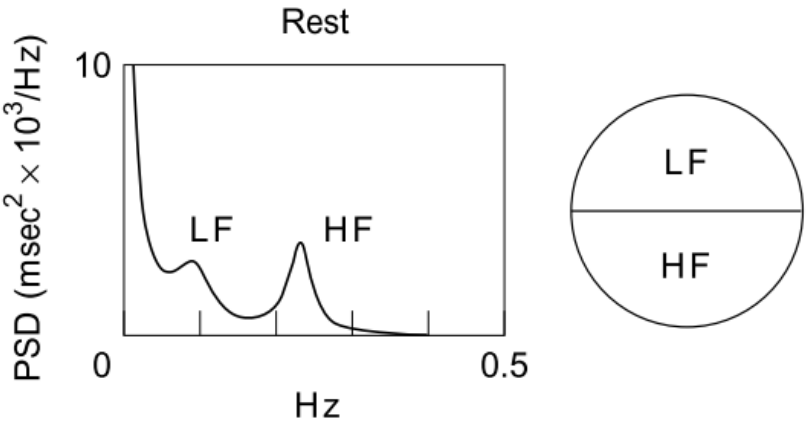


Figure 2.5 *Spectral analysis of RR interval in supine position(Auto regressive)*[68]

Table 2.4 *Spectral analysis of stationary supine 5-min recording* [68]

Parameter	Formula	Range in control
TP (total power) (ms^2)	VLF+LF+HF	3466±1018
LF (ms^2)		1170±416
HF (ms^2)		975±203
VLF (ms^2)		2524±931 [49]
LF (normalizes unit)	LF/(TP-VLF) x 100	54±4
HF (normalizes unit)	HF/(TP-VLF) x 100	29±3
LF/HF		1.5-2

Interpretation: A large body of literature suggests that the HF spectrum reflects oscillations of heart rate occurring with respiration and it may be a reliable marker of the parasympathetic function at unstressed conditions. Furthermore, the LF component is thought to be a marker of sympathetic activity, or both sympathetic and parasympathetic influence if the setting is relatively stressful. In addition, for those that hold the opinion that the LF component of HRV contains information on both the sympathetic and parasympathetic activity, the LF/HF ratio is thought to represent the influence of the cardiac sympathetic nervous system alone[1,55,60].

Limitations: Cubic spline or linear interpolation methods have the advantage of being fast and non-parametric. However, all interpolation methods rely on the assumption of the form of underlying changes in the RR tachogram. For example, linear interpolation is a poor approximation and cubic spline usually provide wrong interpolation when one RR interval is unusually longer that its previous one [74]. Also, there are debated concerns about specificity and reproducibility of the information provided by the FFT[1,76].This is mainly because of the over simplifying assumption of HRV stationarity, and the incorrect assumption of linearity of cardiovascular responses. Spectral analysis of HRV may be a better marker for tracking changes of cardiovascular autonomic function than the mean neural autonomic activity [1,60]. However, FFT-based methods for HRV are the methods employed in the great majority of

studies to date and is the technique for HRV analysis recommended by the task force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology[68].

2.4.1.6 Baroreflex Sensitivity (BRS)

Several techniques exist to measure baroreflex gain. The main concept of all non-invasive methods is finding the relative changes in lengthening/shortening of RR intervals to increasing/decreasing of SBP in consecutive beats. Considering the mechanism of the baroreflex feedback loop, this measurement evaluates the sensitivity of baroreceptors in the maintenance of circulatory homeostasis[16]. Altogether, the techniques of BRS measurement include: injection of vasodilator/vasoconstrictor drugs, applying positive or negative neck chamber pressure, natural challenge of Valsalva in phase II and IV, backward tilt table test, and measuring BRS from spontaneous changes of HR and BP analyzing consecutive pulse sequences of BP and HR change or using spectral methods[15,16,67,77,78].In this study, BRS was calculated from spontaneous changes of SBP and HR.

a) Baroreceptor Sensitivity by the Sequence Technique

The sequence technique is based on the identification of sequences with three or more consecutive beats in which, progressive increase/decrease in SBP is followed by progressive lengthening/shortening of RR interval. A minimum threshold for changes in SBP (1mmHg) and RR interval (6 ms) was used for classification as a sequence[15,16,78]. For each sequence a line is interpolated (linear regression) and then the average slope of eligible sequences is calculated and used as the value of BRS[78]. Figure 2.6 illustrates one sequence for baroreflex measurement and related regression line (increase of SBP for 4 consecutive beats).

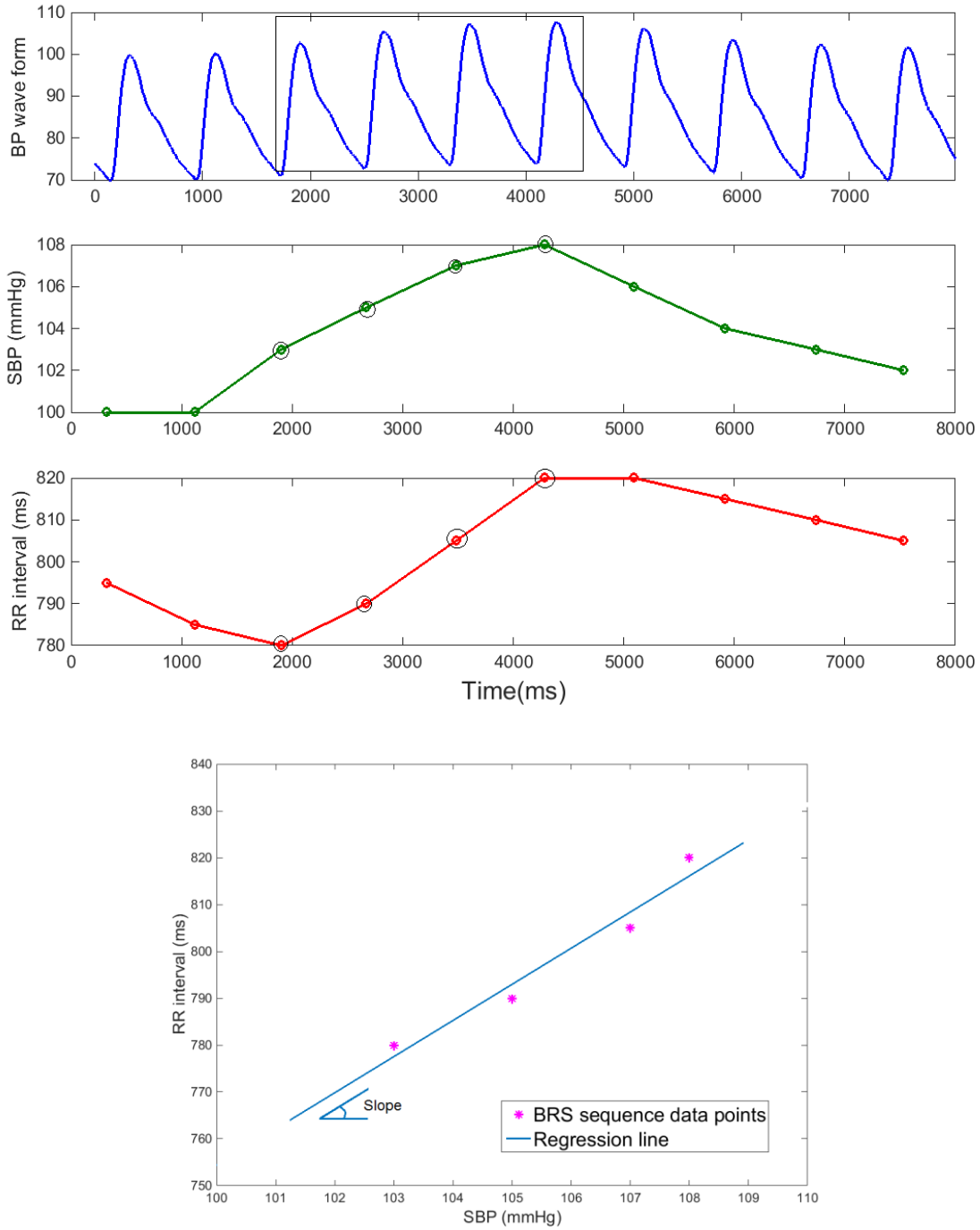


Figure 2.6 *Four consecutive beats with progressive increase in SBP for at least 1mmHg and progressive lengthening of RR interval for at least 6 mmHg, and fitted line for slope calculation.*

Further parameters are extracted from the sequence technique, as well. For instance, BRS for different lags are calculated, i.e.changes in SBP result in changes of RR of the same beat (Lag 0),next beat (Lag1), after 2 beats (Lag2) and after 3 beats (Lag3)[79]. Also, positive and negative sequences are considered individually, and their BRS and number of sequences are calculated separately[77,80]. In Figure 2.6, one positive sequence of BRS in Lag 0 is displayed.

Table 2.5 displays the BRS parameters of sequence technique calculated from 1134 healthy subjects and their average values and ranges for a zero lag[80]. Studies show that spontaneous BRS significantly declines with age[16,67,80] .

Table 2.5 *BRS lag 0 (L0) parameters from 10 minute signal , 1134 healthy subjects*[16,80]

	18-29 year	**30-39**	**40-49**	**50-59**
Total BRS L0	14 (13.1:14.9)	10.3(9.7:11)	7.8(7.4:8.2)	6.8(6.2:7.3)
No. of Slopes BRS L0	26.9(23.3:30.5)	19(16.8:21.2)	19.1(17.5:20.8)	17(14.4:19.7)
BRS -PI/-SBP	13.9(12.3:15.3)	10.8(10.1:12.2)	8.2(7.6: 8.6)	7(6.9 :8.3)
No. of Slopes -PI/-SBP	14.5(12.8:16.8)	10.1(8.8 : 11.5)	11.1(10.1 :12.2)	10.1(8.5 :11.6)
BRS +PI/+SBP	13.7(12.7:14.7)	9.8(9.1 :10.4)	7.1(6.8: 7.5)	6.2(5.6 :6.7)
No. of Slopes +PI/+SBP	12.8(11:14.6)	9.6(5.6:10.7)	9(8.2 :9.8)	7.9(6.6: 9.3)

b) Baroreceptor Sensitivity by Frequency Technique

BRS can be calculated by spectral methods as well. It implies that each spontaneous oscillation of SBP induces an oscillation in RR interval at the same frequency which is the result of baroreflex activity [16]. To this aim, HRV and SBP variability in the frequency domain calculated in part 2.4.1.5 are normalized so that the total area under each curve equals the variance of the spectrum divided by the square of the mean of spectrum (equation 2.7). Then the normalized spectra are used to provide a modulus, or gain function (equation 2.8) [15,16,77,81]:

$$SBP\_V_{norm} = SBP\_V * \left(\frac{var\ (PSDSBP)}{Average\ (PSDSBP)^2} * \frac{1}{TPSBP\_V} \right) \quad \textbf{Eq. 2.7}$$

$HRV_{norm} = HRV * \left(\frac{var\ (PSDHRV)}{Average\ (PSDHRV)^2} * \frac{1}{TPHRV}\right)$

$$TP_{norm} = \sum HRV_{norm}[f] = \sum HRV * \left(\frac{var\ (PSDHRV)}{Average\ (PSDHRV)^2} * \frac{1}{TPHRV}\right)$$
$$= TPHRV * \left(\frac{var\ (PSDHRV)}{Average\ (PSDHRV)^2} * \frac{1}{TPHRV}\right)$$
$$= \frac{var\ (PSDHRV)}{Average\ (PSDHRV)^2}$$

Modulus function [f] = $\sqrt{\frac{\mathrm{HRV}[f]}{\mathrm{SBP\_V}[f]}}$ **Eq. 2.8**

In Figure 2.7 normalized spectrums of HRV and SBPV and Modulus function are displayed (parts a&b).

However, calculation of BRS from Modulus differs in different studies and in this thesis results using two alternative approaches are reported:

(i) Modulus in each band of LF and HF is averaged only if the coherence between RR and SBP time series is not low. It is equivalent to the regression coefficients in regression analysis. So the modulus values become unreliable if the coherence is low. The threshold of accepting or rejecting is the modulus of frequencies where the coherence is greater than or equal to 0.5[16,81]. This threshold is chosen to guarantee reliable BRS estimates. The coherence function and the modulus segment with coherence >0.5 in the LF band are illustrated in Figure 2.7 (parts c & d). The BRS for each frequency bands of LF and HF (usually referred as α index in LF and HF)are calculated as below:

$$\alpha\_LF = Avererage(Modulus(0.04 < f_n < 0.15)|Coherenceinf_n > 0.5\ ) \quad \textbf{Eq.2.9}$$
$$\alpha\_HF = Avererage(Modulus(0.15 < f_n < 0.4)|Coherenceinf_n > 0.5\ )$$

Table 2.6 shows the parameters of BRS measured by frequency technique with coherence acceptance criteria as well as their ranges calculated for healthy controls in two studies[15,67].

(ii) The use of coherence threshold is being criticized in some studies mentioning this criterion does not guarantee reliable BRS measurements[82]. First, the coherence is shown to tend to zero when baroreceptor sensitivity is depressed in pathological subjects. Second, the number of points included in the modulus averaging, alters between subjects, thus affecting the reliability of measurement. Therefore new criterion is used based on the average of the transfer function over the whole LF band, and HF band, regardless of coherence values. This method has been shown to provide the best trade-off between measurability and accuracy[16,82]. The average criterion of BRS calculation is illustrated in Figure 2.7 (part e).

Table 2.6 *LF and HF $\alpha index$calculated from healthy subject*[15,67]

Parameters	**BRS by spectral technique (18 subjects, average age of 32)**	**BRS by spectral technique (10 subjects, average age of 42)**
α_LF (Coherence>0.5)	15.4±5[67]	7±4[15]
α_HF (coherence>0.5)	25.1±8.3[67]	11±4[15]

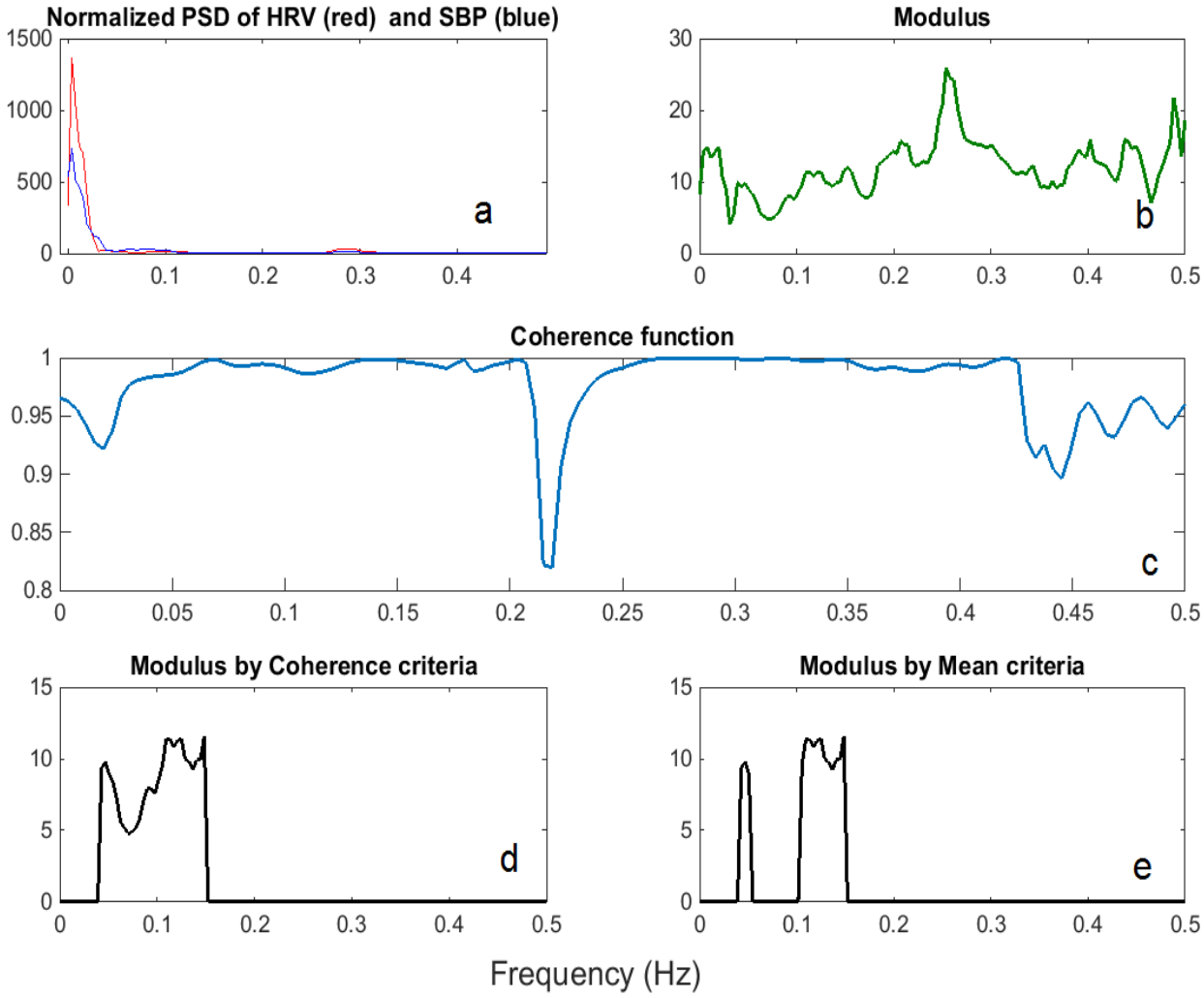


Figure 2.7 *(a) Normalized PSD of HRV and SBP by Welch periodogram method and normalization by Eq.2.7, (b) Modulus function calculated by Eq.2.8, (c) Coherence function, (d) Modulus function in LF band with the Coherence>0.5, and (e) Modulus function in LF band where Modulus> average of modulus in LF band.*

Interpretation: BRS is an important factor in the homeostatic regulation of the cardiovascular system that can provide information about both sympathetic and parasympathetic function[83]. Rise in BP activates baroreceptors and increases parasympathetic and decreases sympathetic activity, which is followed by bradycardia. Conversely, decrease in BP deactivates baroreceptors and cause enhancement of sympathetic activity and parasympathetic inhibition, which is followed by tachycardia [16]. One of the advantages of the sequence technique is the separate identification of baroreceptor activation and deactivation[81]. However, since the time delay of parasympathetic response is significantly shorter than sympathetic response (200-

600ms vs. 2-3 seconds), therefore it seems lower lags of BRS mostly represent parasympathetic function [16]. BRS has a significant correlation with age, HR, body mass index, SBP, DBP and gender[61,80].

Limitation: There are debated concerns about reproducibility of BRS [67], but BRS calculated by α-index in the LF and HF bands showed similar results with BRS calculated through induced blood pressure changes with intravenous phenylephrine injection. Thus the BRS α-index is a good substitute for invasive methods of baroreflex evaluation. However, measuring intrinsic BRS is based on the assumption that relative changes of BP and HR (conditional) represents only baroreflex function. However, progressive changes in BP and HR are not always accompanied by baroreflex, and secondly, the relative changes of BP and HR are used to provide single slope estimation, regardless of the blood pressure value[72,77].

2.4.2 Deep Breathing

2.4.2.1 Data Collection

Deep breathing test is one of the common CANS reflex tests. For this test, the subject in supine position is instructed to perform a few consecutive deep breaths at the rate 6 breaths/min. As is shown in Figure 2.8, each cycle of deep breathing should last 10 seconds with 5 seconds inspiration and 5 seconds expiration. Breathing should be performed through the nostrils with a closed mouth. Usually 6 cycles of deep breathing in the supine position is performed, however, in some studies, three, five or eight cycles are evaluated, or the test is performed in the sitting position [44,56,84]. A normal HR in deep breathing test should resemble a wave form as it is displayed in Figure 2.8.

Two parameters measured from deep breathing are as follows:

Respiratory sinus Arrhythmia (RSA, E-I difference) = $\frac{\sum(maxHR_{insp} - minHR_{exp})}{6}$ **Eq. 2.10**

Average HR variation INS/EXP (E:I ratio) $=\sum(\frac{HR_{INS}}{HR_{EXP}})\ /6$ **Eq. 2.11**

RSA is defined as the average of maximum heart rate variations in inspiration and expiration, and E:I ratio is the average of the ratio of max heart rate in inspiration to min HR of expiration.[44,56]

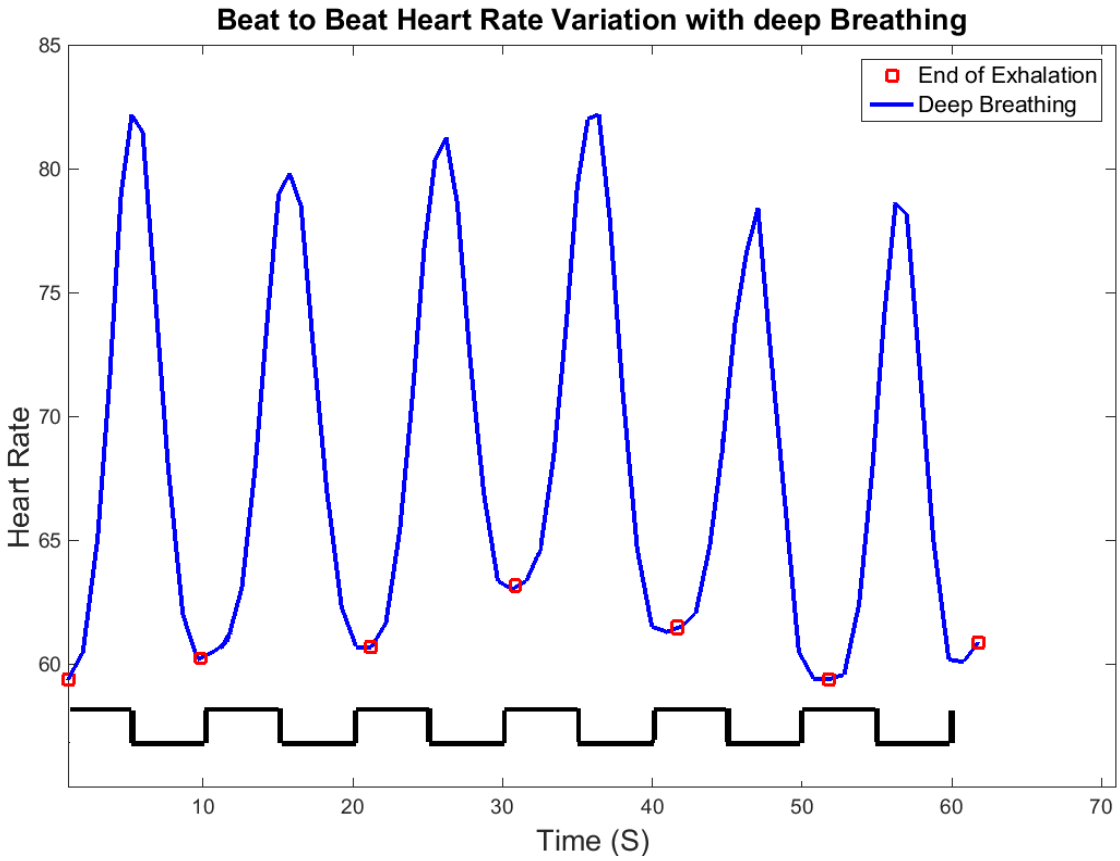


Figure 2.8 *HR changes with deep breathing resemble a wave form. The square wave shows breathing pattern. The upper line of square wave is inspiration and lower line show expiration. The red square markers on the HR wave form are the beginning and end points of each cycle.*

This test is based on the fact that changes in respiration results in rapid responses in heart rate and normally, the heart rate rises during inspiration and falls during expiration. To standardize the test, sudden inhalation/exhalation, holding the breath, breathing through the mouth and hyperventilation should be avoided. To avoid hyperventilation monitoring of CO_2 is recommended[85,86].

2.4.2.2 Normal Values

Deep breathing measurements are age-dependent, with both parameters (RSA and E:I ratio) decreasing with age. The normal values in some studies are provided in Table 2.7

Table 2.7 *Normal age dependent ranges for deep breathing measurements*[71,87]

Age range (years)	Minimim for Normal E:I ratio	Minimum for normal RSA (beat/min)
20-24	1.17	14
25-29	1.15	
30-34	1.13	12
35-40	1.12	
40-44	1.10	10
45-49	1.08	
50-54	1.07	9
55-59	1.06	
60-64	1.04	7
65-69	1.03	
70-75	1.02	NA

However, in some studies one threshold is considered for all ages. For example, E:I ratio <1.2 is considered as abnormal, RSA >15 is interpreted as normal, and RSA<10 is classified as abnormal for all ages [41,71,85].

2.4.2.3 Data Interpretation

This test is the most widely used index of cardiac parasympathetic function because heart rate response to deep breathing is mediated by the vagal system[55,87,88]. With the normal parasympathetic function, inspiration causes increase in HR and expiration causes decrease in HR[57]. However, the result of this test also depends on the following factors:

1- *Subject position and rate of breathing*: the vagal tone is greatest in the supine position and the variation of instant heart rate is maximally provoked by the rate of 6 breaths/min (or between 5 and 10 breaths/min as suggested in some studies). So supine positions and 6 breaths/min is standard-setting for this study[1,7,55,71].

2- *Tidal volume* which is the volume difference after a normal inhalation-exhalation affect the magnitude of RSA and E:I[89].

3- *Hyperventilation*: in case of hyperventilation, the carbon dioxide reduces in blood, leading to rise of blood Ph and initiating constriction of the blood vessels which supply the brain in order to increase brain perfusion. So, HR and sympathetic activity will increase, and may reduce heart rate variability[55].

4- *Age*: as age increases, HR variation in deep breathing declines linearly (3–5 beats/min decrease per decade in control subjects)[88].If a single normative value for all ages applied, the interpretation may results in false negative error in younger patients and false positive error in older patients[55].

Altogether, the result of this test is usually interpreted along with the results of Valsalva ratio and 30:15 ratio (both described in subsequent sections) for assessment of parasympathetic activity[1].

2.4.2.4 Limitations

Although this test is simple, standardizing the test to prevent hyperventilation needs CO_2 monitoring, which hasn't been monitored in many previous studies. However, the breathing pattern and chest movement in deep breathing should be monitored by the investigator to avoid hyperventilation.

2.4.3 Valsalva Maneuver

2.4.3.1 Data Collection

The Valsalva maneuver is a common CANS reflex test. For performing the Valsalva maneuver the subject takes a deep breath and exhales into the mouthpiece of a manometer to keep the pressure at 40 mmHg for 15 seconds. The tube or the mouthpiece should have a small air leak to avoid closing of the glottis[1,55,87,90].The position of the subject is supine with 30° head-up tilt.

Usually, the Valsalva maneuver is repeated 2-3 times (with 3 minutes rest between each maneuver), and the best maneuver is selected for evaluation [87].

Prior to the test, subjects practice the Valsalva maneuver to know how create a seal with the mouthpiece and how to perform the test. During the test, subjects should be able to see the expiratory pressure to adjust the strain, while the feedback about how many seconds left and when to stop the test is given to them.

In some studies Valsalva maneuver is performed in the sitting position[42,56]or with the tilt table elevated to an angle of 20°. Also it has been reported that a rubber clip is attached over the nose to help limit airflow to the mouthpiece[40].

2.4.3.2 Normal Values

A normal Valsalva response is illustrated in Figure 2.9. This maneuver includes two main phases: a strain phase (15 seconds of exhalation into mouthpiece) and a post-strain phase (normal breathing following strain phase). In the strain phase tachycardia and reduction in arterial pressure is observed. In the post-strain phase, a reflex bradycardia and rise in arterial blood pressure is observed [57].

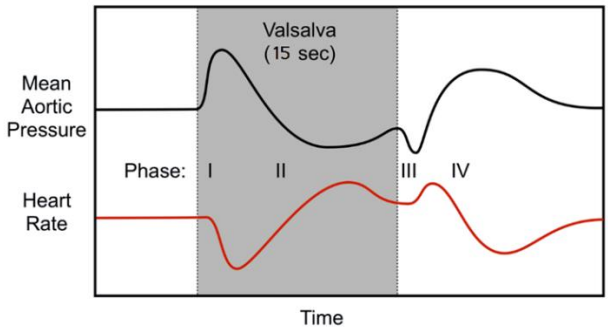


Figure 2.9*Schematic changes of HR and BP in 4 sub-phases of Valsalva maneuver*[91]

Also 4 distinct sub-phases are usually specified for further analysis [1,55]:

Phase I: Initial pressure rise

At the beginning of the strain phase the intra-thoracic pressure is transmitted to the intra-thoracic and abdominal blood vessels forcing blood out of the pulmonary circulation to the left atrium and cause a mild rise in stroke volume. Therefore, a transient rise in BP and a fall in HR are observed in the first few seconds of strain. However, the changes in this sub-phase is mostly due to mechanical reasons rather than sympathetic activity [1,59].

Phase II: Fall in BP and compensation tachycardia

Due to the elevation of intrathoracic pressure, and propulsion of the blood into peripheral circulation, the return of blood into the right atrium is also reduced in the strain phase. Then

the cardiac output also decreases and stroke volume falls. This happens after about 5-14 seconds of strain. As a result, the baroreceptor activity increases HR (compensatory tachycardia) and vascular resistance. Again, at the end of strain phase, mean arterial pressure is restored[57,59].

Phase III: Pressure release

The third sub-phase is releasing the pressure on the chest at the beginning of the post-strain phase. Initially venous return is increased causing an overshoot BP and reduction in HR due to the baroreflex response. Then a small drop in BP and increase in HR is observed, because the expiration is still paused (unintentionally) and the pulmonary vessels and the aorta are able to re-expand[57,59].

Phase IV: Return of cardiac output to baseline

Finally, the venous return returns to normal, and because of residual vasoconstriction and persistence of sympathetic activity, blood pressure overshoots above the baseline before returning to normal value. Also a reflex baroreceptor activity bradycardia is observed before returning to normal values. With return of blood pressure, the pulse rate returns towards baseline[1,55,87].

The parameter extracted from this test are the following:

(a) Valsalva ratio (VR) : VR presents maximum changes in heart rate in the Valsalvamanouver which is calculated by (equation 2.12) the ratio of the longest R-R interval in post-strain phase (within 40 beats after strain, bradycardia in phase IV), to the shortest R-R interval in strain phase (tachycardia in phase II)[1,38,56,57]. VR decreases with aging. Table 2.8 shows the normal VR values published from 2 studies[1,55,65,87].

$$\text{Valsalva Ratio}=\frac{\max HeartRateinStrain}{\min HeartRateinpost-strain}=\frac{longestR-Rintervalinpost-strain}{shortedR-Rintervalinstrain} \quad \textbf{Eq. 2.12}$$

Table 2.8 *Normative values for Valsalva ratio*

Age range (years)	**Minimum for normal VR**[87]		**Minimum for normal VR**[1]
	Women	**Men**	
10-29	1.46	1.59	1.5
30-39	1.50	1.52	
40-49	1.51	1.44	1.45
50-59	1.47	1.36	
60-69	1.39	1.29	1.35

However, in some studies VR> 1.21 is considered to be normal for all ages. The range between 1.11-1.20 is considered as borderline, and any VR<1.10 is interpreted as an abnormal Valsalva ratio [57,88].

(b) BP changes in 4 sub-phases: For a normal Valsalva response, all 4 sub-phases should be observed.

1- In phase I and phase II, initial rise and drop of BP, provides the parameter of maximal drop of MBP in early phase II.
2- At the end of phase II and beginning of phase III, BP has a small rise. MBP in the recovery is also measured.
3- In phase IV, an overshoot in BP is observed that is followed by gradually decrease in BP until reaching the normal level. The rise of BP over baseline value is measured in phase IV.

The normative values for BP changes in Valsalva are not well established. However, the values in Table 2.9 provided by a standard autonomic clinic are used in this study[55,87].

Table 2.9 *Normative values for Valsalva maneuver in blood pressure.*[87]

Parameters	**Normal values**
Maximal drop of the MBP during the early phase II	Normal value< 20 mmHg
MBP at late phase II (recovery)	≥ baseline (mmHg)
MBP at phase IV (overshoot)	> baseline (mmHg)

(c) *BRS*: BRS also, can be measured from Valsalva maneuver by calculating the relative changes of SBP and RR interval in phase II and Phase IV. In phase II, tachycardia and vasoconstriction reflex and fall of BP are mediated by baroreceptor deactivation. On the other hand, in phase IV, the overshoot of blood pressure resulted by activating on sinoaortic baroreceptors, leads to bradycardia. Mostly, BRS values are measured from phase IV only. For this, a linear regression is performed for changes of SBP and RR interval. However, most studies showed limited clinical applicability in patients with advanced heart diseases. Also the correlation between the BRS value measures from Valsalva and phenylephrine challenge test varied by the range of 0.21-0.91 and raise the question of the reliability of this technique[16].

2.4.3.3 Data Interpretation

The Valsalva maneuver as a respiratory procedure is thought to provide a guide to the integrity of the autonomic neural pathways involved by the cardiovascular reflex to changes in intra thoracic pressure. Deviation from the normal response implies either abnormal heart function or abnormal autonomic nervous control of the heart[7].

The blood pressure response to the Valsalva maneuver is a means to evaluate sympathetic functions (sympathetic pathways to the heart and to the vascular tree), HR response evaluates parasympathetic functions (parasympathetic pathways to the heart), and considering both variation, baroreceptor function is evaluated [7,55,87].

Among Valsalva measurements, VR is a widely used indirect, sensitive, and reproducible measure of parasympathetic function along with the result of deep breathing test. On the other hand, the sympathetic dysfunction is suspected if overshoot in phase IV is absent [40]. For Baroreflex sensitivity, however, the simultaneous rise in heart rate and drop of blood pressure is required to show the extent of the sympathovagal activity.

2.4.3.4 Limitations

The result of this test may be affected by the following confounders: the disability of the subject to attain and keep a 40mmHg expiratory pressure (ex. due to muscle impairment), subject's cooperation and strength, drug therapy that might affect heart rate, and the position of subject (lying or sitting)[1].

2.4.4 Postural change

2.4.4.1 Data collection

An orthostatic challenge (postural change) can be induced by using a manual or electrically operated tilt table, or by making the subject initially sit and then stand or stand as quickly as possible [1]. Prior to the challenge, the subject is kept supine for at least 5-10 minutes and the stability of BP and HR is determined. A tilt table is advantageous, especially in subjects who have neurological disabilities or severe postural hypotension. Also it enables the operator to rapidly and safely return the subject to the horizontal level if symptoms occur [1,37]. On the other hand, in tilt-table testing, the fall of blood pressure may be exaggerated. In active

standing, the BP will not drop dramatically, as it is compensated by a slight increase in HR and constriction of blood vessels in the legs[55].

2.4.4.2 Normal Values

After postural change, normally tachycardia is followed by reflex bradycardia. Maximum heart rate approximately 15 beats after standing represent tachycardia peak and the relative bradycardia occurs at approximately 30 beats after standing. The maximum and minimum heart rate, or longest R-R interval of beats 20-40 and the shortest R-R interval of beats 5-25 are used to calculate 30:15 ratio as it is shown in equation 2.13 [71].

$$30{:}15\ \text{ratio} = \frac{\max HR\ around 15\ sec}{\min HR\ around\ 30\ sec} = \frac{\max RR\ interval\ around\ 30\ sec}{\min RR\ interval\ around\ 15\ sec} \qquad \textbf{Eq. 2.13}$$

A typical normal value for the 30:15 ratio is generally greater than 1.03. Table 2.10 displays age-dependent normal ranges for the 30:15 ratio published in one study[71].Apart from changes in HR, a drop in BP with a rapid correction is observed. In healthy controls, the SBP falls minimally after 1-2 min of standing. So, the fall of systolic blood pressure measured 2 minutes after standing should be less than 10mmHg. The borderline response is when fall of SBP is 10-29 mmHg, and abnormal is a SBP fall of > 30 mmHg with symptoms[87]. One typical normal response of HR and BP after standing is shown in Figure 2.10.

Table 2.10 *normal age dependent range of 30:15 ratio* [71]

Age	**21–30**	**31–40**	**41–50**	**51–60**
Normal range of 30:15 ratio	1.15–1.12	1.12–1.10	1.10–1.08	1.08– 1.07

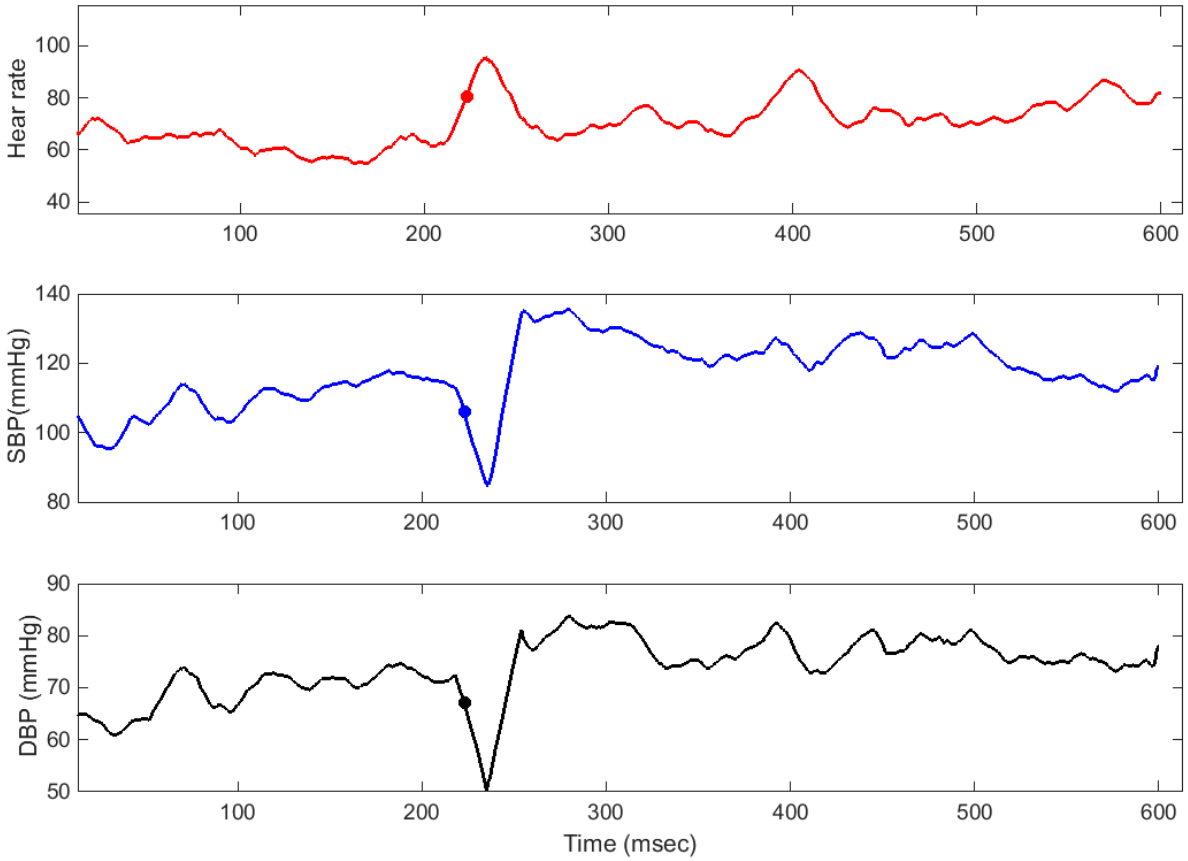


Figure 2.10*Normal responses of BP and HR after standing*

Finally, HRV in the frequency domain is also calculated as described in part 2.4.1.5. The spectral frequency analysis is performed on RR intervals from a 5 minute ECG recording, and parameters of VLF, LF, HF, total power and their normalized units are calculated. The expected measurements are the dominancy of normalized LF power component in comparison with normalized HF power. However, reduced total power is expected compared to HRV in the supine position. HRV measured by the Welch periodogram for a healthy control and the schematic HRV components for 5 min 90° tilt test is displayed in Figure 2.11[68].

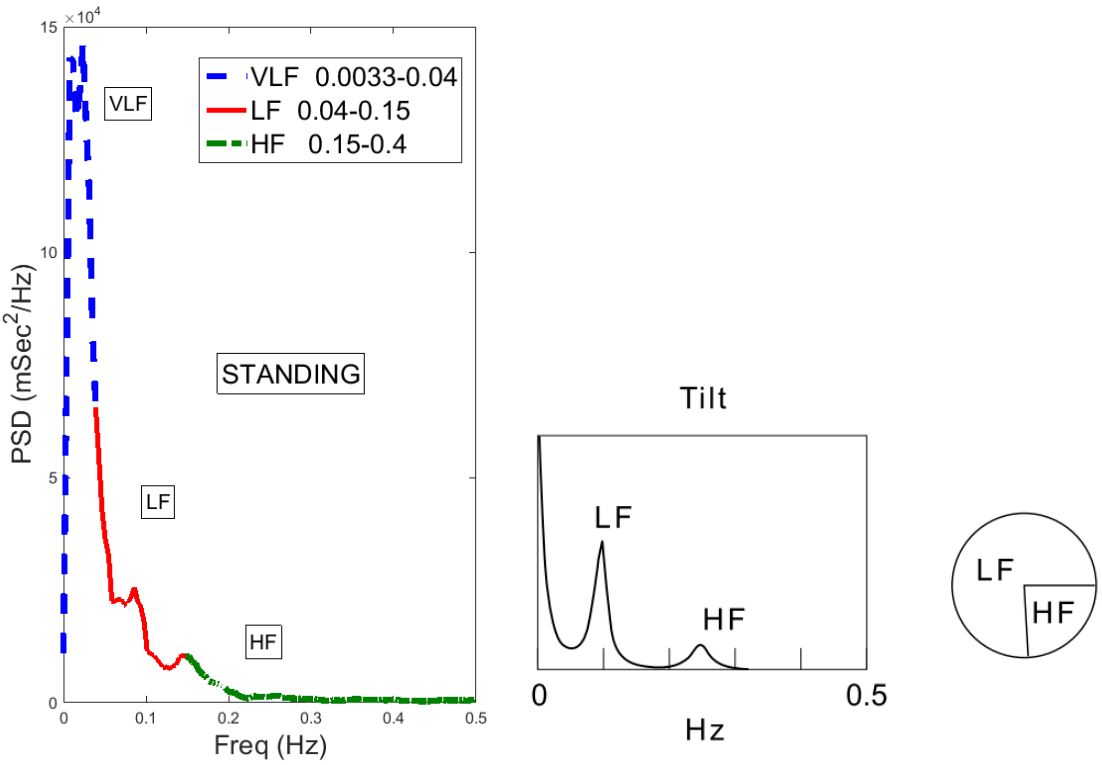


Figure 2.11 *Spectral analysis of HRV by Welch periodogram method and active standing (left),and by autoregressive method and 90° tilt (right)*[68]*. The dominancy of LF shows higher sympathetic activity than parasympathetic activity in standing.*

2.4.4.3 Data Interpretation

Orthostatic challenge is the most commonly performed measure of autonomic function and is used as a separate measurement for some particular dysfunctions[55].Itprovides information about short-term cardiovascular regulation. This test is a commonly performed CANS test and able to evaluate baroreflex function and the sympathetic and parasympathetic nervous system[55,92]. Standing results in translocation of 300-800 cm^3 of blood from the central intravascular section to lower extremities (such as legs, buttocks, pelvis and splanchnic circulation). Then, cardiac stroke volume, arterial blood pressure and blood flow to the brain will decrease. This leads to discharge the baroreceptors in the carotid and aortic walls and as a consequence, sympathetic activation and parasympathetic withdrawal occurs through baroreflex mediated autonomic regulation. Peripheral vasoconstriction recovers BP by decrease in peripheral blood flow. [92,93]

The increase of HR in 1-2 cardiac cycles (3 s) into the standing position is due to parasympathetic withdrawal. Then a further slow increase in HR occurs in 6-8 cardiac cycles (12 s) because of increase in vascular resistance, vascular tone, and cardiac contractility as a result of sympathetic activation in addition to further parasympathetic inhibition. The explained tachycardia in addition to the peripheral vasoconstriction prevents a significant fall in arterial pressure. In normal subjects, SBP falls minimally after 1-2 minutes, DBP increases by approximately 10 mmHg and HR increases by about 10 beats/minutes[1,55,94,95].

Any excessive change or lack of change in BP or HR should be suspected for autonomic disorders[1]. For instance, orthostatic hypotension is defined as a drop in systolic blood pressure of at least 20 mmHg or diastolic blood pressure of at least 10 mmHg within 3 min of standing [96], or postural orthostatic tachycardia syndrome (POTS) is characterized by an exaggerated increase in heart rate of greater than 30 beats per minutes which occurs during 10 minutes of tilt or standing, without orthostatic hypotension in a patient with a history of specific symptoms[97].More severely affected POTS patients may have an increase of HR greater or equal to 120 beats/minutes[55]. On the other hand, if HR does not change in standing in the presence of substantial fall of SBP, it indicates baroreflex abnormality due to afferent baroreceptor lesions or both sympathetic and parasympatheticfailure[1]. A rapid fall in BP is observed sometimes, due to inability to activate sympathetic vasoconstrictor pathways in response to postural change which is usually followed by partial recovery of BP[1].

Altogether, the 30:15 ratio provides a measure of cardiac vagal function and abnormal changes in BP is mainly indicative of sympathetic failure[1,55,98]. HRV at standing, on the other hand may provide valuable information, as the failure to increase LF may be a reflection of impaired sympathetic response or depressed baroreceptor sensitivity[68].

2.4.4.4 Limitations

It is difficult to compare active standing results with tilt table results due to different responses. In passive tilting, the procedure of postural change is slow (5-10 sec) thus regulatory response is activated before the subject is fully tilted. Also, limited muscle activity is required and tilt angle is not 90° (gravity force differs). In active standing, on the other hand, the process is fast (1-5 sec) and requires active muscle contraction. As a result, an immediate increase in HR is observed due to muscle contraction in initiate standing. This increase in HR occurs before the initial drop in blood pressure and is possibly activated by combined effects of vestibular and central command stimulation of muscle sympathetic nerve activity[1,92].

Another limitation is that, the postural change test should be terminated in the presence of any discomfort, chest pain, shortness of breath, dizziness, orsyncope[44].

Finally, there are some doubts about sensitivity and correlation of postural change with other autonomic reflexes. For instance the30:15 ratio and deep breathing which should resemble the parasympathetic activity, have low correlation which suggest involvement of different physiological mechanism in two tests[1,71,94].

2.4.5 Isometric Exercise

2.4.5.1 Data Collection

An isometric exercise test is performed by using either a dynamometer or a partially inflated brachial cuff (conventionally used for blood pressure measurement) attached to a pressure gauge. For this test the subject (in sitting position) squeezes the handgrip with the dominant hand for a few seconds to establish a maximum effort. After at least three minutes rest to

obtain baseline values of BP, the subject is asked to sustain the handgrip constantly to 30% of the maximum effort for 3-5 minutes or until exhaustion.

2.4.5.2 Normal Values

The normal response for diastolic blood pressure is a rise of greater than 16 mmHg in the other arm [1,55], while the range between 10-15 mmHg is usually considered as borderline and less than 10 mmHg increase is assumed to be abnormal[57]. Table 2.11 provides the ranges and average values of changes in DBP for 122 healthy control in different age bands[1].

Table 2.11 *Normative values of changes of DBP, SBP and HR in isometric exercise test studied on 122 control subjects in different age bands* [1].

Age	**20-29**	**30-39**	**40-49**	**50-59**	**60-69**	**>70**
Δ DBP (mean ± STD) Range	13±2 (9:17)	11±2 (7:15)	12±2 (8:14)	11±3 (5:17)	11±2 (7:15)	11±1 (9:13)
ΔSBP (mean ± STD) Range	15±3 (9:21)	13±2 (9:17)	15±3 (9:21)	19±3 (13:25)	17±3 (11:23)	2±4 (14:30)
ΔHR (mean ± STD) Range	7±1 (5:9)	7±2 (3:11)	9±2 (5:13)	7±2 (3:11)	4±1 (2:6)	6±1 (4:8)

2.4.5.3 Data Interpretation

In this test, blood pressure, heart rate and sympathetic nerve activity increase. Two responsible mechanisms for this include: pressor reflex (neurological reflex) that constricts small blood vessels during muscular contraction and thereby increases the blood pressure and central command (CNS) which increases efferent sympathetic activity due to activation of cardiovascular centers involved in initiation of somatomotor activity[55,99]. Usually the rise in DBP is used as a measure of sympathetic evaluation in isometric exercise test.

2.4.5.4 Limitations

Although these changes in blood pressure and heart rate have been used as a clinical test of sympathetic function[7,55,65], some factors independent of the autonomic nervous system may affect the responses. First, the response may be variable due to difficulty in standardizing the muscular effort [99]. In addition, in trained muscles, lower increase in BP will be obtained because of reduced sensitivity of muscle afferents to accumulated metabolites. Finally, decrease in metabolite accumulation also may reduce the muscle chemoreceptor afferent activity and decrease BP enhancement. Altogether, since the sensitivity and specificity of this test is low, its results usually evaluated along with changes of BP in postural change and Valsalva maneuver[55].

2.5 Other Common CANS tests

The other tests for evaluation of cardiac autonomic nervous system include sweat test, tilt table, pressor stimuli group (such as isometric exercise), and Coetaneous cold (immersing the hand for up to 2 minutes in ice slush, usually just below 4°C)[68].

Chapter 3
Methods

In this section, information about subject recruitment protocol, the conditions needed to participate in CANS test in order to obtain universally approved and reproducible results, capture data, data collection instrument and processing software are described. The most common tests for CANS assessment (including those used in the study presented in this thesis) are explained in detail. Also, the expected output and interpretation of out of range results are described.

3.1 Data Collection

3.1.1 Patient recruitment protocol

The patients who participated in this study were diagnosed with definite multiple sclerosis according to Poster's classification and they were regularly attending the MS clinic at Royal North Shore Hospital (RNSH), Sydney, NSW[100,101]. Patients were recruited at the MS clinic after the MS neurologist informed the patients about this test. If the patient was interested in participating, a one-hour study time was arranged, at the same day of their. Participation in this study was entirely voluntary, and subjects were informed that their decision to participate would not affect their treatment and their relationship with the staff. Subjects were given a

written information sheet about the study, and written, informed consent was obtained from all participants. This study had the ethics approval of the Northern Sydney Local Health District (Ethics Committee reference number: HREC/12/HAWKE/397). The project was also approved by the Macquarie University Human Ethics Committee, (reference number 5201600002) acknowledging the approval of the Northern Sydney Local Health District ethics committee.

3.1.2 Control data collection

Control (healthy normal) subjects were recruited from the Macquarie University staff and student base. Written informed consent was obtained from all participants. The data collection in control subjects was approved by Macquarie University Human Ethics Committee (reference number 5201300055).

The collected data for the control group included only supine-rest test. Although in Section 2.3, it is suggested that each study, should have their own control subjects, in this study the results of reflex tests in MS subjects were compared to normal ranges for healthy controls published in previous studies (Appendix A). Control subjects with matched age and sex with each MS subject were collected from the control dataset.

3.1.3 Demographic information collection

Demographic information was collected including age, sex, height, weight, dominant hand, date of birth and BMI as calculated by the relation of $\frac{weight\ (kg)}{height^2\ (m2)}$.

3.1.4 Clinical Information Collection

All clinical data were collected and provided by Dr. John Parratt and Dr. Yi-ching Lee, the neurologists of MS clinic at RNSH. The Clinical information includes the following:

1- **Disease duration:** Calculated from date of test and onset of disease.Onset of disease is set as the first symptom of MS patients experienced. For most patients, they seek treatment after the first symptom and so the onset is recorded in their medical history. For some patients with long disease duration, usually their MS was not diagnosed after the first symptom or either it was overlooked. So, patient's own recall of the first symptom is considered as onset

2- **Number of lesions:** The number of lesions as imaged in MRI scans of brain and spine, counted by Dr Yi-ching Lee. The number of lesions was updated based on the most recent MRI scans with the date of scans.

3- **EDSS & MSSS scores:** The EDSS and MSSS scores were updated with the most recent MRI scans. The EDSS quantifies disability in eight functional systems (FS). The FS include pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, cerebral and other. The EDSS scale is from 0 (normal neurological exam) to 10 (death due to MS) with steps of 0.5 score. The MSSS score is calculated from the EDSS and corrects it for disease duration by compare patient's disability score with the distribution of scores in a database for same disease durations[32,33].

4- **Topography:** The number of lesions in each of deep white matter, brainstem, spine and cortical are counted. Then if the lesions are localized in one section of CNS, the topography is named after that section as dwMS, bcMS, sMS and cMS respectively. If the lesions were spread in the whole CNS, the topography type is considered as general MS (gMS).

5- **Progress of disease:** This is defined by the neurologists, based on identification of remitting or progressive relapses, and primary or secondary mode of progressive MS.

6- **Treatment delay:** The treatment for a patient indicate the relative time that treatment is postponed after onset of disease. Four groups available in this clinical variable are:

    - (N) Never treated.

- (E) Treatment started early, within 2 years after onset and still continuing till now (based on the investigation showed that number of relapse in the first two years correlates with severity of disease)[102,103].
- (L) Treatment late, postponed more than 10 years after onset.
- (M) Any kind of treatment delay except that E and L. (i.e. treatment starts after 2 years but before 10 years of onset, or started within 2 years after onset and stopped)

3.2 Cardiovascular autonomic nervous system evaluation

3.2.1 Test Requirements

Subject's condition for CANS test as explained in Section 2.1 was followed for both MS and control subjects.

A continuous and noninvasive measurement of finger arterial pressure was obtained using a Nexfin monitor by Finapres methodology (Edwards Nexfin®, Amsterdam, The Netherlands). The non-invasive Finapres method is an established substitute for invasive intra-arterial BP measurements in both clinical and research settings[101,104–109]. The Finapres system measures beat-to-beat BP using a finger cuff[50,96]. The cuffs were available in three sizes and were wrapped around the middle finger of the non-dominant hand as shown in Figure 3.1. An electrocardiogram (ECG) was acquired simultaneously using 4 standard ECG limb electrodes attached to the patient's upper body, the signal acquired using the Nexfin device. The ECG and arterial finger blood pressure were continuously measured. The test was performed in a sound/noise proof room to minimize any arousal stimuli, and the temperature was kept at 22°C with central air conditioning.

In addition to online monitoring of HR and BP changes in different reflex tests, the data was stored for off-line analysis. During the test, start and stop points of each test were marked

manually on the monitor. The data was extracted and converted to files readable in MATLAB and Excel using the program FrameInspector.exe (provided by the Edwards Lifescience company). The data generated from FrameInspector comes in two parts. The first part (Excel) contains beat-to-beat information of HR, RR interval, SBP, DBP, MBP, cardiac output, and stroke volume in which the index of marked points are also provided. This format is used for generating CANS values and figures. The second part provides the continuous ECG waveform (at 1000Hz sampling rate) and the BP waveform (at 200Hz sampling rate) in binary format, which is readable by MATLAB for further morphological illustrations.

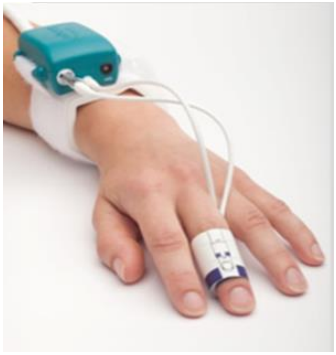


Figure 3.1 *Finger cuff system used by Nexfin* [110].

3.2.2 Data Collection

With the subject in the supine position, 4 ECG electrodes were attached to the upper body in the standard positions, and the BP cuff and height correction transducer cuff (that adjusts for the hydrostatic pressure difference between the position of the finger relative to the position of the heart) were wrapped around middle and index fingers of non-dominant hand. All tests were done in the following order:

3.2.2.1 Supine Rest Test

The first test in this study was supine rest. The subject lay down with closed eyes for 8-10 minutes, in the quiet, dark test room. The subject's breathing should be normal, and the body should be kept as still as possible, especially his/her non-dominant hand (on which cuff are

attached). From the recorded data, the latest 5 minutes NN RR tachogram was extracted for analysis, and the first few minutes was discarded (adequate time needed for BP and HR to adapt to supine position)[49,68,80,81,111,112]. The calculated measurements of this test are explained in Section 2.4.1 and listed in the Table 3.1 (variables 1 to 64).

3.2.2.2 Deep Breathing Test

After 8-10 minutes of rest, the subject was still kept in the supine position. The lights were turned on and kept on for the rest of tests. A few practice deep breaths were completed by the subject, as lead by the investigator. Then after 2-3 minutes rest, the subject was instructed to perform 8 consecutive deep breaths as explained in section 2.4.2. Monitoring of CO_2 to avoid hyperventilation was not feasible with this study's equipment. However, the chest movement was controlled. The data was labelled at the beginning and end of each cycle for offline analysis. The two variables extracted from this test calculated by equation 2.10 and 2.11 in section 2.4.2 and listed as variables 65 and 66 in Table 3.1.

3.2.2.3 Valsalva maneuver

After the deep breathing test, the subject was asked to perform one or two Valsalva maneuvers as practice. After 2-3 minutes rest after practice, the subject was instructed to perform a Valsalva maneuver in the supine position for 15 seconds as explained in section 2.4.3. A disposable syringe (5-10 ml) connected to the mercury column of a sphygmomanometer was used as the mouthpiece in this study. This test was repeated 2 times and 2-3 minute rest was given to the subjects in between tests. Increasing and keeping the pressure to 40 mmHg was difficult for some subjects. For those, any pressure between 20mmHg and 40mmHg was considered sufficient, as it is reported to result in necessary changes in previous studies[1]. Four variables were measured from the Valsalva maneuver as explained in section 2.4.3 and listed in Table 3.1 (variables 67 to 70).

3.2.2.4 Postural change

The subject was given 3-4 minutes to rest after the Valsalva manoeuvre. The subject was then asked to perform active standing. This test could not be performed by disabled participants (2 subjects). In this, subjects stood up as quickly as possible. In addition to monitoring BP to identify any risk of symptoms, the subjects were informed that in case of dizziness they should lean to the bed or sit down. Any discomfort, chest pain, shortness of breath or dizziness did not present in the subjects studied. Therefore, orthostatic intolerance was not expected, and participants continued standing quietly and as still as possible for 5-6 minutes. The 16 variables extracted from this test explained in section 2.4.4 are listed in the Table 3.1 (variables 71 - 86).

3.2.2.5 Hand Grip Test

The last test in this study was isometric exercise. In this test the subject was kept in the sitting position for 2-3 minutes after the postural change before performing isometric exercise as described in section 2.4.5. As a hand grip, a partially inflated brachial cuff (conventionally used for blood pressure measurement) attached to a pressure gauge was used. One variable was extracted from this test which is explained in section 2.4.5 and listed as the 87^{th} variable in the Table 3.1.

Table 3.1 *The variables extracted from CANS tests (Explained in Section 2.4).*

Parameter number	Test	Parameter
1	supine rest	SBP
2	supine rest	DBP
3	supine rest	MBP
4	supine rest	HR
5	supine rest	Mean RR
6	supine rest	SDNN
7	supine rest	rMSSD
8	supine rest	PNN50%
9, 10	supine rest	TP of HRV_{supine}&$SBPV_{supine}$
11, 12	supine rest	VLF of HRV_{supine}&$SBPV_{supine}$
13, 14	supine rest	LF of HRV_{supine}&$SBPV_{supine}$
15, 16	supine rest	HF of HRV_{supine}&$SBPV_{supine}$
17, 18	supine rest	LF_{nu} of HRV_{supine}&$SBPV_{supine}$
19, 20	supine rest	HF_{nu} of HRV_{supine}&$SBPV_{supine}$
21, 22	supine rest	LF/HF of HRV_{supine}&$SBPV_{supine}$
23	supine rest	α_{LF}
24	supine rest	Number of data points included in α_{LF}
25	supine rest	α_{HF}
26	supine rest	Number of data points included in α_{HF}
27	supine rest	BRS_{LF} by mean value
28	supine rest	Number of data points included in BRS_{LF}
29	supine rest	BRS_{HF} by mean value
30	supine rest	Number of data points included in BRS_{HF}
31	supine rest	Mean value of $BRS_{Modulus\ LF}$
32	supine rest	Mean value of $BRS_{Modulus\ HF}$
33	supine rest	Mean value of $BRS_{Modulud}$

Table 3.1 continued. *The variables extracted from CANS tests (Explained in Section 2.4).*

Parameter number	Test	Parameter
34, 35, 36, 37	supine rest	BRS lag 0,1, 2 and 3
38,39,40,41	supine rest	Number of BRS sequences lag 0, 1, 2, 3
42,43,44,45	supine rest	BRS lag 0,1, 2, 3 positive slopes
46, 47, 48, 49	supine rest	No. of BRS lag 0,1, 2,3 positive slopes
50, 51, 52, 53	supine rest	BRS lag 0,1,2,3 negative slopes
54,55,56,57	supine rest	No. of BRS lag 0,1,2, 3 negative slopes
58	supine rest	Total BRS gain
59	supine rest	Total BRS positive slope sequences
60	supine rest	Total BRS negative slope sequences
61	supine rest	PQ interval
62	supine rest	QRS interval
63	supine rest	QT interval
64	supine rest	QT_c interval
65, 66	Valsalva maneuver	RSA & E:I ratio
67	Valsalva maneuver	VR ratio
68	Valsalva maneuver	Maximal drop of MBP in early phase II
69	Valsalva maneuver	MBP at late phase II
70	Valsalva maneuver	MBP at late phase IV
71	Postural change	30:15 ratio in postural change test
72	Postural change	SBP change 2 minutes post postural change
73, 74	Postural change	TP of HRV_{supine}&$SBPV_{supine}$
75, 76	Postural change	VLF of HRV_{supine}&$SBPV_{supine}$
77, 78	Postural change	LF of HRV_{supine}&$SBPV_{supine}$
79, 80	Postural change	HF of HRV_{supine}&$SBPV_{supine}$
81, 82	Postural change	LF_{nu} of HRV_{supine}&$SBPV_{supine}$

Table 3.1 continued. *The variables extracted from CANS tests (Explained in Section 2.4).*

Parameter number	Test	Parameter
83, 84	Postural change	HF_{nu} of HRV_{supine}&$SBPV_{supine}$
85, 86	Postural change	LF/HF of HRV_{supine}&$SBPV_{supine}$
87	Hand grip test	Changes in DBP in hand grip test

3.3 Data analysis

Three types of analysis were done in this study. For all analyses, MATLAB software (version R2016a) was used. The threshold for significant results was considered as $p<0.05$. The analyses are explained in the following with the relative statistics used for each test.

3.3.1 Comparing MS to controls

As only 5-minute supine rest was available for controls (and not the reflex tests), the first 60 features of Table 3.1 were extracted from controls. Also, 5 demographic parameters of control and MS patients were included in the study. Except gender, which is categorical and matched for controls and patients, each feature was compared between MS and control groups. The normality of distribution of each feature was tested and rejected by Kolmogorov-Smirnov test (‘*kstest’* function in MATLAB). The Mann Whitney U test (‘*ranksum’* function in MATLAB) was applied as it does not require the assumption of normal distribution. The features with significant p-values (p-value<0.05) were selected as the input of multi logistic regression. The dependent variable of the multi logistic regression method however is a column with the values of 0 and 1 representing MS and control class. The function ‘*mnrfit’* in MATLAB was used for multi logistic regression and the reported result are the independent features significantly different between MS and controls.

3.3.2 Comparing Autonomic Variables with Clinical Variables

All of the 87 variables listed in Table 3.1, as well as demographic information of patients, provide a data set of 92 features for MS subjects. Among those, 3 features of sex, changes of MBP at late Phase II and changes of MBP at phase IV of Valsalva maneuver are categorical and the rest are numeric variables. All 92 features were compared within 7 clinical variables. These comparisons are provided with the two following methods for continuous and categorical clinical variables respectively:

3.3.2.1 Comparing Autonomic Variables with Continuous Clinical Variables

The continuous clinical variables included disease duration, EDSS, MSSS and number of lesions, the groups within each were compared for all autonomic variables. Linear regression analysis (‘*regress’* function in MATLAB) was performed between each MS feature continuous clinical variables. The variables with significant p-values of regression lines were collected as the input to step wise regression analysis (‘*stepwisefit*’ function in MATLAB). The independent significant variables from the stepwise linear regression are reported for linear regression modelling of the clinical variable.

3.3.2.2 Comparing Autonomic Variables with Categorical Clinical variables

The categorical clinical variables include topography, progress type and treatment delay. The variable treatment delay can be assumed as hierarchical categorical variable while the other two are nominal. An ANOVA test was performed to identify the features that were significantly different among categories. Then the features with significant p-values were assigned as the input of post-hoc comparison method to find the corrected p-values and distinct independent variables.

3.3.3 Comparing MS subjects with normal and abnormal cardiac autonomic reflexes

The features from 4 challenge studies of autonomic test were checked with the normal ranges of these variables, as described in Appendix A. These four tests and their features include:

- Deep breathing (RSA (Respiratory sinus Arrhythmia), E:I ratio (Average HR variation inspiration/expiration))
- Valsalva maneuver (VR (Valsalva ratio: HR_{max}/HR_{min}), changes of MBP in Valsalva, MBP in late phase II, MBP at phase IV)
- Postural challenge (30:15 ratio (max RR around 30 sec/min RR around 15 sec), changes of SBP 2 minutes after standing), and
- Isometric exercise (change of DBP in hand grip test).

Almost all of these features are age dependent and their normal ranges are available for different age bands. So if subjects are classified as normal (1) or abnormal (0) in each test, the age factor is already being considered. Based on the standard autonomic testing, RSA, VR and 30:15 ratio are used for parasympathetic scoring. However for sympathetic scoring, only ΔSBP 2 min after standing and ΔDBP in hand grip test were used [55,93]. The total scores of 5 tests were calculated that could vary from 0 to 5. If 2 or more tests were found as abnormal, the CAD was diagnosed[23]. However, since the measurements of sympathetic activity by Valsalva maneuver parameters were also available, the scoring in this study was expanded. Therefore, 3 tests were included for sympathetic scoring. The parameters included in sympathetic and parasympathetic scoring are illustrated in Table 3.2. As suggested in previous studies the sympathovagal score is calculated by accumulation the sympathetic and parasympathetic score[86,87,93]. This measurement can vary from 0 to 6 with equal share of sympathetic and parasympathetic parameters. The sympathovagal score below 4 (more than 2

abnormal test) was considered as abnormal. This score was compared to clinical variables by chi-square test.

Also, to investigate the sympathetic and parasympathetic activity individually, the two scores were also compared to clinical variables. The scores vary from 0 to 3, and based on interpreting sympathovagal score, the individual scores <2 (more than 1 abnormal results) was considered as sympathetic or parasympathetic dysfunction, respectively.

Table 3.2 Parasympathetic and Sympathetic scores can vary from 0-3.

Parasympathetic features	Coefficients of parasympathetic	Sympathetic features	Coefficients of sympathetic scoring
RSA	1	ΔBP in Valsalva	1: ΔMBP Valslava 0.5 MPB in late phase II 0.5
VR	1	ΔSBP 2 min after standing	1
30:15 ratio	1	ΔDBP in hand grip test	1

Chapter 4
Results

By following the protocol explained in Chapter 3, 53 patients with clinically diagnosed Multiple Sclerosis (MS) were studied. The demographic information of MS subjects in this study is illustrated in Table 4.1 and Figure 4.1**.**

Table 4.1 *Demographic information of MS participants in this study.*

	Females	Males	all subjects	range
Number	34 (64%)	19 (36%)	53	
Age (year)	47.7±13.8	47.2±13.6	47.5±13.5	19-69
Age at Onset (year)	35.6±11.4	31±11	33.9± 11.5	11-59
Disease duration (years)	13.4±11.5	13.3±8.2	13.4±10.4	1-47
EDSS	2.1±1.8	2.1±1.7	2.1±1.7	0-7
MSSS	2.3±1.8	2.4±2.2	2.3±2	0.01-7.33
Total Number of lesions	36±20	35±16	36±20	4-83

Data represented as mean ± standard deviation. EDSS: Expanded Disability Status Scale. MSSS: Multiple Sclerosis Severity Score

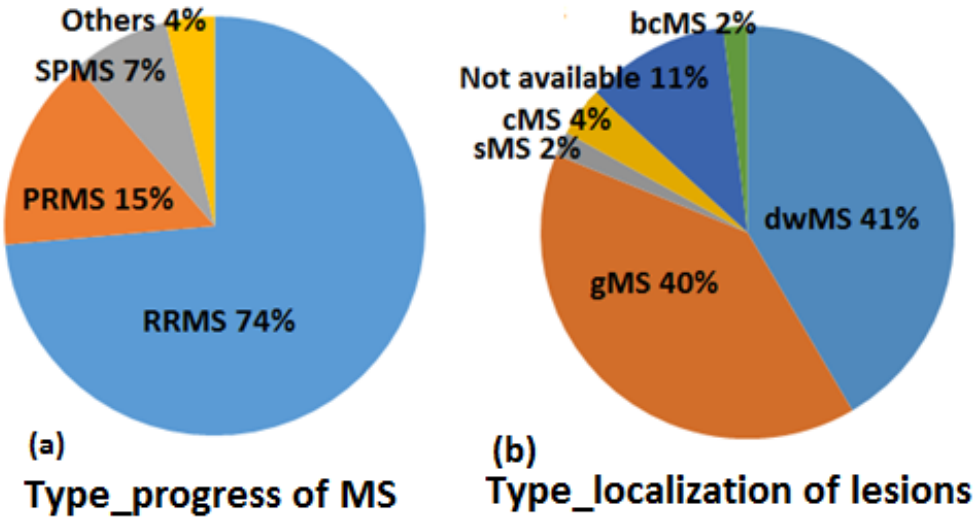


Figure 4.1 *Prevalence of MS types in this study.**(a)** Progress-based classification: RRMS (Relapsing Remitting MS), PRMS (Progressive Relapsing MS), SPMS (Secondary Progressive MS), Others (1 subject: not available, 1 subject: CIS (Clinically Isolated Syndrome) that one episode of symptoms is detected), **(b)** Lesion's topography-based classification: dwMS (deep white matter MS), gMS (generalized MS), sMS (spinal MS), cMS (cortical MS),bcMS (brain stem/cerebellar), and Not available.*

4.1 Multiple Sclerosis differences from control subjects

23 control subjects with the same age and sex as 23 MS subjects were selected. Among all 92 cardiac autonomic features, 65 features extracted from 5-minute supine rest were compared between these 2 groups. Demographic information of 23 MS subjects and their matched controls are shown in Table 4.2. Of all the measured parameters, there were 11 that were significantly different between MS and control subjects (Table 4.3). Entering these variables (with the exception of HF SBPV in normalized units, supine position, which is equal to 1 minus LF SBPV in normal units, supine position and therefore removed) into the multinomial logistic regression resulted in three of the variables remaining significant predictors. The features with significant p-values in the model and yet independent from each other include BRS by frequency technique (coherence criteria) in HF band (α_{HF}) (p=0.0391) and mean of

BRS Modulus in HF band (0.0412). These two features have lower values in MS comparing to control group. Also height was higher in MS, though with p=0.0500. Figure 4.2 illustrates the distribution of three mentioned features in two groups of MS and control.

The Mann Whitney U test (function '*ranksum'* in MATLAB) was used to compare each variable between the two independent groups of MS and control with significance set for p-value<0.05. The variables that were significantly different between the groups were entered in a multinomial logistic regression ('*mnrfit'* function in MATLAB) and the significant predictor variables noted.

Table 4.2 *Demographic information of 23 MS subjects and matched controls.*

Demographic features	23 MS subjects	23 controls
Age (year)	37.5±11.8 (19-65)	37.7±11.9(20-66)
Female/Male	15/8	15/8
Height (cm)	171±10.7	164±9.3
Weight (kg)	71±17	60-10
BMI (kg/m^2)	24±4.5	22.2±2.8

Data represented and mean ± standard deviation (range).

Table 4.3 *Variables those were significantly different between MS and control.*

Variable	MS	Control	P
Lower in MS			
LF/HF ratio SBPV, supine position(unit less)	3.9±3.17	8.9±6.8	0.006
LF SBPV in normalised unit, supine position(unit less)	69.5±18.8	82.1±14.3	0.006
BRS (coherence criteria) in HF band(α_{HF})(ms/mmHg)	15.5±9	28.7±16.7	0.003
BRS (mean criteria) in HF band(ms/mmHg)	21.7±12.5	39.2±25	0.007
Mean of BRS Modulus, HF band(ms/mmHg)	16.1±9.3	28.6±17	0.006
Mean of BRS Modulus(ms/mmHg)	14.8±8	24.8±15.4	0.003
Higher in MS			
Height(cm)	171.4±10.8	164.4±9.3	0.035
Weight(kg)	71.2±17.3	60.1±9.6	0.026
HF SBPV in normalised units, supine position(unit less)	30.5±18.8	17.9±14.3	0.006
BRS negative sequences of at Lag 3(ms/mmHg)	16.2±7.4	11.6±9.5	0.045

BRS: baroreceptor sensitivity. LF: low frequency. HF: high frequency. SBP: systolic blood pressure

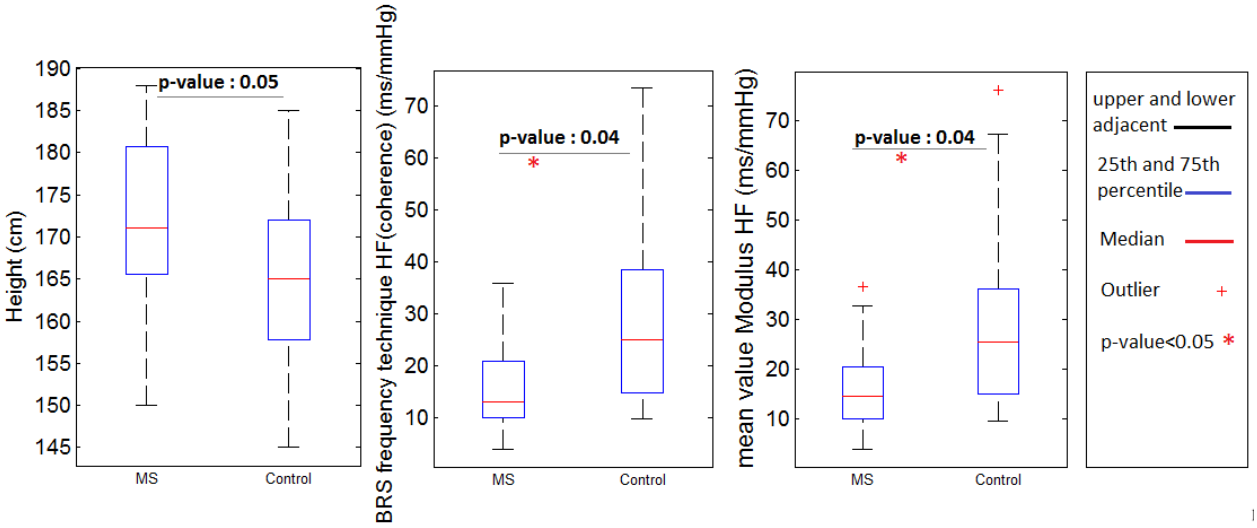


Figure 4.2 *Three features with significant differences between MS and control. The upper adjacent and the lower adjacent show the maximum and minimum data, after excluding outliers in each group. The outliers are the data points outside the rage of +-2.7 sigma for a normal distribution (this rage cover 99.3% of data in a normal distribution)*

Multinomial logistic regression finds independent predictor variables, and it is therefore supposed that BRS by frequency technique (coherence criteria) in HF band (α_{HF}) and mean of BRS Modulus in HF band are not predicted by height. However, to show their independency, their scatter plots together with height are demonstrated in Figure 4.3. Since the regression lines for control and MS are not parallel, the linear regression was not significant ($p>0.05$) and R^2 values were all small, it shows that height was independent of both BRS measures in this data set.

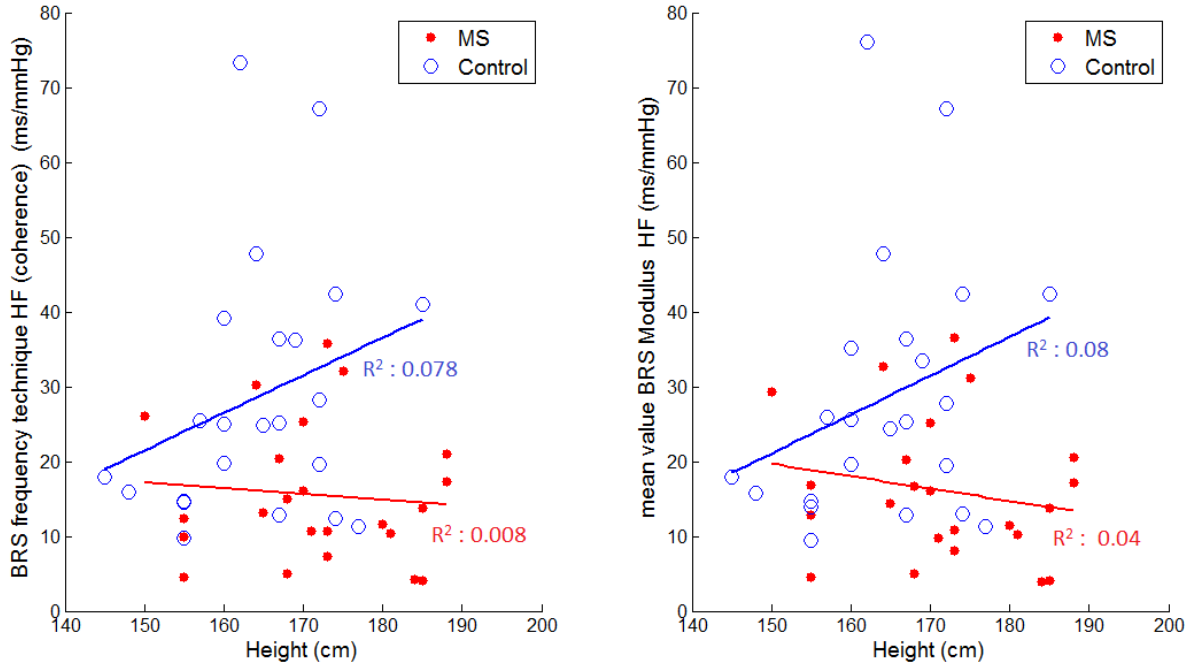


Figure 4.3 *The regression lines had low goodness of fit (R^2) reject the hypostasis that height was a predictor of BRS by frequency technique (coherence criteria) in HF band or mean of BRS Modulus in HF band.*

4.2 Sub-group analysis within Multiple Sclerosis patients

4.2.1 Disease duration

The parameter of disease duration was available in all but one patient, who was eliminated in this component of the analysis. There was a significant linear relationship between disease duration and eighteen individual variables (Table 4.4).Among these 18 features, variables calculated from BRS by sequence technique (features 9 to 18) are strongly inter-dependent and largely measure the same thing. Therefore, to reduce the error of too many input variables for stepwise regression, only variable number 9 (BRS at lag 0) was selected from the last 10 features and placed into the stepwise linear regression with the first 9 variables. The resultant model remained with age (years) and LF/HF ratio of SBPV in the supine position (ratio, unit less) as predictors of disease duration (years) (R^2=0.40, $p<0.001$, equation 4.1).Scatter plots and linear regression lines between the individual output and input variables resulting from the stepwise linear regression are shown in Figure 4.4. Also, the two resulting independent features are plotted against each other in Figure 4.5 to show their relationship.

$$\text{Disease duration} = 10.2 + 0.43 \times \text{age} + 0.74 \times \text{LF/HF}_{\text{SBPV}} \qquad \textbf{Eq. 4.1}$$

Table 4.4 *The features that had a significant linear regression with disease duration.*

Variable	slope	intercept	R^2	P
positive slope				
age (years)	0.43	-7.16	0.32	0.000
SBP (mmHg)	0.21	-13.34	0.14	0.006
DBP (mmHg)	0.46	-16.25	0.14	0.007
LF/HF SBPV, supine position (unit less)	0.86	9.88	0.09	0.029
PQ interval (ms)	0.09	-2.61	0.08	0.056
negative slope				
BRS (coherence criteria) LF band (α_{LF}) (ms/mmHg)	-0.07	19.47	0.10	0.021
BRS (mean criteria) LF band (ms/mmHg)	-0.52	19.24	0.10	0.021
Mean BRS modulus LF band (ms/mmHg)	-0.75	19.83	0.11	0.017
BRS at Lag 0 (ms/mmHg)	-0.43	18.69	0.10	0.025
BRS negative sequences Lag 0 (ms/mmHg)	-0.41	18.66	0.10	0.019
BRS at Lag 1 (ms/mmHg)	-0.47	18.82	0.09	0.028
BRS positive sequences Lag 1 (ms/mmHg)	-0.44	18.40	0.09	0.030
BRS negative sequences Lag 1 (ms/mmHg)	-0.47	18.71	0.12	0.010
BRS at Lag 3 (ms/mmHg)	-0.38	18.75	0.07	0.049
BRS negative sequences Lag 3 (ms/mmHg)	-0.49	19.62	0.13	0.010
BRS number of Lag 3 negative sequences	-0.48	16.28	0.09	0.032
BRS total gain, sequence technique (ms/mmHg)	-0.48	19.34	0.10	0.024

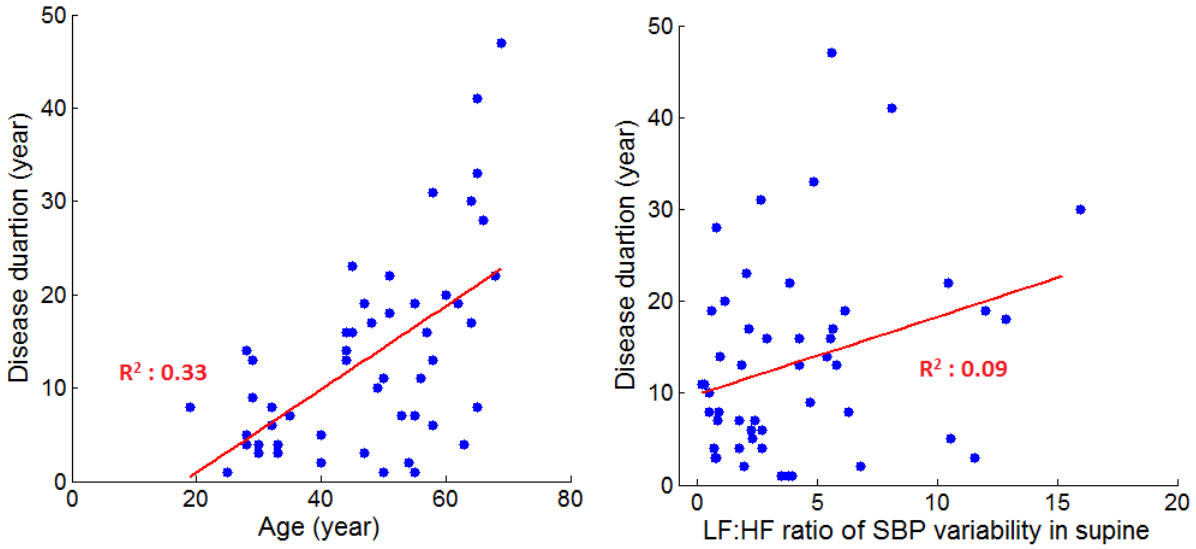


Figure 4.4 *Age showed a high positive correlation with disease duration (p=0.000).LF/HF ratio of SBP variability in the supine position showed a lower goodness of fit (R^2=0.09), buy was positively correlated with disease duration (p=0.035).*

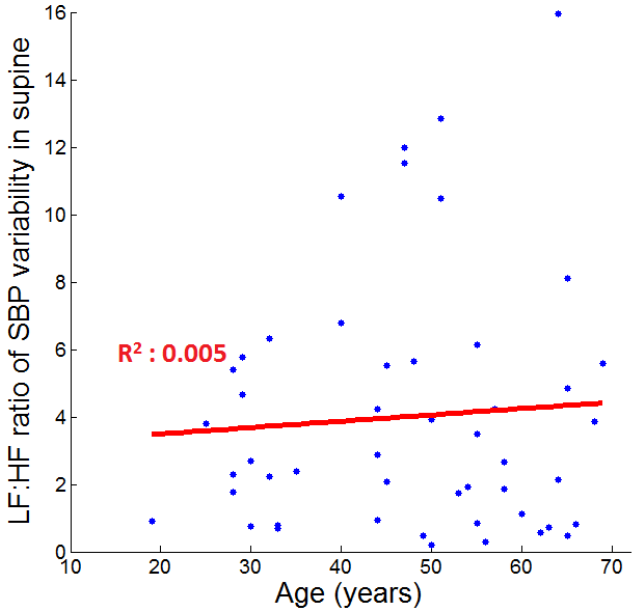


Figure 4.5 *Age and LF/HF ratio of SBP variability in the supine position were not correlated (p=0.63).*

The linear regression between age and LF/HF ratio of SBP variability in the supine position was weak but significant (Figure 4.4). However, the regression between age and disease duration was also significant (Figure 4.4).That age is a dominant confounder of disease duration is not entirely surprising. The analysis presented here is a post-hoc application to the data and this study was not designed to find the consequences of disease duration on autonomic features. For that aim, as study design should control for age by selecting subjects of the same age and different disease durations.

4.2.2 Total number of lesions

The total number of lesions in the CNS was not available for 7 subjects. Linear regression showed two variables were significantly related to number of lesions: changes of MBP in the Valsalva maneuver and change of SBP 2 minutes after standing. These two features were both negatively correlated with total number of lesions, multiple linear regression giving the model in Equation 3.2 (R^2=0.22, p=0.010).

$$\text{total lesions} = 35.0 + 0.46 \times \Delta\text{MBP}_{\text{Valsalva}} + 0.44 \times \Delta\text{SBP}_{\text{2 minutes standing}} \qquad \textbf{Eq. 4.2}$$

The two independent features are representing changes of BP compared to the baseline value in response to a cardiovascular challenge. Therefore, the meaning of negative coefficients is that as the number of lesions increases, the BP drop is greater in response to the cardiovascular challenge. The scatter plot of these two predictor variables against each other is illustrated in Figure 4.6, with a significant relationship between the variables. As a consequence, for the subjects who are unable to perform either Valsalva maneuver or postural change, one parameter may be estimated from the other one, and both give some information as to the total number of lesions in MS patients, as shown in the linear regressions presented in Figure 4.7.

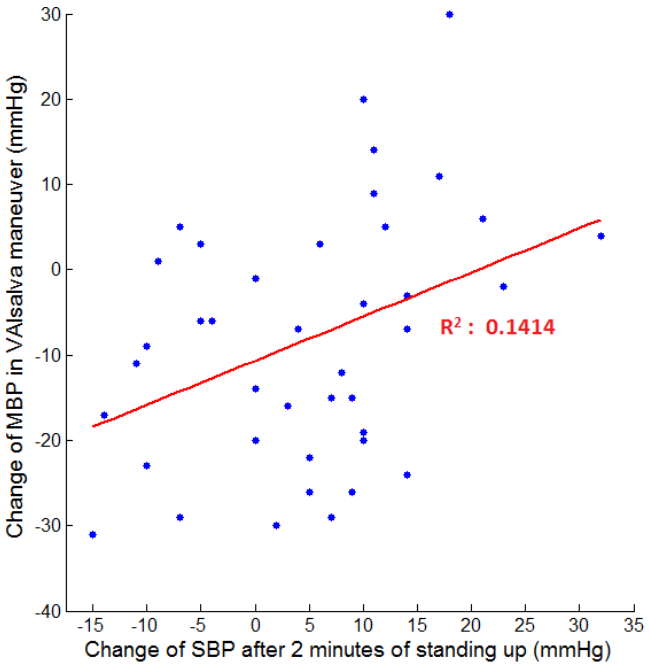


Figure 4.6 *The change of MBP in the Valsalva maneuver and the changes of SBP 2 minutes after standing are strongly correlated with each other(p=0.014).*

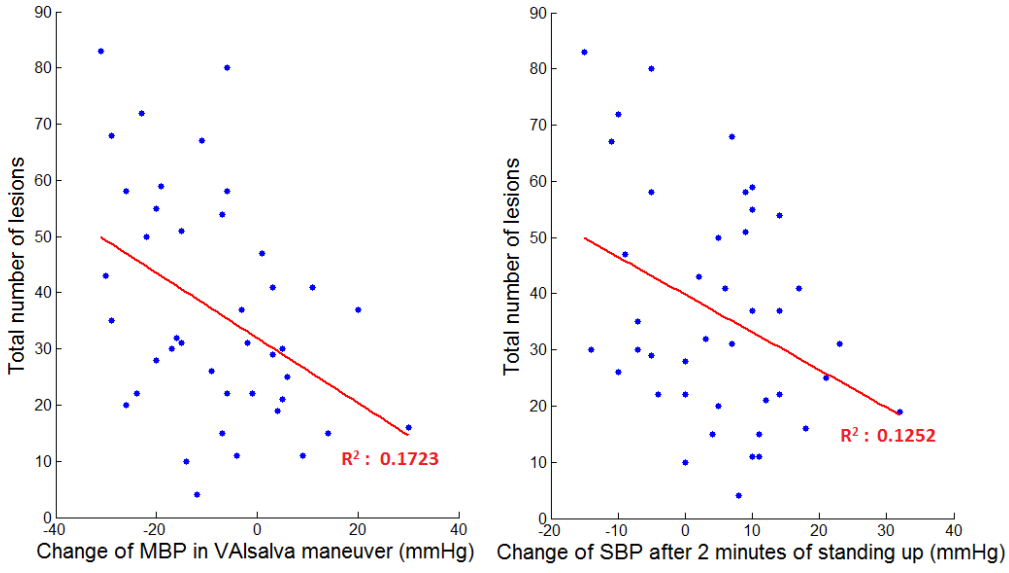


Figure 4.7 *Negative slope of the linear regression between the total number of lesions and the predictor variables, change in mean blood pressure (MBP) in response to a Valsalva maneuver (p=0.007), and change in systolic blood pressure (SBP) at 2 minutes standing after being seated (p=0.023).*

4.2.3 Expanded Disability Status Score (EDSS)

EDSS for one subject was undefined and this subject was excluded from the analysis. The features with significant p-values of regression lines are as listed in Table 4.5.Applying stepwise method to find the true independent significant variables results in only 3 significant features with the model presented in Equation 4.3 (R2=0.36, p<0.001).The linear regression between each selected feature and EDSS score are illustrated in Figure 4.8.

$$EDSS = -5.0 + 0.33 \times age + 0.07 \times DBP + 0.06 \times BRS_{\text{number positive sequences lag 3}} \quad \textbf{Eq. 4.3}$$

Table 4.5 *Variables with significant linear regression with EDSS.*

variable	slope	intercept	R^2	p
age (years)	0.05	-0.09	0.13	0.008
SBP (mmHg)	0.04	-2.86	0.18	0.002
DBP (mmHg)	0.08	-4.06	0.21	0.001
BRS number of lag 3 positive sequences	0.05	1.56	0.07	0.045

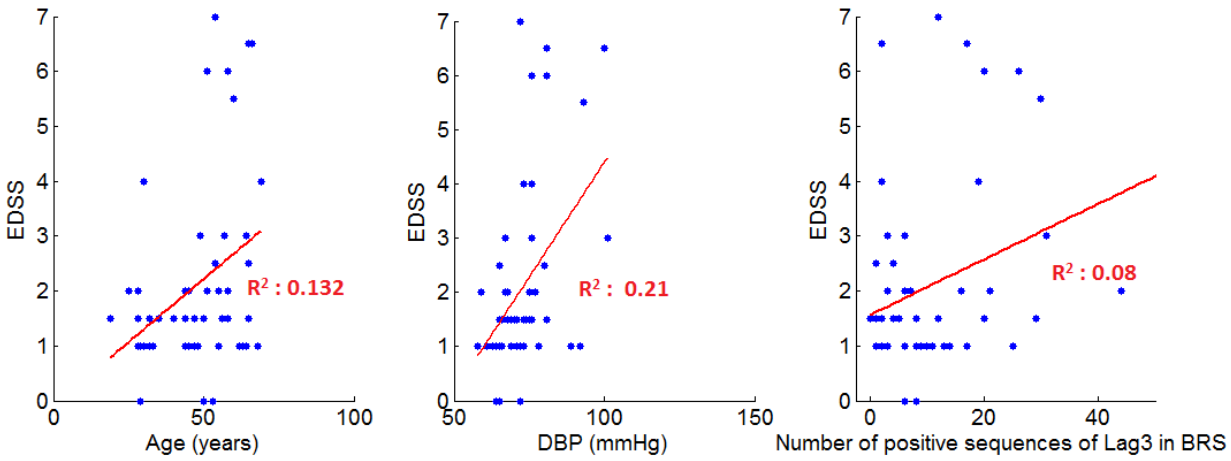


Figure 4.8 *Expanded Disability Severity Score (EDSS) relationship with age (p=0.008), diastolic blood pressure (DBP, p=0.0006) and BRS positive sequences at lag 3 (p=0.044).*

4.2.4 Multiple Sclerosis Severity Score (MSSS)

The value of MSSS was not available for two of the 53 subjects. Excluding those two subjects, the features with a significant regression with the MSSS are described in Table 4.6. Entering these three variables into a stepwise linear regression resulted in all variables being retained (R^2=0.21, p=0.009, Equation 4.4).However, the individual scatter plots between these three features and MSSS (Figure 4.9) showed low R^2 values.

$$MSSS = 0.22 - 0.08 \times BMI + 0.05 \times DBP + 0.19 \times BRS_{\text{number negative sequences Lag 2}} \quad \textbf{Eq. 4.4}$$

Table 4.6 *Variables with significant linear regression with MSSS.*

variable	slope	intercept	R^2	p
positive slope				
DBP (mmHg)	0.06	-2.01	0.08	0.043
BRS number of lag 2 negative sequences	0.20	1.82	0.085	0.038
negative slope				
BMI (kg/m^2)	-0.10	4.99	0.08	0.042

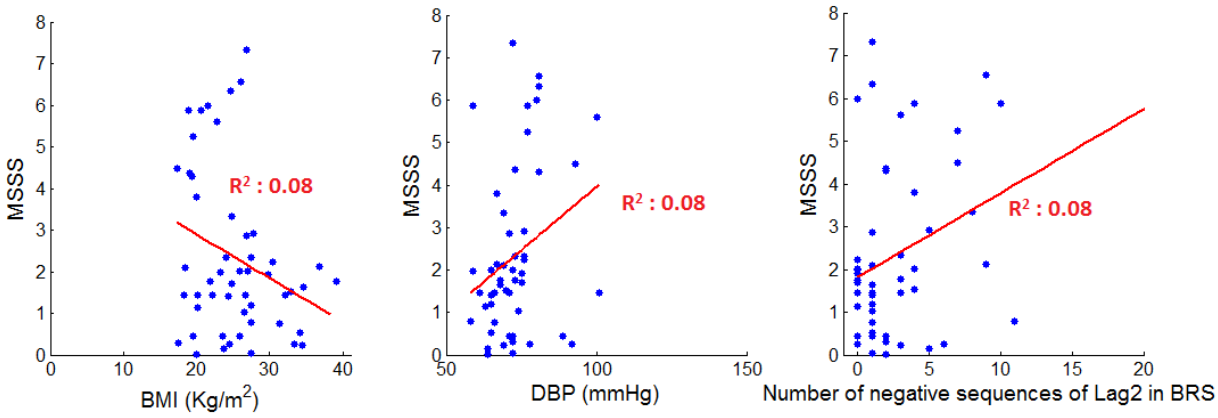


Figure 4.9*The goodness of fit (R^2) are low for the regression between MSSS and the predictor variables body mass index (BMI, p=0.042), diastolic blood pressure (DBP, p=0.043) and number of BRS negative sequences of lag 2 in BRS (p=0.038).*

4.2.5 Type of MS-localization (Topography)

There was missing data for type of MS based on localization of lesions (Topography) in 6 subjects. The type of MS-localization for the remaining 47 subjects is provided in Table 4.7. As 3 of the categories include only 1 or 2 subjects, and there is no meaningful similarity to merge them as one category, the 3 categories of bcMS, cMS and sMS were excluded. Thus in the 43 remaining subjects, the differences between two categories of dwMS and gMS were studied.

Student's t-tests on each variable between the two groups (gMS and dwMS) showed no significant differences. Only the feature LF of SBP variability in the standing position was borderline with the p-value of 0.050. This variable's distribution in the two groups is displayed in Figure 4.10.

Table 4.7*The major proportion of subjects are classified as dwMS (deep white matter MS) and gMS (generalized MS).*

Topographical groups	bcMS	cMS	dwMS	gMS	sMS	total
Number	1	2	22	21	1	47

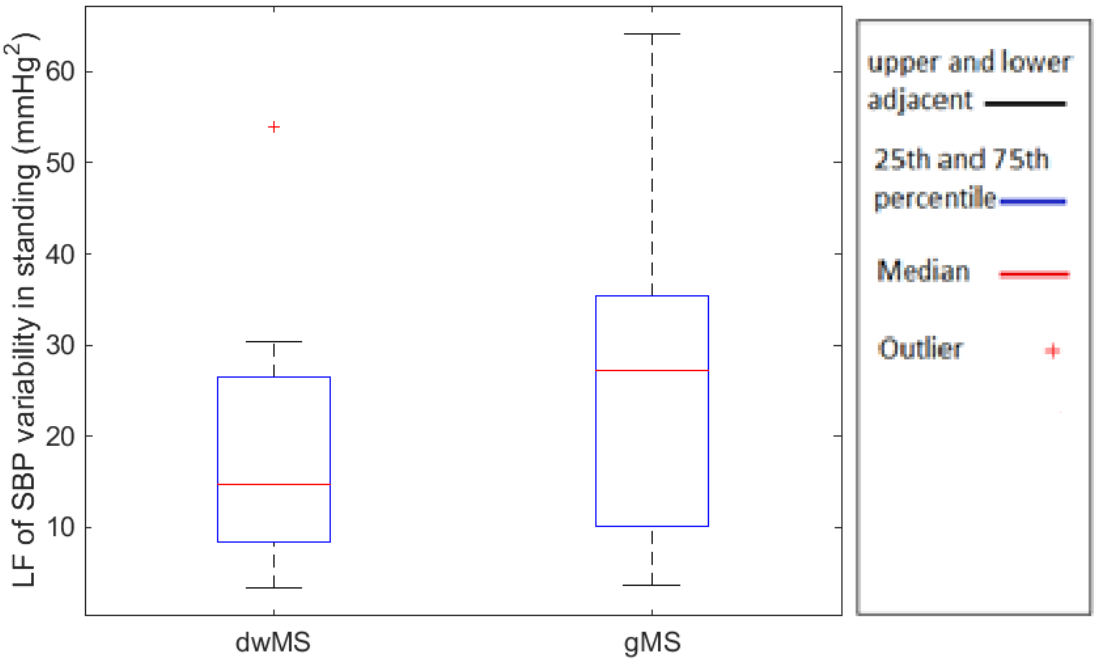


Figure 4.10*No significant differences between dwMS and gMS were found. The lowest p value amongst all variables was for low frequency (LF) of systolic blood pressure (SBP) variability in the standing position (p=0.050).*

4.2.6 Type of MS-progress

The type of MS based on the progress of disease was not available for one subject. The distribution of the remaining subjects amongst the MS progress type is given in Table 4.8.

After excluding CIS from the analysis due to the small sample size (n=1), ANOVA across the three remaining MS-progress types was significant forage, PQ interval and QT_c interval. The scatter plots in Figure 4.11 shows the distribution of subjects across the three predictor variables in the three MS-progress type categories. The distribution of three variables between the three MS-type categories and their corrected p-values are illustrated in Figure 4.12.

Table 4.8*Major proportion of subjects was in the early stable phase of disease (RRMS) with the remaining subjects being distributed amongst the other disease progression types.*

Type of MS-Progress	CIS	PRMS	RRMS	SPMS	Total
Number	1	4	39	8	52

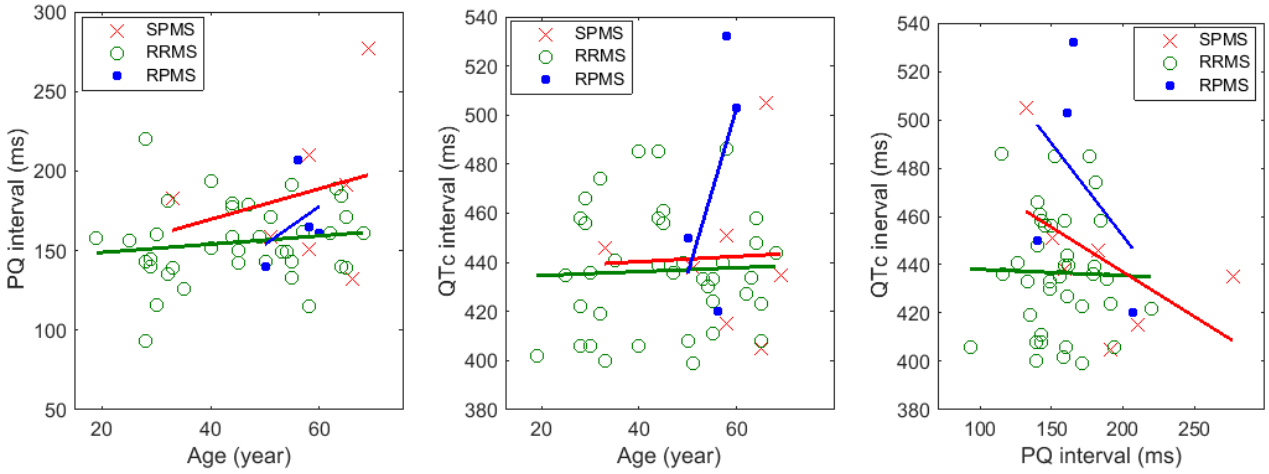


Figure 4.11 *R^2 of PQ interval vs. Age: RRMS=0.13, SPMS=0.33, RPMS=0.18. R^2 of QT_cinterval vs. Age: RRMS=0.06, SPMS=0.003, RPMS=0.3, R^2 of QT_c interval vs. PQ interval: RRMS=0.02, SPMS= 0.002, RPMS=0.0006.*

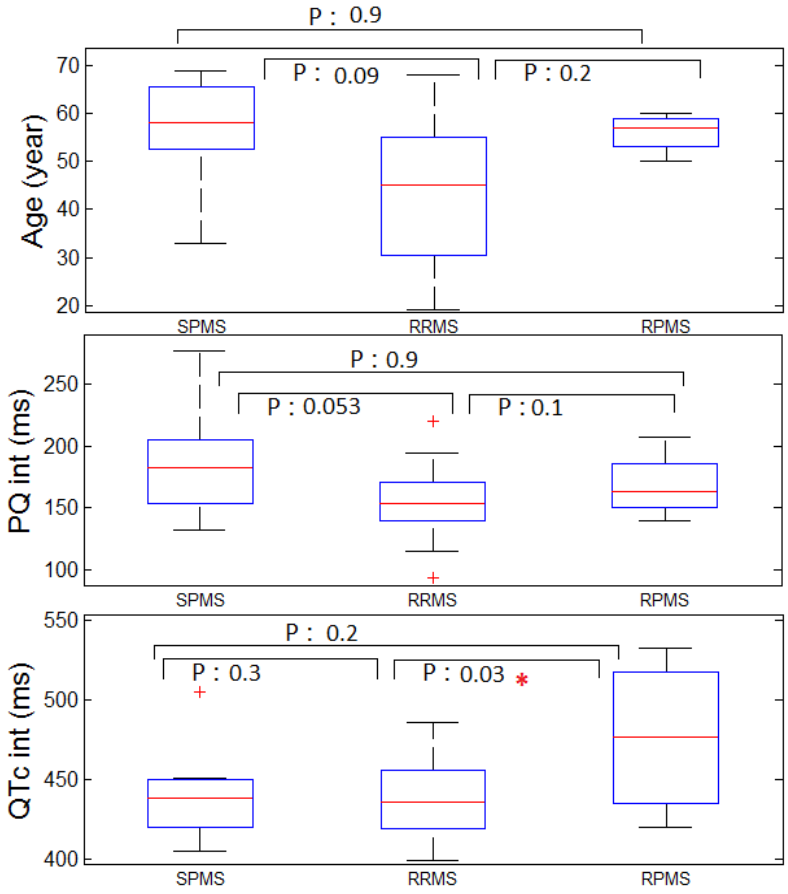


Figure 4.12 *Corrected p-values of each pair of groups in 3 different features, shows significant difference of RRMS and RPMS with longer QT_c in RPMS.*

4.2.7 Treatment delay

Treatment delay category was unavailable for one subject. Table 4.9 displays the number of subjects in each group for the remaining subjects.

ANOVA showed that age, height, SBP and changes of DBP in the hand grip test were significantly different between the four categories (Figure 4.13). The four variables were assigned as hierarchical inputs in a multinomial logistic regression (in order: none, late, middle and early). The multiple comparison corrected p-values are provided in Table 4.10.

Age was the only significant distinction between the subjects who were never treated (N) and those under any treatment (L, M, E) as subjects never treated were older. The subjects who started treatment late differed significantly in height and changes of DBP in the hand grip test compared to subjects treated before that (M and E). The late treated subjects were taller and had a smaller change in DBP during the hand grip test. The middle treated group had a higher SBP than the early treated group.

Table 4.9*Number of subjects in the four different categories of treatment delay.*

Treatment groups	Early	Middle	Late	Never
number	18	18	9	7

Table 4.10*Hierarchical multinomial logistic regression p values for the treatment delay categories none (N), late (L), middle (M) and early (E)for the variables found significant by ANOVA (age, height, systolic blood pressure (SBP) and change in diastolic blood pressure (ΔDBP) in the handgrip test).*

	N vs L,M,E	L vs M,E	M vs E
age	**0.042**	0.839	0.394
height	0.073	**0.017**	0.380
SBP	0.635	0.839	**0.036**
ΔDBP hand grip test	0.375	**0.015**	0.061

Analysis was also conducted considering the treatment onset as independent groups instead of a hierarchical variable. The resulting significant differences between groups are displayed in Figure 4.13. Post-hoc analysis using non-hierarchical methods showed no significant differences between none and early treated groups. No treatment and middle-onset treatment only differed in age. Early and middle onset treatment differed in systolic blood pressure. Late onset treatment differed all other groups in height and ΔDBP in the hand grip test. Results are shown in Figure 4.13 and multiple comparison adjusted p-values given in Table 4.11.

Table 4.11*Multiple comparison adjusted p-values for none (N), late (L), middle (M) and early (E) treatment treated as non-hierarchical variables for the variables found significant by ANOVA (age, height, systolic blood pressure (SBP) and change in diastolic blood pressure (ΔDBP) in the handgrip test).*

	L vs N	L vs E	L vs M	M vs N	M vs E	N vs E
Age	0.075	0.949	0.631	**0.029**	0.429	0.052
Height	**0.004**	**0.011**	**0.028**	0.081	0.290	0.240
SBP	0.854	0.225	0.709	0.541	**0.032**	0.292
ΔDBP hand grip test	**0.014**	**0.005**	**0.038**	0.234	0.053	0.840

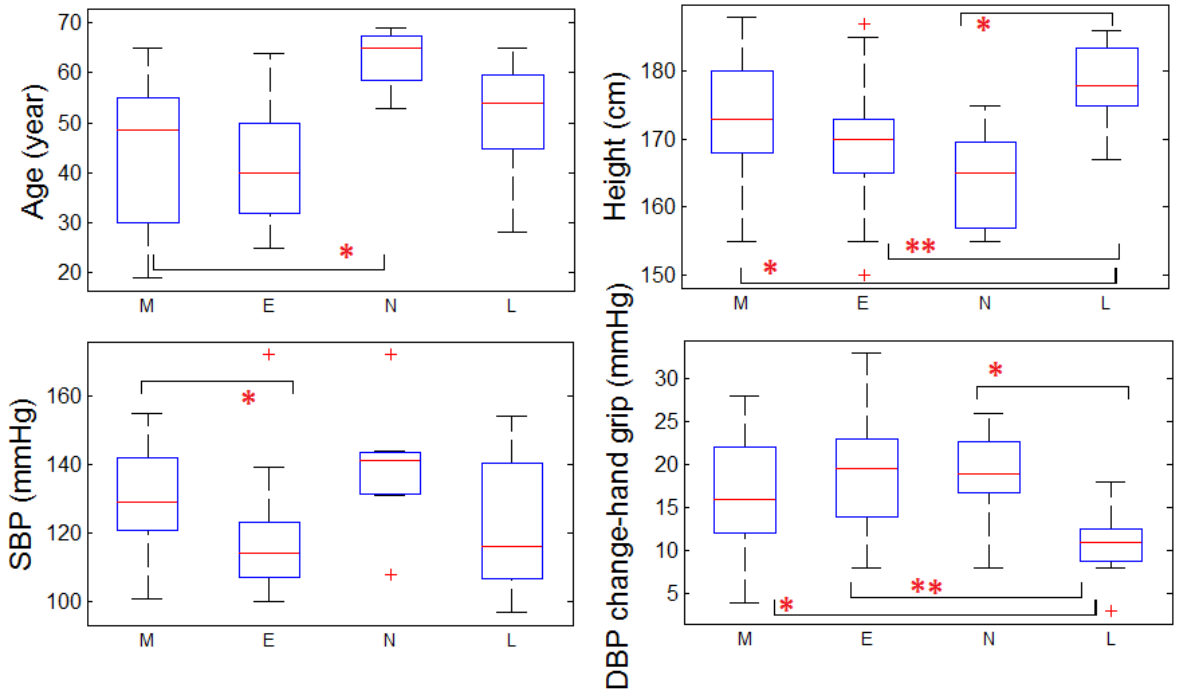


Figure 4.13*Variables that were significantly different between treatment onset categories were age, height, systolic blood pressure (SBP) and change in diastolic blood pressure (DBP) in the hand grip test. Age was significantly different only between no (N) and middle-onset (M) treatment. Height was greater in late-onset (L) treatment than in all other groups. SBP only differed between M and early-onset (E) treatment. Changes of DBP in the hand grip test were significantly lower in L group compared to all other groups.*

4.3 Multiple Sclerosis clinically abnormal autonomic reflex test results

The autonomic function reflex tests for each MS patient were rated as either in the normal or abnormal range, as described in the Methods (Section 3.2). The rates of abnormalities for each feature are shown in Table 4.12.Among the 9 autonomic reflex test parameters, parasympathetic activity was predominantly evaluated by RSA, VR and the 30:15 ratio. The sympathetic activity was predominantly represented by changes of MBP in Valslava, MBP at late phase II Valsalva, MBP at phase IV Valsalva, changes of SBP 2 minutes after standing and changes of DBP in hand grip test.

Table 4.12*The 9 features from 4 reflex cardiac autonomic tests giving total numbers in the normal and abnormal range and not available (NA) values. Whether the parameter reflects predominantly sympathetic or parasympathetic response is given in the right hand column.*

Variables	Normal	Abnormal	Number of
Predominantly parasympathetic-response variables			
RSA (beats/min)	34 (64%)	19 (36%)	0
E:I	50 (94%)	3(6%)	0
VR	29 (58%)	21 (42%)	3
30:15 ratio	30 (59%)	21(41%)	2
Predominantly sympathetic-response variables			
changes of MBP in Valslava (mmHg)	23 (46%)	27 (54%)	3
MPB in late phase II(mmHg)	30 (60%)	20 (40%)	3
MBP in Phase IV (mmHg)	49 (98%)	1 (2%)	3
ΔSBP 2 min after standing (mmHg)	37 (72%)	14 (28%)	2
ΔDBP in hand grip test (mmHg)	26 (49%)	27 (51%)	0

RSA, respiratory sinus arrhythmia. E:I, inspiration: expiration average heart rate variation. VR, Valsalva Ratio. MBP, mean blood pressure. SBP, systolic blood pressure. DBP, diastolic blood pressure.

The E:I ratio and the MBP in Phase IV of Valsalva produced normal results in a large majority of patients. In addition, in standard autonomic tests, between two parameters of deep breathing tests, RSA ratio is mostly used for CANS scoring[93]. Also, . Since the aim in this part is to compare the MS subjects together and the two variables of E:I ratio and MPB in Phase IV does not produce informative information (normal for all subjects) they are excluded from scoring the autonomic test as they do not produce informative information.

4.3.1 Parasympathetic score and sympathetic score

The total parasympathetic scores and sympathetic scores (Methods Section 3.2) were individually studied in order to find any relation between them and the clinical variables of

EDSS, MSSS, disease duration, total number of lesions, topography, progress type and treatment delay. No significant differences were found except that sympathetic score was significantly lower in late onset treatment compared to not late treatment (either N+M+E or M+E). Figure 4.14 shows the distribution of sympathetic scores in the two groups of Late and Not late treatment.

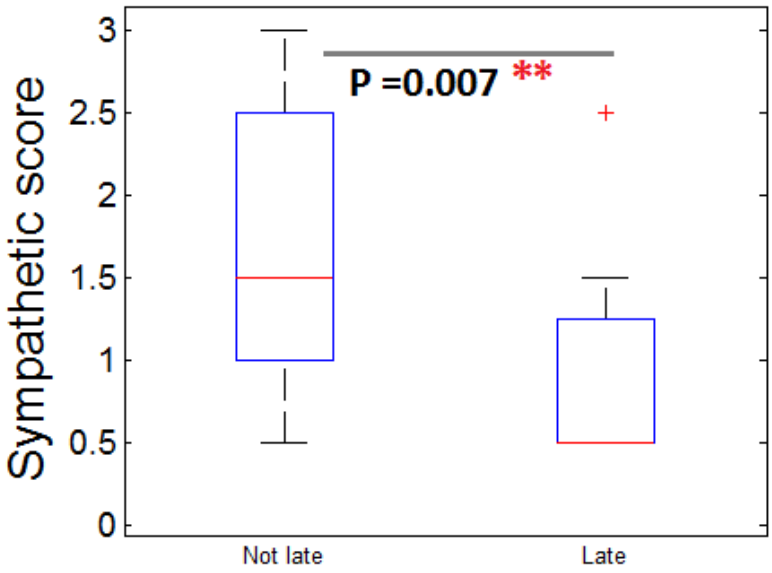


Figure 4.14*Distributions of sympathetic score in Late and Not Late treatment onset. Considering or excluding group 'N' (no treatment) from Not Late group did not change the result*

4.3.2 Combined parasympathetic score and sympathetic score

The combination of the scores of both sympathetic and parasympathetic scores (sympathovagal score) (Methods Section 3.2) was studied to find any relation between this parameter and clinical variables. To this aim, the new feature was calculated by accumulation of sympathetic score and parasympathetic score. The results showed significant difference of this parameter between late-onset treatment and not late treatment. Figure 4.15 shows the result of significantly lower values of this score in late treatment. Also, this variable was inversely correlated with disease duration with borderline p-value of 0.05 (Figure 4.16).

However, if the sympathovagal score is divided in to two groups of scores <4 and scores>=4, as it is shown in Figure 4.17, disease duration is significantly different between two groups.

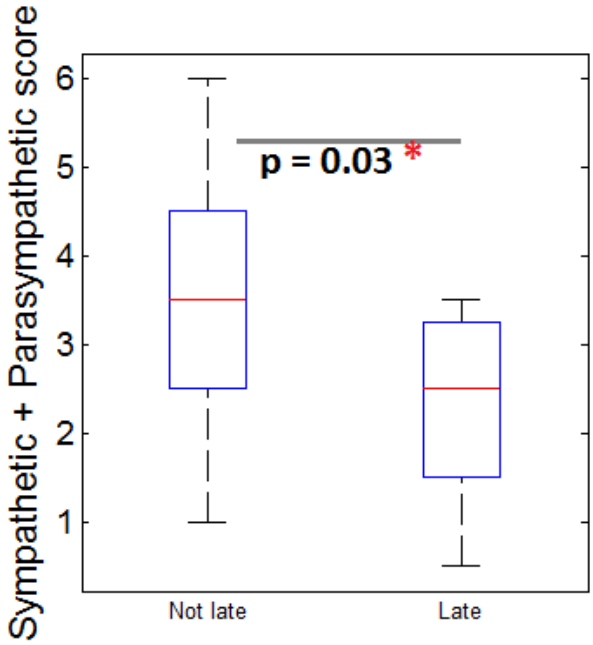


Figure 4.15*Significant lower mean values of sympathovagal scores in two treatment groups of late and not late-onset treatment.*

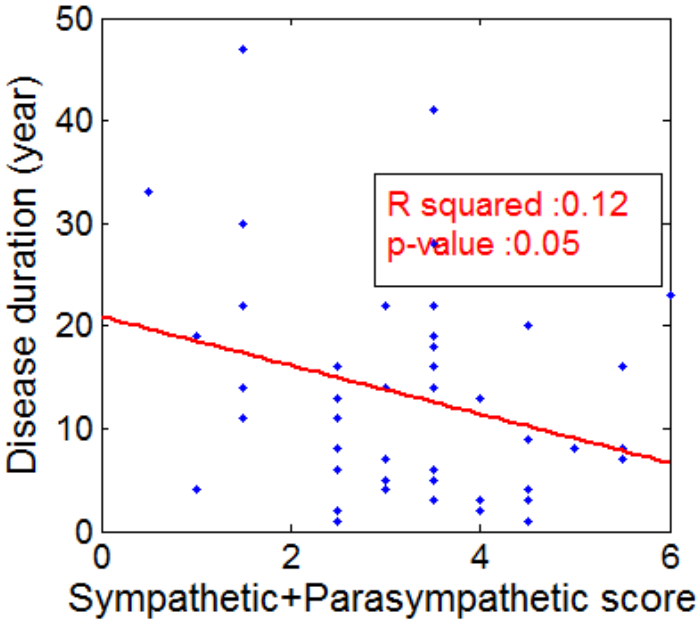


Figure 4.16*Disease duration and sympathovagal score had a borderline significant linear regression with p-value of 0.05.*

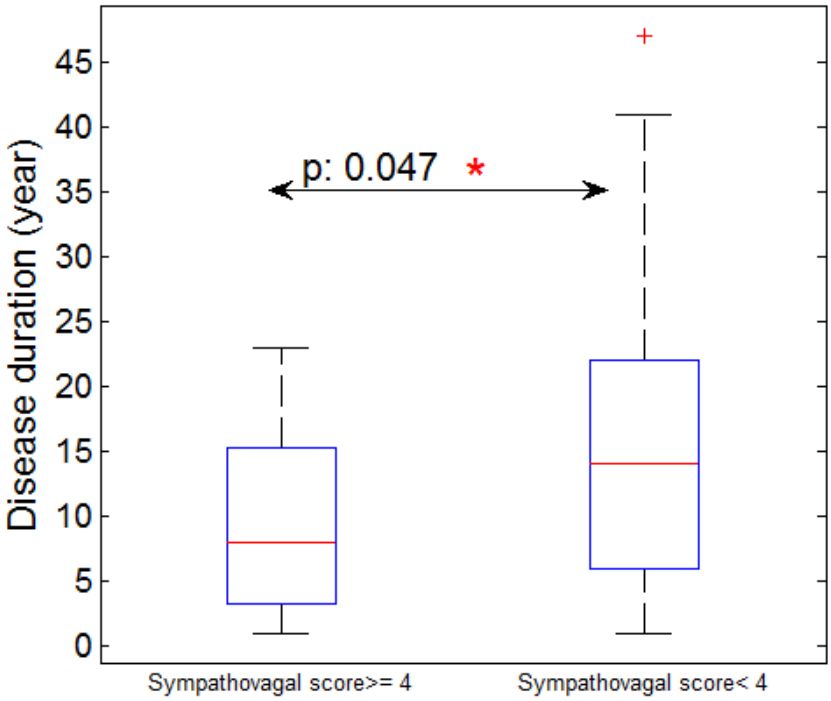


Figure 4.17*Distribution of disease duration in groups of sympathovagal score≥4 or <4.*

Chapter 5
Discussion

CAD is frequent in MS patients and its impact on quality of life is substantial. It might be the reason of sudden death in MS patients, while being hidden for years until progressive impairment of cardiac autonomic balance[41,49]. Characterization of CAD can lead to identification of cardiovascular risk and treatment planning. However CAD is not very well described in MS patients and it is usually overlooked in calculating disease severity and progress scores[8,86].

In this thesis a comprehensive cardiac autonomic test was performed on 53 MS patients. All distinctions between MS and control and all relations between CANS results and MS clinical variables were investigated.

The standardized cardiovascular reflex measurements were used in studying CANS in MS. The reflex tests included deep breathing, Valsalva manoeuvre, postural change and isometric exercise. In addition, a rarely used comprehensive analysis of short term (5minutes) spontaneous changes of HR and BP was performed. The analysis included HRV in time and frequency domains, SBPV in the frequency domain and BRS by both sequence and frequency techniques.

Both reflex tests and analysis of spontaneous variability of HR and BP proved to be informative about distinction of CAD in MS, as well as being descriptive of MS type, severity and progress.

5.1 Subject selection

The selection of MS subjects in this study was completely random, and they were not collected based on their age, sex or type of MS. As a result, the proportion of female subjects to male subjects and their disease progress types was similar to demographic make-up of the MS population worldwide. In Table 5.1, the demographic information of MS patients in this study and MS population in the world (published in MS Atlas 2008 & 2013[113,114]) illustrate this similarity.

Table 5.1*Demographic information of MS subjects in this study and MS patients in the world*[113,114]

Subjects' demographic information	MS group in this study	MS population in the world
Women/men	64% / 36%	67% / 34%
Average age at onset (year)	34	29
RRMS	74%	69%
PRMS	15%	19%
SPMS	7%	5%
PPMS	0	7%

Selection of controls was first based on finding control subjects with matched age and sex with each MS subject. Among the list of 68 healthy controls, only 23 were found to be matched with 23 MS subjects of this study. For a few cases, there was more than one option to choose. Therefore, the matched controls were selected based on the similar BMI. As shown in Appendix B and Table 4.2, the BMI of two groups were similar, but the heights and weights of MS subjects were significantly higher than controls. The reason could be the race difference between the two groups, as the MS subjects were all Caucasian and controls were

from a mix of races. It should be taken into consideration that different races can result in significant different HF, LF and LF/HF ratio of HRV. In a study performed on 1984 healthy persons, the parameters LF and LF/HF ratio of HRV were reported to be significantly higher, and HF of HRV to be significantly lower in Caucasians compared to age and sex matched African-Americans[115].

5.2 MS and Controls Comparison

Comparing autonomic features of MS with controls in this study was only feasible for 65 first features extracted from 5 minutes supine rest, which can be discussed in the four following categories:

HRV in the Time Domain: This study found no significant differences of short term (5 minutes) analysis of RR interval in the time domain between MS and control. The mean values and ranges of these 3 parameters are shown in Appendix B for both MS and control groups. Although the ranges of these three variables are wider in controls compared to MS, the difference between them is not significant.

In previous studies, however, long term (24 hour) calculation of SDNN, rMSSD and PNN50% in MS subjects were reported to be significantly lower comparing to controls[49,116]. No other studies to date have performed short term analyses of spontaneous HRV in the time domain in MS patients, the study presented in this thesis being the first to investigate HRV in this manner.

HRV in the Frequency Domain: For comparison of short term HRV in the frequency domain between MS and control subjects, results between studies are not consistent. For instance in one study on 10 MS subjects and matched controls, no differences were observed

in VLF, LF and HF of HRV, and only LF/HF ratio and LF of HRV in normalised units were found significantly lower in MS[36].In another study (on 16 MS and matched controls), only TP, VLF and LF of HRV were reported significantly lower in MS[47]. Also, in a study on 39 MS subjects and matched controls, all HRV parameters calculated from 24 hour ECG was reported to be significantly lower in MS, except LF/HF ratio of HRV with no significant difference[49]; while the exact opposite results (significantly higher results in MS) were reported in another 24 hour study on MS[117]. Another study of 13 MS subjects and controls, found no significant differences between short term HRV parameters[46].

In this study however, the parameters of HRV in frequency domain were not significantly different from controls. As can be seen in Appendix B and Table 5.2, the average of these variables in MS group are higher than control group and the ranges are wider, but not significantly different and the p-values indicate that that the two groups are not close to being significantly different.

Variability between studies may be for several reasons. Firstly, the method of measuring HRV is different between studies, some using short term (minutes) measurement of heart rate and others using longer term (24 hour) measurement of heart rate. Secondly, the sample sizes vary between studies, with some having quite small sample sizes. Another reason for non-consistent results could be different statistical methods applied. In this study, Mann Whitney U test is applied (as in one of previous studies[46]). However, if the independent t-test is applied (as in two of previous studies[36,47], requiring a normal distribution unlike the Mann Whitney U test) the p-values will be still insignificant but close to significant. Table 5.2 shows the p-values calculated with two different tests indicating the importance of choosing the correct statistic test in data interpretation.

Table 5.2 *The p-values calculated from Mann Whitney U test and student t-test for parameters of HRV in frequency domain.*

HRV in frequency domain parameters	p-value of Mann Whitney U test (distribution free)	p-value of Independent t-test (normal distribution assumption)
TP (ms^2)	0.4	0.08
VLF (ms^2)	0.1	0.09
LF (ms^2)	0.9	0.08
HF (ms^2)	0.4	0.2

In addition, race differences, as previously mentioned in Section 5.1 may affect the results. Another reason for different results could be due to large variation in control values in this study. This problem was reported in some previous studies as well, and considered as a limitation in clinical application of spectral methods[50].

SBPV in Frequency Domain: In this study, LF power in normal units and LF/HF of SBPV variability were found to be significantly lower in MS subjects compared to the control group. Also, HF power in normalised units and HF of SBPV were significantly higher in MS. In two similar previous studies, no significant differences of SBPV parameters were reported between MS and control[36,50].

The interpretation of SBPV differs from HRV. The underlying cause of BPV in the HF band is due to respiration that often occurs around 0.25 Hz, and in the LF band is due to oscillations in baroreceptor and chemoreceptor reflex control systems (Mayer waves) at around 0.1 Hz[50].

The power in the LF band (0.077-0.15Hz) of SBPV is interpreted to be related to both sympathetic function and the function of smooth muscles in blood vessels to keep the blood flow constant (myogenic vascular function).For this, the smooth muscles react to stretching or contraction in vessels which is caused by increase and decrease of blood pressure in order to

maintain blood flow. However, in most studies lower values of SBPV in the LF band is interpreted as reduced sympathetic vasomotor outflow[50].

The mechanism underlying in the HF band (0.15–0.40Hz) of BPV relates to smooth muscle relaxation (via endothelial-derived nitric oxide release) and the hormone system for regulating BP and fluid balance (renin-angiotensin system). However it has not been adequately explained despite several studies indicating that it is a mechanical consequence of respiration and thus originated from parasympathetic functiononly[50].The large variation of SBPV values of controls is also considered as a limitation in clinical application of spectral methods as well[50]. However as can be seen in Appendix B, in the current study only the values of VLF and TP of SBPV in the control group had large variation.

Altogether, in this study significantly lower values of LF power of SBPV in normalised units is considered as an indication of lower sympathetic function as well as lower baroreceptor reflex control system in MS compared to control subjects[118,119].

a) BRS by Sequence and Frequency Techniques:

- **Sequence Technique**

In this study BRS calculated by the sequence technique didn't show any significant differences compared to the control group. This result was consistent with one previous study which suggested that BRS for MS subjects with low EDSS (2.1 ± 0.5) does not differ from controls[111]. In the current study the average EDSS score for 23 MS subjects was 1.7±1.24.

- **Frequency Technique**

This study showed significantly diminished BRS in the HF band by both mean and coherence criteria in the MS group compared to controls. These results are consistent with one previous study of BRS calculated by the frequency technique for MS patinets[50].

In previous studies, calculating BRS by the frequency technique and coherence criteria was shown to have similar results compared to measurement of BRS measurement by phenylephrine injection (vasoconstrictor agent)[16,46]. However, since non-invasive methods (frequency and sequence techniques) measure spontaneous function of BRS and the invasive method measures the strength of BRS reflex to an blood pressure stimulation requiring invasive methods, the non-invasive techniques cannot substitute the invasive method for diagnosis purposes[16]. Diminished BRS in the LF band is usually interpreted as baroreflex impairment. But abnormal BRS in the HF band is doubted to have only a BRS origin. In animal experiments, after baroreceptor denervation, the measure in the HF band is persevered, its impairment is thought to be related to central impairment rather than vagal dysfunction of BRS[16]. This could be due to brainstem alteration with disturbed cardiorespiratory coupling in MS patients[50].

5.3 Rate of Abnormal Results in Reflex Tests

Most cardiac autonomic studies done on MS patients compared the CANS reflex tests in MS and control groups. In previously published studies, the abnormalities of reflex tests in MS groups were reported to be significantly higher than in the control groups[39,41]. However, in this study CANS reflex tests were only performed on MS subjects and the results were compared to age-dependant normal ranges of each test published in previous investigations[8,55,71,87] (Appendix A).

In this study, the rate of abnormalities in deep breathing (RSA parameter) was 36%, and two other predominantly parasympathetic variables (Valsalva ratio and the 30:15 ratio of standing) produced the abnormality rate of 42% and 41%. The range of deep breathing abnormalities published in a meta-analysis study of CANS on MS is reported to be from 13.2%–40.4% abnormality with the average of 25.6%. It is suggested that since among parasympathetic evaluation variables this test has the most consistent ranges of abnormalities,

this is a good marker of parasympathetic function[39,120,121].In previous studies results of abnormal VR and 30:15 ratio were reported to vary between 0to 31% of MS patients[37,38,40,43,47,56,85,86,120]. However it is still suggested that the Valsalva ratio and the 30:15 ratio are of great importance to be included in parasympathetic assessment[49]. For instance, the 30:15 ratio was reported to be the only reflex index that showed a reduction with disease progression in longitudinal studies of MS[56,122]. In the study presented in this thesis, among the 53 subjects out of 3 parameters of reflex tests for assessment of parasympathetic function, 18 had more than 1 (34%) abnormal results. Among those with more than1 abnormal result, only 3 had normal RSA while in the other 35 subjects, 33 had normal RSA. The chi-square p-value is equal to 0.0006, showing the ability of the deep breathing test to be a good representative of parasympathetic function.

Among predominant sympathetic reflex tests assessed for MS patients, the hand grip test showed the most consistent abnormal results in previous studies with the abnormality range up to 44%[38,47,120,121]. In the current study, the hand grip test presented 51% rate of abnormality in MS subjects. Changes of MBP in Valsalva are less frequently used because of the difficulty in identification of the 4 phases in Valsalva, as well as normal response validation. However, in this study, only overshoot in late Phase II and the adequate fall in MBP in early Phase II (4 Phase Valsalva response)were considered as normal. Assessment of changes of SBP in postural challenge however, is of great importance to detect systolic orthostatic hypotension or hyper tension. Orthostatic intolerance is reported to occur in up to 49% of MS subjects[39]. In this study 28% of subjects had abnormal changes in SBP 2 min after standing, including either more than 20 mmHg decreases or increase in SBP. Altogether, in this study the subjects with more than 1 abnormal sympathetic test were 31 (58%). Among those 31 subjects, 6 had normal hand grip result, and among 22 with 1 or no abnormal tests 20 had normal hand grip result. The p-value of chi-square test is equal to 0.003, showing the efficiency of handgrip test to be representative of sympathetic function.

Motor deficit may be the reason of impaired respiration force in deep breathing test and weakened hand grip and may be a dominant confounder of abnormal results in these two tests[120,121]. However, by including 3 tests for sympathetic and parasympathetic function assessment, the final scores are reliable.

In previous investigations, sympathetic function was reported to be more damaged than parasympathetic function[123]. The current study, showed 34% abnormal results in parasympathetic and 58% sympathetic that complies with the previous studies.

5.4 Comparing Autonomic Results with Clinical Variables

5.4.1 Disease duration

Many investigations aimed to find the relation between disease duration and CANS tests parameters. In previous studies, decreased HRV parameters in the frequency domain[49], and higher rate of abnormal reflex tests were reported to be significantly correlated with disease duration but not with severity score[34,49,86].

In this study, age is shown to be a confounder for disease duration. However, the sympathovagal score calculated from reflex tests was shown to be negatively correlated with disease duration. Since this score is calculated based on age-dependent normal values thus normalising for age, this correlation is independent of age. The linear regression between disease duration and sympathovagal score (Figure 4.16 in Results chapter) has a borderline significant p-value (0.050).

Also the subjects with more than two abnormal variables (sympathovagal score<4) had significantly longer disease duration (Figure 4.17 in Results chapter). This implies that the whole sympathovagal function is significantly diminished with longer disease duration and this reduction is not related to age.

However, this study was not designed for studying the effect of disease duration on CANS parameters. For that aim, subjects with the same age and different disease durations should be studied.

5.4.2 Number and Localization of Lesions

With the improvement of imaging techniques many studies aim to relate the location, load and number of lesions to CAD and clinical variables of MS. While some studies found no relation between CANS results and localisation of lesions and MS lesions load[44,47,90], in other studies midbrain and brainstem[124], or brainstem and spinal cord were suggested to be related to CAD[43]. Most investigations, including longitudinal studies, failed to find any relation between disease severity scores and number of lesions[56].

In the current study, the topography of lesions in two groups of dwMS and gMS were compared and no relation was found with CANS parameters. However, the total number of lesions is concluded to be significantly correlated with the results of two sympathetic parameters of reflex tests. In this study, the changes of MBP in the Valsalva manoeuvre and changes of SBP2 minutes after postural change were found to be significantly correlated with total number of lesions. The higher the change in BP in these two challenges indicated more impairment of sympathetic function, and less control of sympathetic system to recover BP in challenges such as standing. Therefore, higher numbers of lesions is accompanied with more sympathetic dysfunction.

Usually CANS tests are solely labelled as normal or abnormal. However, the findings in this study showed the advantage of evaluating the relation of raw values with clinical variables. This part has the potential for future further investigation. Comparing the number of lesions in different locations with CANS parameters, for instance brain vs. spine lesions or cortical vs. deep white matter lesions is one avenue that could be explored further.

5.4.3 Progress Type

In previous investigations, the frequency of AD is reported to be more in PPMS than SPMS and RRMS[42].In this study, only 3 types of RRMS, SPMS and RPMS were included. Among these 3 types the only significant difference was longer QTc in RPMS vs. RRMS. However, it is reported by the U.S Food and Drug Administration (FDA) that Fingolimod (Gilenya), taken by some MS subjects including a subset in this study, is associated with a lengthening of the QTc[125]. This medication is predominantly used by patients in RPMS progress. Future studies of a larger sample size could consider excluding patients with this type of medication or compare patients using Fingolimod with those not on the medication.

5.4.4 EDSS and MSSS

Most studies didn't find any relationship between CAD and EDSS[42,63,86]. EDSS is only found to be worsened by disease duration in longitudinal studies[56].

In this study EDSS was correlated with age but not correlated with any of the CANS reflex parameters or spectral results. MSSS also was not correlated with any of CANS parameters. However, there was a negative linear relation between MSSS and BMI. As can be seen in Figure 4.9, there is a significant negative linear regression between MSSS and BMI but the regression is not strong (R^2=0.08). However, if subjects are categorised to 4 groups of underweight (BMI<18.5), normal (18.5<BMI<24.9), overweight (25<BMI<30) and obese (BMI>30), patients in obese group had borderline significant lower MSSS (p=0.053) as shown in Figure 5.1.But4 categories are not significantly different in MSSS values as shown in Figure 5.2. In addition, if subjects are classified based on the average value of MSSS (average MSSS= 2.33), the subjects in higher MSSS group have significantly lower BMI (p=0.01) as shown in Figure 5.3. However, there are no studies currently in the same field for comparison of this finding and this investigation has the potential for future studies designed

specifically to investigate this phenomenon. However, both weight loss and weight gain is reported as a consequence of MS, originated from fatigue, swallowing problem, medications or immobility. Therefore, for further investigations, the history of weight loss or weight gain also should be considered.

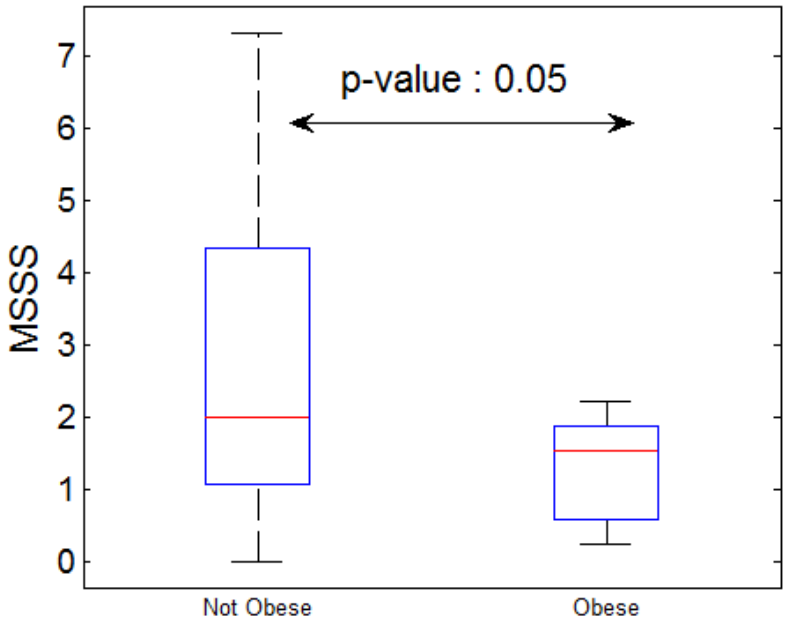


Figure 5.1 *Obese patients (BMI>30) and not obese patients (BMI<30) had borderline significant differences in MSSS.*

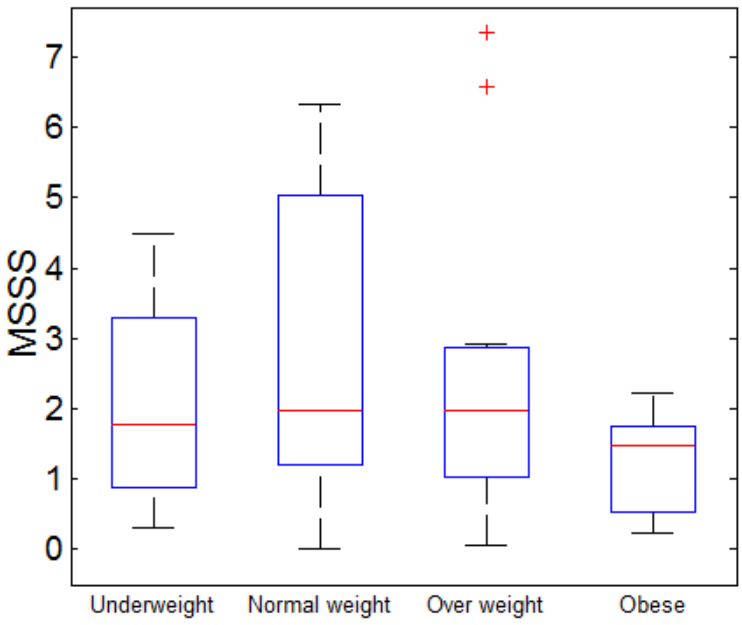


Figure 5.2 *Four categories of BMI are not significantly different in their MSSS values.*

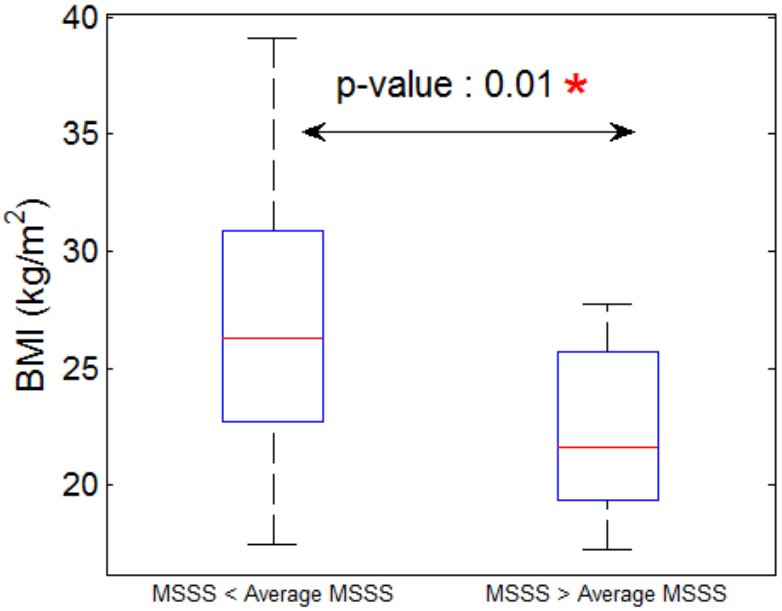


Figure 5.3 *36 subjects with MSSS<2.33 (Average MSSS) have significantly higher BMI comparing to 15 subjects with MSSS>2.33*

5.4.5 Treatment delay

The effect of treatment delay on different CANS parameters was studied and the main distinction was related to the subjects who started medication after 10 years of disease onset compared to the other patients. This late treatment group had significantly lower changes of DBP in the hand grip test indicating damaged sympathetic function. Also, the sympathetic and sympathovagal scores calculated based on age-dependent normal values in reflex tests were significantly lower in the late treatment group compared to others. This implies impairment of sympathetic function and overall sympathovagal balance with majorly delayed treatment.

5.5 General limitations

The main limitations of this study include the following:

1. The number of age and sex matched controls was low.
2. The racial makeup of control subjects was different to that of MS subjects and is a likely confounder in the HRV parameters.
3. Spontaneous BP and HR tests were conducted but reflex tests were not done for controls. Therefore, the normal ranges for reflex tests had to be considered using normal values from other studies. Ideally, normal values measured from controls in the same clinic, by the same investigators, would be used and this is a possible expansion of the current project for a more detailed comparison of the current MS cohort with control subjects.
4. The diversity of MS patients in terms of topography, progress type and severity levels, combined with some missing values in the clinical data made investigation impossible for some categories due to insufficient sample size. Inclusion of these categories would require a study of much larger sample size due to the lower prevalence of these categories in MS.

5.6 Future work

1- The control group study could be expanded and the complete autonomic test performed.

2- The number and load of lesions in different locations of CNS could be investigated.

3- The MS group study could be expanded to include sufficient number of subjects in each topographical, or progress type categories.

4- The exaggerated responses of parasympathetic tests (vasovagal syndrome) seen in some patients can be studied. Since in CANS tests usually there is only a lower normal limit (and not upper normal limit), those exaggerated responses are not necessarily considered as abnormal.

5- In this study the whole statistics were based on the criteria of significant p-value. However, feature selection methods such as principle component analysis (PCA) also could be used in addition to the criteria based on p-value.

6- The changes of some parameters within MS group and within control group could be measured and the new variables could be compared between MS and control. For example, the changes of spectral parameters of HRV in supine and standing for each group could be calculated and then compared between groups

7- Many factors such as medication and the antibody levels in blood tests could also be included in the investigation.

5.7 Conclusions

The main conclusions of the current study are:

1- The LF of SBPV in normalised units significantly was lower in MS compared to control, indicating impaired sympathetic function and diminished baroreceptor reflex control system in MS patients.

2- BRS in the HF band (α_{HF}) was significantly lower in MS compared to the control group. The same result in previous studies is interpreted as brainstem alteration with disturbed cardiorespiratory coupling in MS patients[50].

3- Deep breathing and isometric exercise tests were found to be the most representative parameters of parasympathetic and sympathetic dysfunction in MS patients.

4- Age corrected sympathetic score and sympathovagal score of reflex tests significantly decreased with longer disease duration.

5- MS patients had a higher rate of sympathetic impairment compared to parasympathetic damage based on reflex tests.

6- Total number of lesions was significantly correlated with more damaged sympathetic system based on abnormal changes of BP in Valsalva manoeuvre and orthostatic challenge.

7- MS patient with late treatment (more than 10 years after onset) had significantly less sympathetic control derived from isometric exercise test.

References

1. Mathias, C. J. & Bannister, R. *Autonomic failure A Textbook of Clinical Disorders of the Autonomic Nervous System*. (Oxford Univ., 2013).

2. Kandel, E. R. *Essentials of Neural Science and Beha*vior (McGraw-Hill 1995).

3. Freeman, W. Tutorial on Neurobiology: From Single Neurons to Brain. *Chaos. Int. J. BifurcationChaos. Int. J. Bifurc.* **2,** 451 (1992).

4. Goldstein, D. S., et al. Dysautonomias: clinical disorders of the autonomic nervous system. *Ann. Intern. Med.* **137,** 753-763 (2002)

5. Walker, C. [Available online:] http://www.biologyreference.com/Oc-Ph/Peripheral-Nervous-System.html (2015).

6. Bannister, R. Treatment of autonomic failure. *Curr. Opin. Neurol. Neurosurg.* **5,** 487–491 (1992).

7. Kahn, R. Autonomic Nervous System Testing. *Diabetes Care* **15,** 1095–1103 (1992).

8. Lensch, E. & Jost, W. H. Autonomic disorders in multiple sclerosis. *Autoimmune Dis.* **2011,** 803841 (2011).

9. Biaggioni, I. Parkinson's disease: Autonomic neuronopathy with impaired cardiovascular regulation. *Hypertension* **49,** 21–22 (2007).

10. Spyer, K. M. Central nervous mechanisms contributing to cardiovascular control. *J. Physiol.* **474,** 1–19 (1994).

11. Assadi, R. [Avaiable online:] http://emedicine.medscape.com/article/1923077-overview (2016)

12. Gale, C. Neuroendocrine aspects of thermoregulation. *Annu. Rev. Physiol.* **35,** 391–430 (1973).

13. Koizumi, K., Terui, N. & Kollai, M. Effect of cardiac vagal and sympathetic nerve activity on heart rate in rhythmic fluctuations. *J Aut. Nerv Syst* **12,** 251–259 (1985).

14. Bishop, B. & Bachofen, H. Comparative influence of proprioceptors and chemoreceptors in the control of respiratory muscles. *Acta Neurobiol. Exp. (Wars).* **33,** 381–390 (1973).

15. Parati, G., Di Rienzo, M. & Mancia, G. How to measure baroreflex sensitivity: from the cardiovascular laboratory to daily life. *J Hypertens* **18,** 7–19 (2000).

16. La Rovere, M. T., Pinna, G. D. & Raczak, G. Baroreflex sensitivity: measurement and clinical implications. *Ann. Noninvasive Electrocardiol.* **13,** 191–207 (2008).

17. Boron, W. F. & Boulpaep, E. . *Medical physiology : a cellular and molecular approach,. MMED 8120 Cardiovascular system.* (2005).

18. [Avaiable online:] http://images.slideplayer.com/16/4920073/slides/slide_43.jpg (2016)

19. Pinterr, A., Cseh, D., Sarrkozi, A., Illigens, B. M. & Siepmann, T. *Autonomic dysregulation in multiple sclerosis*. *International Journal of Molecular Sciences* **16,** 16920-16952 (2015).

20. Goldenberg, M. M. Multiple sclerosis review. *P T* **37,** 175–84 (2012).

21. Chari, D. M. Remyelination in multiple sclerosis. *Int. Rev. Neurobiol.* **79,** 589–620 (2007).

22. [Avaiable online:] https://en.wikipedia.org/w/index.php?title=Neuron&oldid=688226872 (2016)

23. [Avaiable Online:] http://humanphysiology.tuars.com/program/section1/1ch6/s1ch6_11.htm(2015)

24. Hauser, D. S. G. Multiple sclerosis and other demyelinating diseases. *Harrison's Princ. Intern. Med.* **18,** 473–492 (2011).

25. Compston, A. & Coles, A. Multiple sclerosis. *Lancet* **372,** 1502–1517 (2008).

26. Nakahara, J., Maeda, M., Aiso, S. & Suzuki, N. Current Concepts in Multiple Sclerosis: Autoimmunity Versus Oligodendrogliopathy. *Clin. Rev. Allergy Immunol.* **42,** 26–34 (2012).

27. Ramagopalan, SV. *Genetics and epidemiology of multiple sclerosis.* 15-29 (Oxford University Press; 2011)

28. Morgen, G. [Available online:] http://www.hendess.net/ms_e.htm (2016)

29. Craelius, W. Comparative epidemiology of multiple sclerosis and dental caries. *J. Epidemiol. Community Health* **32,** 155–165 (1978).

30. Leach, Tom. [Available online:] http://almostadoctor.co.uk/content/systems/neurology-psychiatry/neurology/multiple-sclerosis-ms (2016).

31. [Available Online:] http://www.webmd.com/multiple-sclerosis/magnetic-resonance-imaging-mri-of-multiple-sclerosis (2016)

32. Kurtzke, J. F. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* **33,** 1444–1452 (1983).

33. Roxburgh, R. H. S. R. *et al.* Multiple Sclerosis Severity Score: using disability and disease duration to rate disease severity. *Neurology* **64,** 1144–1151 (2005).

34. Adamec, I. & Habek, M. Autonomic dysfunction in multiple sclerosis. *Clin. Neurol. Neurosurg.* **115,** S73–S78 (2013).

35. Flachenecker, P. *et al.* Fatigue in MS is related to sympathetic vasomotor dysfunction. *Neurology* **61,** 851–853 (2003).

36. Keselbrener, L. *et al.* Is fatigue in patients with multiple sclerosis related to autonomic dysfunction? *Clin. Auton. Res.* **10,** 169–175 (2000).

37. Flachenecker, P., Reiners, K., Krauser, M., Wolf, A. & Toyka, K. V. Autonomic dysfunction in multiple sclerosis is related to disease activity and progression of disability. *Mult. Scler.* **7,** 327–34 (2001).

38. Vita, G. *et al.* Cardiovascular autonomic dysfunction in multiple sclerosis is likely related to brainstem lesions. *J. Neurol. Sci.* **120,** 82–86 (1993).

39. Racosta, J. M. *et al.* Cardiovascular autonomic dysfunction in multiple sclerosis: A meta-analysis. *Mult. Scler. Relat. Disord.* **4,** 104–111 (2015).

40. McDougall, a. J. & McLeod, J. G. Autonomic nervous system function in multiple sclerosis. *J. Neurol. Sci.* **215,** 79–85 (2003).

41. Acevedo, a R., Nava, C., Arriada, N., Violante, a & Corona, T. Cardiovascular dysfunction inmultiple sclerosis. *Acta Neurol. Scand.* **101,** 85–88 (2000).

42. De Seze, J. *et al.* Autonomic dysfunction in multiple sclerosis: cervical spinal cord atrophy correlates. *J. Neurol.* **248,** 297–303 (2001).

43. Sterman, a B., Coyle, P. K., Panasci, D. J. & Grimson, R. Disseminated abnormalities of cardiovascular autonomic functions in multiple sclerosis. *Neurology* **35,** 1665–1668 (1985).

44. Nordenbo, a M., Boesen, F. & Andersen, E. B. Cardiovascular autonomic function in multiple sclerosis. *J. Auton. Nerv. Syst.* **104,** 129–134 (1991).

45. Arata, M. & Sternberg, Z. Transvascular autonomic modulation: a modified balloon angioplasty technique for the treatment of autonomic dysfunction in multiple sclerosis patients. *J. Endovasc. Ther.* **21,** 417–428 12p (2014).

46. Sanya, E. O. *et al.* Abnormal heart rate and blood pressure responses to baroreflex stimulation in multiple sclerosis patients. *Clin. Auton. Res.* **15,** 213–218 (2005).

47. Frontoni, M. *et al.* Power spectrum analysis contribution to the detection of cardiovascular dysautonomi in multiple sclerosis. *Acta Neurol. Scand.* **93** 241–245 (1996).

48. Keller, D. M. *et al.* Reduced spontaneous sympathetic nerve activity in multiple sclerosis patients. *J. Neurol. Sci.* **344,** 210–214 (2014).

49. Mahovic, D. & Lakusic, N. Progressive impairment of autonomic control of heart rate in patients with multiple sclerosis. *Arch. Med. Res.* **38,** 322–325 (2007).

50. Linden, D., Diehl, R. R., Kretzschmar, A. & Berlit, P. Autonomic evaluation by means of standard tests and power spectral analysis in multiple sclerosis. *Muscle and Nerve* **20,** 809–814 (1997).

51. Riudavets, M. A. *et al.* Causes of Unexpected Death in Patients With Multiple Sclerosis. *Am. J. Forensic Med. Pathol.* **26,** 244–249 (2005).

52. Hengstman, G. & Kusters, B. Sudden cardiac death in multiple sclerosis caused by active demyelination of the medulla oblongata. *Mult. Scler.* **17,** 1146–1148 (2011).

53. Bramow, S. *et al.* Fatal neurogenic pulmonary edema in a patient with progressive multiple sclerosis. *Mult. Scler.* **14,** 711–5 (2008).

54. Sternberg, Z. Promoting sympathovagal balance in multiple sclerosis; pharmacological, non-pharmacological, and surgical strategies. *Autoimmun. Rev.* **15,** 113-123 (2016).

55. Freeman, R. Assessment of cardiovascular autonomic function. *Clin. Neurophysiol.* **117,** 716–730 (2006).

56. Nasseri, K., Tenvoorde, B. J., Adèr, H. J., Uitdehaag, B. M. J. & Polman, C. H. Longitudinal follow-up of cardiovascular reflex tests in multiple sclerosis. *J. Neurol. Sci.* **155,** 50–54 (1998).

57. Kodounis, A., Stamboulis, E., Constantinidis, T. S. & Liolios, a. Measurement of autonomic dysregulation in multiple sclerosis. *Acta Neurol. Scand.* **112,** 403–408 (2005).

58. Billman, G. E. The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Front. Physiol.* **4,** (2013).

59. Clifford, G. D. & Tarassenko, L. Quantifying Errors in Spectral Estimates of HRV Due to Beat Replacement and Resampling. *IEEE Trans. Biomed. Eng.* **52,** 630–638 (2005).

60. Melkonian, D., Korner, a., Meares, R. & Bahramali, H. Increasing sensitivity in the measurement of heart rate variability: The method of non-stationary RR time–frequency analysis. *Comput. Methods Programs Biomed.* **108,** 53–67 (2012).

61. Clifford, G. D. Signal Processing Methods for Heart Rate Variability. PhD dissertation, University of Oxford, Department of Engineering Science, (2002).

62. Pop-Busui, R. Cardiac autonomic neuropathy in diabetes: A clinical perspective. *Diabetes Care* **33,** 434–441 (2010).

63. De Seze, J. *et al.* Cardiac repolarization abnormalities in multiple sclerosis: Spinal cord MRI correlates. *Muscle and Nerve* **23,** 1284–1286 (2000).

64. Rami, J. [Available online:] http://pubs.sciepub.com/bse/2/1/3/figs.(2015)

65. Flachenecker, P. & Reiners, K. Twenty-four-hour heart rate power spectrum for evaluation of autonomic dysfunction in Guillain-Barré syndrome. *J. Neurol. Sci.* **165,** 144–153 (1999).

66. Wagner, G. S. *et al.* AHA/ACCF/HRS Recommendations for the Standardization and Interpretation of the Electrocardiogram. Part VI: Acute Ischemia/Infarction A Scientific Statement From the American Heart Association Electrocardiography and Arrhythmias Committee, Council on Clini. *J. Am. Coll. Cardiol.* **53,** e235–e240 (2009).

67. Davies, L. C. *et al.* Reproducibility of methods for assessing baroreflex sensitivity in normal controls and in patients with chronic heart failure. *Clin. Sci. (Lond).* **97,** 515–522 (1999).

68. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation, and clinical use. *Eur Heart J.* **17**, 354-381 (1996).

69. [Online.Available:] http://www.bloodpressureuk.org/BloodPressureandyou/Thebasics/Bloodpressurechart (2015).

70. Nunan, D., Sandercock, G. R. H. & Brodie, D. a. A quantitative systematic review of normal values for short-term heart rate variability in healthy adults. *Pacing Clin. Electrophysiol.* **33,** 1407–1417 (2010).

71. Ziegler, D. *et al.* Assessment of Cardiovascular Autonomic Function: Age-related Normal Ranges and Reproducibility of Spectral Analysis, Vector Analysis, and Standard Tests of Heart Rate Variation and Blood Pressure Responses. *Diabet. Med.* **9,** 166–175 (1992).

72. Campos, L. a. *et al.* Mathematical biomarkers for the autonomic regulation of cardiovascular system. *Front. Physiol.* **4,** 1–9 (2013).

73. Clayton, R. H. Comparison of Autoregressive and Fourier Transform Based Techniques for Estimating. *Comput. Cardiol.* **24,** 379–382 (1997).

74. Ebden, M. A Comparison of HRV Techniques : The Lomb Periodogram versus The Smoothed Pseudo Wigner-Ville Distribution. *Submitted to: Prof. Lionel Tarassenko* (2002).

75. [Available online :] http://www.gaussianwaves.com/forums/topic/how-to-find-psd-of-a-signal (2015).

76. Cohen, L. Time-Frequency Distributions --- A Review. *Proceedings of the IEEE* **77,** 941–981 (1989).

77. Di Rienzo, M. *et al.* Baroreflex effectiveness index: an additional measure of baroreflex control of heart rate in daily life. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **280,** R744–R751 (2001).

78. G. Bertinieri, M. Di Rienzo, A. Cavallazzi, A. U. Ferrari, A. Pedotti, and G. M. Evaluation of baroreceptor reflex by blood pressure monitoring in unanesthetized cats. *Am. J. Physiol. - Hear. Circ. Physiol.* **254,** H377–H383 (1988).

79. Persson, P. B. *et al.* Time versus frequency domain techniques for assessing baroreflex sensitivity. *J. Hypertens.* **19,** 1699–1705 (2001).

80. Kardos, A., Menezes, R. De & Casadei, B. Determinants of Sopntaneous Baroreflex Sensitivity in a Healthy Working Population. *Hypertension.* **37**, 911-916 (2001).

81. Hindrik W. J. R. *et al.* Assessment of Baroreceptor reflex sensitivity by Means of Spectral Analysis Techniques. *Hypertension* **10,** 538–544 (1987).

82. Pinna, G. D. & Maestri, R. New criteria for estimating baroreflex sensitivity using the transfer function method. *Med. Biol. Eng. Comput.* **40,** 79–84 (2002).

83. Michas, F. *et al.* Baroreceptor reflex sensitivity is associated with arterial stiffness in a population of normotensive and hypertensive patients. *Blood Press. Monit.* **17**, 155–159 (2012).

84. Freeman, R. *Heart rate variability : the time domain*. Clin Auton. Disord 297–308 (Lipincott Raven Publishers, Philadelphia 1997).

85. Senaratne, M. P., Carroll, D., Warren, K. G. & Kappagoda, T. Evidence for cardiovascular autonomic nerve dysfunction in multiple sclerosis. *J. Neurol. Neurosurg. Psychiatry* **47,** 947–952 (1984).

86. Gunal, D. I., Afsar, N., Tanridag, T. & Aktan, S. Autonomic dysfunction in multiple sclerosis: Correlation with disease-related parameters. *Eur. Neurol.* **48,** 1–5 (2002).

87. Novak, P. Quantitative autonomic testing. *J. Vis. Exp. JoVE* 227–236 (2011).

88. Low, P. *Clinical Autonomic Disorders: Evaluation and Management*. (Lippincott Williams & Wilkins, 1997).

89. Freeman, R., Cohen, R. J. & Saul, J. P. Transfer function analysis of respiratory sinus arrhythmia: a measure of autonomic function in diabetic neuropathy. *Muscle Nerve* **18,** 74–84 (1995).

90. Ferini-Strambi, L. *et al.* Cardiac autonomic function during sleep and wakefulness in multiple sclerosis. *J. Neurol.* **242,** 639–643 (1995).

91. Klabunde, R. [Online. Available]:www.cvphysiology.com/Hemodynamics/H014.htm (2016).

92. Mette S. Olufsen, Alston, A. V. & Tran, and H. T. Modeling Heart Rate Regulation—Part I: Sit-to-stand Versus Head- up Tilt. *Card. Eng* **8,** 73–87 (2008).

93. Ewing, D. J. & Clarke, B. F. Diagnosis and management of diabetic autonomic neuropathy. *Br. Med. J. (Clin. Res. Ed).* **285,** 916–918 (1982).

94. Freeman, R. *et al.* Consensus statement on the definition of orthostatic hypotension, neurally mediated syncope and the postural tachycardia syndrome. *Clin. Auton. Res.* **21,** 69–72 (2011).

95. Smit, A. a J., Halliwill, J. R., Low, P. a. & Wieling, W. Pathophysiological basis of orthostatic hypotension in autonomic failure. *J. Physiol.* **519,** 1–10 (1999).

96. Madden, K. M. & Lockhart, C. Arterial baroreflex function in older adults with neurocardiogenic syncope. *Clin. Invest. Med.* **32,** E191–E198 (2009).

97. Adamec, I. *et al.* Postural orthostatic tachycardia syndrome associated with multiple sclerosis. *Auton. Neurosci.* **173,** 65–8 (2013).

98. Rodrigues, E. a & Ewing, D. J. Immediate heart rate response to lying down: simple test for cardiac parasympathetic damage in diabetics. *Br. Med. J. (Clin. Res. Ed).* **287,** 800 (1983).

99. Mark, A. L., Victor, R. G., Nerhed, C. & Wallin, B. G. Microneurographic Studies of the Mechanisms of Sympathetic Nerve Responses to Static Exercise in Humans. *Circ Res* **57,** 461–469 (1985).

100. Saari, a. *et al.* Cardiovascular autonomic dysfunction correlates with brain MRI lesion load in MS. *Clin. Neurophysiol.* **115,** 1473–1478 (2004).

101. Poser, C. M. *et al.* New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann Neurol* **13,** 227–231 (1983).

102. Scalfari A., Neuhaus A., Degenhardt A., Rice G., Muraro P., Daumer M, et al. The natural history of multiple sclerosis: a geographically based study 10: relapses and long-term disability. *Brain* **133,** 1914–1929 (2010).

103. Weinshenker, B. G. *et al.* The natural history of multiple sclerosis: a geographically based study. 2. Predictive value of the early clinical course. *Brain* **112,** 133–146 (1989).

104. Broch, O. *et al.* A comparison of the Nexfin ® and transcardiopulmonary thermodilution to estimate cardiac output during coronary artery surgery. *Anaesthesia* **67,** 377–383 (2012).

105. Akkermans, J. *et al.* Continuous non-invasive blood pressure monitoring, a validation study of Nexfin in a pregnant population. *Hypertens. pregnancy* **28,** 230–42 (2009).

106. Nowak, R. M. *et al.* Noninvasive continuous or intermittent blood pressure and heart rate patient monitoring in the ED. *Am. J. Emerg. Med.* **29,** 782–789 (2011).

107. Martina, J. *et al.* Noninvasive continuous arterial blood pressure monitoring with Nexfin. *Anaesthesiology* **116,** 1092–103 (2012).

108. Eeftinck Schattenkerk, D. W. *et al.* Nexfin Noninvasive Continuous Blood Pressure Validated Against Riva-Rocci/Korotkoff. *Am. J. Hypertens.* **22,** 378–383 (2009).

109. Chen, G. *et al.* Comparison of noninvasive cardiac output measurements using the Nexfin monitoring device and the esophageal Doppler. *J. Clin. Anesth.* **24,** 275–283 (2012).

110. Critchley, L. a. H. Minimally Invasive Cardiac Output Monitoring in the Year 2012. *Artery Bypass* (2012).

111. Konecny, L. *et al.* Baroreflex Sensitivity in Multiple Sclerosis. *Scr. Med. (Brno).* **81,** 231–238 (2008).

112. Acharya, U. R., Joseph, K. P., Kannathal, N., Lim, C. M. & Suri, J. S. Heart rate variability: A review. *Med. Biol. Eng. Comput.* **44,** 1031–1051 (2006).

113. Msif. Atlas of MS 2013: Mapping Multiple Sclerosis Around the World. *Mult. Scler. Int. Fed.* 1–28 (2013).

114. World Health Organization. Atlas: Multiple sclerosis resources in the world. *Vasa* (2008).

115. Liao, D. *et al.* Age, race, and sex differences in autonomic cardiac function measured by spectral analysis of heart rate variability-The ARIC study. *Am. J. Cardiol.* **76,** 906–912 (1995).

116. Tombul, T., Anlar, O., Tuncer, M., Huseyinoglu, N. & Eryonucu, B. Impaired heart rate variability as a marker of cardiovascular autonomic dysfunction in multiple sclerosis. *Acta Neurol. Belg.* **111,** 116–120 (2011).

117. Monge-Argilés, J. a, Palacios-Ortega, F., Vila-Sobrino, J. a & Matias-Guiu, J. Heart rate variability in multiple sclerosis during a stable phase. *Acta Neurol. Scand.* **97,** 86–92 (1998).

118. Höcht, C. Blood Pressure Variability: Prognostic Value and Therapeutic Implications. *ISRN Hypertens.* **2013,** 16 (2013).

119. Stauss, H. M. Identification of blood pressure control mechanisms by power spectral analysis. *Clin. Exp. Pharmacol. Physiol.* **34,** 362–368 (2007).

120. Labuz-Roszak, B. & Pierzchala, K. Difficulties in the diagnosis of autonomic dysfunction in multiple sclerosis. *Clin. Auton. Res.* **17,** 375–377 (2007).

121. Brian pentland, D. J. E. Cardiovascular reflexes in Multiple sclerosis. *Eur. Neurol* **26,** 46–50 (1987).

122. Nasseri, K., Uitdehaag, B., van Walderveen, M., Ader, H. & Polman, C. Cardiovascular autonomic function in patients with relapsing remitting multiple sclerosis: a new surrogate marker of disease evolution? *European journal of neurology* **6,** 29–33 (1998).

123. 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.* **3,** 111–114 (1982).

124. Saari, A. *Autonomic dysfunction in multiple sclerosis and optic neuritis*. PhD dissertation, University Ouluensis, Department of Neurology, August (2010).

125. Highlights of prescribing information: GILENYA® safely and effectively. See Reference ID. 3889413. Food and Drug Administration, FDA. [Online. Available]: http://www.fda.gov/Safety/MedWatch/SafetyInformation/ucm266123.htm.(2016)

Appendix A
Variables' normal range for healthy controls

Feature	Normal Value / range for healthy population
Heart Rate(bpm)	60-100
PQ int (ms)	<200 ms
QRS int (ms)	60-120
QT int (ms)	<440ms
QT_c (Bazett's)	350-460 for women, 350-450 for men
mean RR (ms)	926±90
SDNN (ms)	50±51
rMSSD (ms)	42±42
PNN50%	8.9±7.2
TP (ms2)	3466±1018
VLF (ms2)	2524 ± 931
LF (ms2)	1170±416
HF (ms2)	975±203
LF (nu)	54±4

HF (nu)	29±3			
mean SBP (mmHg)	90-120			
mean DBP (mmHg)	60-80			
Total BRS L0	18-29 year	30-39 year	40-49 year	50-59 year
	14 (13.1:14.9)	10.3(9.7:11)	7.8(7.4:8.2)	6.8(6.2:7.3)
No. of Slopes BRS L0	26.9(23.3:30.5)	19(16.8:21.2)	19.1(17.5:20.8)	17(14.4:19.7)
BRS -PI/-SBP	13.9(12.3:15.3)	10.8(10.1:12.2)	8.2(7.6: 8.6)	7(6.9 :8.3)
No. of Slopes -PI/-SBP	14.5(12.8:16.8)	10.1(8.8 : 11.5)	11.1(10.1 :12.2)	10.1(8.5:11.6)
BRS +PI/+SBP	13.7(12.7:14.7)	9.8(9.1:10.4)	7.1(6.8:7.5)	6.2(5.6 :6.7)
No. of Slopes +PI/+SBP	12.8(11:14.6)	9.6(5.6:10.7)	9(8.2 :9.8)	7.9(6.6: 9.3)
α_LF (ms/mmHg)	15.4±5			
α_HF (ms/mmHg)	25.1±8.3			
E:I	20-29 years	1.16		
	30-39 years	1.125		
	40-49 years	1.09		
	50-54 years	1.065		
	60-64 years	1.035		
	70-75	1.02		
Minimum for normal RSA (beat/min)	20-29 years	14		
	30-39 years	12		
	40-49 years	10		
	50-54 years	9		
	60-64 years	7		
	1.125 years	12		
Maximal drop of the MBP during the early phase II(mmHg)	Normal value< 20 mmHg			

MBP at late phase II (recovery)	≥ baseline (mmHg)
MBP at phase IV (overshoot)	> baseline (mmHg)
Rise of DBP in hand grip test (mmHg)	>16 Normal , <10 Abnormal

Appendix B
Variables' Distributions

Control vs MS

	MS				Control				
	mean	std	min	max	mean	std	min	Max	p-value
Gender	0.35	0.48	0.00	1.00	0.35	0.48	0.00	1.00	1.000
AGE	37.52	11.81	19.00	65.00	37.70	11.87	20.00	66.00	0.956
Height	171.43	10.78	150.00	188.00	164.43	9.35	145.00	185.00	0.035
Weight(kg)	71.17	17.28	44.00	103.00	60.13	9.62	46.00	76.00	0.026
BMI	24.00	4.53	18.31	34.60	22.21	2.78	17.24	27.25	0.219
SBP	118.78	12.16	100.00	155.00	119.52	15.02	96.00	155.00	0.939
Heat Rate	70.78	10.52	58.00	98.00	67.52	9.16	50.00	84.00	0.575
mean RR	871.61	103.44	615.70	1020.63	906.95	123.01	725.47	1186.38	0.553
SDNN	47.25	15.50	15.93	67.73	60.23	31.89	14.82	142.59	0.263
rMSSD	41.28	22.74	11.39	89.39	49.79	37.81	12.11	160.20	0.660
PNN50%	16.23	17.27	0.00	48.68	15.97	15.33	0.00	55.04	0.843
TP (ms2)	1888	1134	222	4675	4615	7128	191	32315	0.368
VLF (ms2)	685	480	35	1816	1981	3445	108	16428	0.109
LF (ms2)	629	530	48	2002	1692	2727	54	9688	0.598
HF (ms2)	574	536	49	2106	942	1388	29	6200	0.568
LF/HF	1.96	2.26	0.35	10.02	2.08	2.59	0.13	10.62	0.660
LF (nu)	53.10	20.69	25.69	90.92	55.29	19.17	11.87	91.40	0.660
HF (nu)	46.90	20.69	9.08	74.31	44.71	19.17	8.60	88.13	0.660
TP SBP	34.63	48.51	3.11	208.98	44.81	60.44	6.85	298.92	0.253
VLF SBP	20.08	31.33	1.00	147.43	35.49	56.26	2.71	276.99	0.141
LF SBP	8.74	9.55	0.87	47.89	8.06	5.83	0.74	22.26	0.792
HF SBP	5.81	16.69	0.42	83.79	1.26	0.81	0.17	3.31	0.004
SBPLF/HF	3.90	3.17	0.48	12.85	8.92	6.85	0.98	23.15	0.006
SBPHFnu	30.49	18.78	7.22	67.59	17.87	14.29	4.14	50.44	0.006
SBPLFnu	69.51	18.78	32.41	92.78	82.13	14.29	49.56	95.86	0.006
α LF Coh	10.44	4.39	3.62	21.17	16.37	12.47	4.06	53.47	0.147
n LF Coh	20.04	8.81	2.00	28.00	22.65	5.84	6.00	28.00	0.679

α HF Coh	15.54	9.00	4.12	35.80	28.74	16.77	9.80	73.28	0.003
n HF Coh	47.48	14.34	14.00	64.00	54.13	11.42	22.00	64.00	0.121
α LF Mean	14.04	6.34	4.90	29.10	22.99	18.77	5.20	85.37	0.124
n LF Mean	12.91	2.59	8.00	18.00	12.74	2.62	8.00	17.00	0.765
mean value	10.73	4.30	3.62	21.17	16.23	11.68	4.07	47.84	0.253
α HF Mean	21.73	12.53	5.26	50.72	39.24	25.05	12.76	115.22	0.007
n HF Mean	27.48	3.99	20.00	34.00	27.78	3.96	21.00	37.00	1.000
mean value	16.15	9.26	3.89	36.61	28.58	17.02	9.43	76.02	0.006
mean	14.77	7.94	4.33	35.00	24.82	15.39	9.33	67.02	0.013
BRS0	15.87	8.03	5.46	39.29	13.25	5.63	0.00	23.94	0.538
NUM0	60.74	26.55	12.00	114.00	56.09	45.32	0.00	169.00	0.292
BRS+0	14.87	7.38	5.42	34.57	13.70	6.34	0.00	32.68	0.947
NUM+0	30.22	14.18	4.00	61.00	30.35	25.64	0.00	88.00	0.435
BRS-0	16.79	8.88	3.43	41.65	13.26	7.10	0.00	29.59	0.235
NUM-0	30.52	13.50	6.00	57.00	25.74	22.42	0.00	83.00	0.127
BRS1	14.05	7.29	3.97	32.73	12.49	5.69	0.00	25.06	0.775
NUM1	28.78	17.89	4.00	58.00	29.61	17.59	0.00	66.00	1.000
BRS+1	14.03	7.36	4.64	32.74	12.63	6.47	0.00	27.14	0.676
NUM+1	20.70	14.02	3.00	49.00	19.13	13.04	0.00	47.00	0.775
BRS-1	13.66	8.82	0.00	37.50	12.60	8.84	0.00	35.89	0.645
NUM-1	8.09	5.77	0.00	23.00	10.48	8.63	0.00	33.00	0.488
BRS2	14.14	9.61	0.00	41.91	13.74	6.81	0.00	24.76	0.801
NUM2	12.30	10.91	0.00	37.00	14.35	12.95	0.00	61.00	0.553
BRS+2	14.66	12.15	0.00	43.04	13.37	7.03	0.00	24.51	0.792
NUM+2	9.26	8.91	0.00	28.00	9.04	7.80	0.00	31.00	0.660
BRS-2	12.55	9.73	0.00	35.60	10.90	9.68	0.00	40.85	0.581
NUM-2	3.04	2.88	0.00	10.00	5.30	7.24	0.00	30.00	0.756
BRS3	16.84	6.17	7.91	28.21	13.57	6.61	0.00	30.93	0.124
NUM3	18.70	10.45	2.00	44.00	17.96	19.56	0.00	85.00	0.180
BRS+3	17.44	8.73	7.90	47.07	13.57	6.44	0.00	28.19	0.219
NUM+3	11.87	7.99	1.00	29.00	12.61	16.07	0.00	75.00	0.367
BRS-3	16.23	7.38	0.00	28.03	11.60	9.48	0.00	37.63	0.045
NUM -3	6.83	4.90	0.00	19.00	5.35	6.03	0.00	24.00	0.158
Total BRS	15.23	6.72	5.66	30.45	13.81	5.08	5.59	23.87	0.708
Total BRS	14.71	6.59	6.49	29.18	13.76	5.30	5.58	24.77	0.829
Total BRS	16.06	7.37	3.64	33.18	14.23	6.43	4.43	31.10	0.447

Disease duration

	Intercept	Slope	R^2	P-value
Gender	13.455	-0.139	0.000	0.964
AGE	-7.157	0.435	0.323	0.000
Height (cm)	-14.000	0.160	0.023	0.279
Weight(kg)	5.934	0.100	0.034	0.191
BMI	6.084	0.289	0.022	0.293
SBP	-13.347	0.213	0.140	0.006
DBP	-16.247	0.406	0.137	0.007
MBP	-16.768	0.321	0.137	0.007
Heat Rate (60-100)	16.310	-0.042	0.004	0.651
mean RR (ms)	8.476	0.006	0.006	0.592
SDNN (ms)	17.383	-0.100	0.034	0.188
rMSSD (ms)	15.896	-0.078	0.031	0.209
PNN50%	14.957	-0.159	0.050	0.110
TP (ms2)	15.506	-0.001	0.055	0.096
VLF (ms2)	14.936	-0.003	0.022	0.294
LF (ms2)	14.824	-0.002	0.043	0.140
HF (ms2)	15.249	-0.004	0.065	0.067
LF/HF	12.409	0.495	0.011	0.462
LF (nu)	9.733	0.067	0.017	0.350
HF (nu)	16.409	-0.067	0.017	0.350
TP SBP	13.767	-0.009	0.007	0.563
VLF SBP	13.753	-0.012	0.011	0.464
LF SBP	12.890	0.057	0.003	0.684
HF SBP	13.288	0.024	0.001	0.849
SBPLF/HF	9.880	0.865	0.092	0.029
SBPHFnu	17.548	-0.134	0.069	0.061
SBPLFnu	4.197	0.134	0.069	0.061
α LF Coh	19.468	-0.729	0.102	0.021
n LF Coh	9.349	0.187	0.019	0.324
α HF Coh	15.899	-0.196	0.026	0.256
n HF Coh	7.872	0.107	0.017	0.359
α LF Mean	19.245	-0.524	0.103	0.021
n LF Mean	7.086	0.473	0.016	0.372
mean value Modulus LF	19.830	-0.753	0.109	0.017
α HF Mean	16.009	-0.149	0.028	0.237
n HF Mean	26.286	-0.467	0.044	0.137
mean val Modulus HF	16.160	-0.211	0.032	0.207
mean modulus	15.628	-0.185	0.018	0.343
BRS0	18.687	-0.429	0.096	0.025
NUM0	16.750	-0.066	0.036	0.181
BRS+0	18.104	-0.397	0.074	0.051
NUM+0	16.380	-0.117	0.030	0.223
BRS-0	18.658	-0.414	0.104	0.019
NUM-0	16.664	-0.128	0.037	0.173
BRS1	18.824	-0.472	0.093	0.028

NUM1	14.091	-0.025	0.003	0.720
BRS+1	18.396	-0.438	0.090	0.030
NUM+1	14.248	-0.042	0.004	0.655
BRS-1	18.709	-0.471	0.125	0.010
NUM-1	13.514	-0.015	0.000	0.944
BRS2	16.616	-0.266	0.045	0.130
NUM2	13.225	0.013	0.000	0.900
BRS+2	15.041	-0.139	0.020	0.322
NUM+2	12.951	0.043	0.002	0.734
BRS-2	15.559	-0.216	0.032	0.202
NUM-2	14.443	-0.397	0.012	0.433
BRS3	18.754	-0.384	0.075	0.049
NUM3	15.906	-0.154	0.028	0.232
BRS+3	17.568	-0.294	0.068	0.061
NUM+3	13.433	-0.003	0.000	0.986
BRS-3	19.629	-0.493	0.127	0.010
NUM -3	16.282	-0.483	0.088	0.032
Total BRS gain	19.345	-0.484	0.098	0.024
Total BRS plus	18.311	-0.406	0.073	0.053
Total BRS minus	20.334	-0.550	0.130	0.009
Avg RSA DB	18.409	-0.376	0.073	0.053
Avg HRV DB	32.503	-15.423	0.068	0.061
Vals Ratio	19.860	-4.132	0.020	0.338
MBP fall Vals(>20)	13.935	-0.058	0.006	0.595
MBP PH2 Vals	13.552	-0.448	0.002	0.774
MBP PH4 Vals	14.708	-1.292	0.001	0.812
RR30:15	24.763	-10.035	0.034	0.197
Fall SBP standing	12.884	0.042	0.002	0.758
HG	17.339	-0.236	0.024	0.272
aTP_STND	12.925	0.000	0.002	0.787
aVLF_STND	13.791	-0.001	0.014	0.408
aLF_STND	14.033	-0.001	0.025	0.269
aHF_STND	12.538	0.000	0.002	0.767
lfhf_STND	13.592	-0.306	0.007	0.558
LFnu_STND	16.492	-0.059	0.013	0.439
HFnu_STND	10.586	0.059	0.013	0.439
aSBPTP_STND	11.339	0.025	0.011	0.478
aSBPVLF_STND	11.343	0.065	0.013	0.436
aSBPLF_STND	11.920	0.031	0.003	0.705
aSBPHF_STND	12.331	0.036	0.005	0.630
SBPLF/HF_STND	12.113	0.135	0.002	0.757
SBPHFnu_STND	13.893	-0.046	0.005	0.639
SBPLFnu_STND	9.321	0.046	0.005	0.639
PQ int(<200)	-2.609	0.098	0.081	0.046
QRS int(<120)	14.632	-0.012	0.001	0.850
QT int	-7.483	0.051	0.035	0.193
QTcint	-14.442	0.063	0.032	0.214

Number of lesions

	slope	Intercept	R^2	P-value
Gender	36.379	-1.615	0.002	0.795
AGE	22.197	0.289	0.038	0.193
Height (cm)	-	0.345	0.030	0.250
Weight(kg)	34.979	0.011	0.000	0.946
BMI	41.262	-0.216	0.003	0.701
SBP	9.264	0.212	0.033	0.228
DBP	8.201	0.378	0.032	0.232
MBP	-1.022	0.394	0.052	0.127
Heat Rate (60-100)	43.942	-0.119	0.009	0.521
mean RR (ms)	48.830	-0.015	0.011	0.493
SDNN (ms)	43.412	-0.187	0.031	0.242
rMSSD (ms)	39.169	-0.108	0.014	0.429
PNN50%	37.024	-0.134	0.009	0.541
TP (ms2)	38.467	-0.002	0.024	0.307
VLF (ms2)	35.459	0.001	0.000	0.917
LF (ms2)	39.076	-0.005	0.063	0.094
HF (ms2)	37.261	-0.004	0.012	0.473
LF/HF	40.784	-2.349	0.072	0.071
LF (nu)	43.029	-0.129	0.018	0.370
HF (nu)	30.107	0.129	0.018	0.370
TP SBP	36.366	-0.014	0.005	0.633
VLF SBP	36.187	-0.014	0.004	0.673
LF SBP	38.947	-0.374	0.030	0.247
HF SBP	35.142	0.192	0.002	0.793
SBPLF/HF	39.709	-0.906	0.029	0.261
SBPHFnu	31.802	0.136	0.018	0.375
SBPLFnu	45.364	-0.136	0.018	0.375
α LF Coh	42.074	-0.745	0.028	0.265
n LF Coh	30.870	0.223	0.007	0.571
α HF Coh	37.310	-0.117	0.002	0.747
n HF Coh	23.441	0.234	0.020	0.355
α LF Mean	43.038	-0.651	0.041	0.179
n LF Mean	49.391	-1.003	0.019	0.367
mean value Modulus LF	42.731	-0.807	0.033	0.230
α HF Mean	38.893	-0.174	0.010	0.514
n HF Mean	45.587	-0.359	0.008	0.566
mean val Modulus HF	38.062	-0.171	0.005	0.633
mean modulus	37.601	-0.148	0.003	0.718
BRS0	40.956	-0.415	0.025	0.299
NUM0	32.153	0.069	0.011	0.498
BRS+0	40.544	-0.400	0.020	0.349
NUM+0	31.991	0.143	0.012	0.471
BRS-0	40.572	-0.369	0.023	0.314
NUM-0	32.759	0.115	0.008	0.554
BRS1	40.636	-0.412	0.020	0.348
NUM1	34.212	0.055	0.003	0.697

BRS+1	38.947	-0.273	0.010	0.509
NUM+1	33.976	0.084	0.005	0.653
BRS-1	35.535	0.021	0.000	0.957
NUM-1	35.237	0.075	0.001	0.865
BRS2	32.330	0.281	0.014	0.429
NUM2	36.776	-0.070	0.003	0.739
BRS+2	33.228	0.217	0.011	0.480
NUM+2	36.118	-0.029	0.000	0.907
BRS-2	36.255	-0.045	0.000	0.894
NUM-2	38.752	-1.148	0.029	0.256
BRS3	39.396	-0.261	0.010	0.512
NUM3	36.009	-0.013	0.000	0.960
BRS+3	35.553	0.016	0.000	0.959
NUM+3	37.371	-0.141	0.005	0.654
BRS-3	43.338	-0.614	0.053	0.122
NUM -3	34.193	0.267	0.008	0.566
Total BRS gain	40.616	-0.389	0.017	0.385
Total BRS plus	40.219	-0.364	0.016	0.408
Total BRS minus	40.814	-0.390	0.018	0.375
Avg RSA DB	39.419	-0.283	0.010	0.518
Avg HRV DB	66.793	-25.064	0.050	0.135
Vals Ratio	40.641	-2.386	0.002	0.777
MBP fall Vals(>20)	33.049	0.492	0.128	0.018
MBP PH2 Vals	37.747	-3.900	0.037	0.216
MBP PH4 Vals	34.036	3.036	0.002	0.768
RR30:15	63.274	-22.898	0.047	0.157
Fall SBP standing	38.896	0.702	0.137	0.013
HG	24.989	0.633	0.044	0.160
aTP_STND	30.887	0.002	0.051	0.142
aVLF_STND	30.618	0.005	0.060	0.109
aLF_STND	32.392	0.003	0.026	0.294
aHF_STND	33.683	0.004	0.025	0.309
lfhf_STND	34.897	0.197	0.001	0.863
LFnu_STND	24.648	0.160	0.017	0.399
HFnu_STND	40.681	-0.160	0.017	0.399
aSBPTP_STND	28.835	0.132	0.037	0.211
aSBPVLF_STND	35.111	0.021	0.000	0.906
aSBPLF_STND	26.734	0.383	0.087	0.052
aSBPHF_STND	32.540	0.408	0.015	0.426
SBPLF/HF_STND	32.512	0.715	0.014	0.438
SBPHFnu_STND	38.176	-0.099	0.005	0.657
SBPLFnu_STND	28.269	0.099	0.005	0.657
PQ int(<200)	8.648	0.171	0.070	0.082
QRS int(<120)	48.743	-0.108	0.016	0.407
QT int(<440)	13.436	0.056	0.013	0.466
QTcint(350-440,460))	-	0.184	0.078	0.066

EDSS

	slope	Intercept	R^2	P-value
Gender	2.106	-0.027	0.000	0.957
AGE	-0.086	0.046	0.132	0.008
Height (cm)	-0.017	0.012	0.005	0.626
Weight(kg)	2.471	-0.005	0.003	0.700
BMI	2.913	-0.032	0.010	0.484
SBP	-2.858	0.039	0.176	0.002
DBP	-4.065	0.085	0.209	0.001
MBP	-4.115	0.066	0.205	0.001
Heat Rate (60-100)	2.459	-0.005	0.002	0.733
mean RR (ms)	1.122	0.001	0.008	0.525
SDNN (ms)	2.115	0.000	0.000	0.970
rMSSD (ms)	2.267	-0.005	0.005	0.607
PNN50%	2.363	-0.027	0.054	0.099
TP (ms2)	2.188	0.000	0.004	0.669
VLF (ms2)	2.084	0.000	0.000	0.962
LF (ms2)	2.147	0.000	0.002	0.756
HF (ms2)	2.243	0.000	0.015	0.389
LF/HF	1.945	0.074	0.009	0.507
LF (nu)	1.480	0.011	0.018	0.345
HF (nu)	2.596	-0.011	0.018	0.345
TP SBP	2.095	0.000	0.000	0.992
VLF SBP	2.094	0.000	0.000	0.983
LF SBP	1.964	0.015	0.008	0.535
HF SBP	2.158	-0.013	0.008	0.538
SBPLF/HF	1.625	0.113	0.056	0.091
SBPHFnu	2.558	-0.015	0.033	0.194
SBPLFnu	1.170	0.013	0.024	0.270
α LF Coh	2.639	-0.065	0.030	0.221
n LF Coh	0.912	0.054	0.059	0.081
α HF Coh	2.289	-0.015	0.005	0.604
n HF Coh	0.799	0.025	0.034	0.193
α LF Mean	2.782	-0.062	0.052	0.104
n LF Mean	1.134	0.071	0.013	0.424
mean value Modulus LF	2.771	-0.079	0.043	0.138
α HF Mean	2.247	-0.009	0.003	0.685
n HF Mean	2.998	-0.033	0.008	0.530
mean val Modulus HF	2.302	-0.016	0.006	0.577
mean modulus	2.306	-0.017	0.006	0.595
BRS0	2.560	-0.037	0.026	0.251
NUM0	2.295	-0.004	0.005	0.634
BRS+0	2.441	-0.029	0.014	0.402
NUM+0	2.177	-0.003	0.001	0.843
BRS-0	2.616	-0.041	0.036	0.177
NUM-0	2.380	-0.011	0.010	0.474
BRS1	2.420	-0.028	0.012	0.446
NUM1	1.906	0.007	0.007	0.563

BRS+1	2.486	-0.034	0.019	0.325
NUM+1	1.923	0.008	0.006	0.591
BRS-1	2.417	-0.029	0.017	0.363
NUM-1	1.964	0.019	0.005	0.603
BRS2	2.216	-0.010	0.002	0.734
NUM2	1.737	0.026	0.044	0.134
BRS+2	2.342	-0.021	0.015	0.383
NUM+2	1.742	0.031	0.049	0.113
BRS-2	2.227	-0.014	0.005	0.630
NUM-2	1.972	0.048	0.007	0.564
BRS3	2.554	-0.033	0.019	0.324
NUM3	1.695	0.024	0.026	0.251
BRS+3	2.406	-0.022	0.013	0.414
NUM+3	1.567	0.051	0.078	0.045
BRS-3	2.827	-0.057	0.061	0.077
NUM -3	2.300	-0.034	0.016	0.366
Total BRS gain	2.560	-0.038	0.021	0.303
Total BRS plus	2.400	-0.025	0.010	0.484
Total BRS minus	2.787	-0.055	0.046	0.128
Avg RSA DB	2.579	-0.036	0.025	0.265
Avg HRV DB	3.121	-0.828	0.007	0.551
Vals Ratio	2.443	-0.232	0.002	0.748
MBP fall Vals(>20)	2.148	-0.009	0.005	0.636
MBP PH2 Vals	2.038	0.196	0.012	0.452
MBP PH4 Vals	1.552	0.552	0.008	0.538
RR30:15	4.281	-1.967	0.057	0.095
Fall SBP standing	1.944	0.007	0.002	0.743
HG	1.629	0.028	0.013	0.416
aTP_STND	2.046	0.000	0.020	0.323
aVLF_STND	2.152	0.000	0.030	0.233
aLF_STND	2.134	0.000	0.030	0.228
aHF_STND	1.959	0.000	0.010	0.500
lfhf_STND	2.030	-0.041	0.006	0.607
LFnu_STND	2.354	-0.007	0.008	0.546
HFnu_STND	1.662	0.007	0.008	0.546
aSBPTP_STND	1.761	0.003	0.006	0.598
aSBPVLF_STND	1.543	0.018	0.043	0.148
aSBPLF_STND	1.782	0.005	0.004	0.674
aSBPHF_STND	1.966	-0.006	0.005	0.610
SBPLF/HF_STND	1.741	0.040	0.008	0.546
SBPHFnu_STND	2.110	-0.008	0.005	0.615
SBPLFnu_STND	1.349	0.008	0.005	0.615
PQ int(<200)	1.470	0.003	0.003	0.691
QRS int(<120)	2.703	-0.006	0.009	0.507
QT int(<440)	-1.332	0.008	0.039	0.169
QTcint(350-440,460))	-2.328	0.010	0.034	0.201

MSSS

	slope	Intercept	R^2	P-value
Gender	2.274	0.158	0.002	0.786
AGE	2.177	0.003	0.001	0.873
Height (cm)	0.641	0.010	0.002	0.735
Weight(kg)	4.009	-0.022	0.046	0.129
BMI	4.998	-0.105	0.081	0.042
SBP	0.188	0.017	0.024	0.273
DBP	-2.011	0.060	0.081	0.043
MBP	-1.185	0.038	0.050	0.114
Heat Rate (60-100)	2.428	-0.001	0.000	0.938
mean RR (ms)	1.335	0.001	0.007	0.571
SDNN (ms)	1.641	0.017	0.028	0.238
rMSSD (ms)	2.057	0.009	0.011	0.474
PNN50%	2.450	-0.012	0.008	0.545
TP (ms2)	1.973	0.000	0.043	0.142
VLF (ms2)	1.938	0.001	0.040	0.159
LF (ms2)	2.053	0.000	0.045	0.133
HF (ms2)	2.158	0.000	0.016	0.379
LF/HF	2.193	0.069	0.006	0.590
LF (nu)	1.941	0.007	0.006	0.602
HF (nu)	2.656	-0.007	0.006	0.602
TP SBP	2.150	0.004	0.047	0.126
VLF SBP	2.178	0.005	0.059	0.087
LF SBP	2.047	0.032	0.028	0.239
HF SBP	2.428	-0.019	0.013	0.418
SBPLF/HF	2.098	0.057	0.011	0.464
SBPHFnu	2.567	-0.008	0.006	0.585
SBPLFnu	1.803	0.008	0.006	0.585
α LF Coh	2.285	0.006	0.000	0.925
n LF Coh	1.411	0.042	0.028	0.241
α HF Coh	2.453	-0.009	0.002	0.779
n HF Coh	1.888	0.009	0.003	0.700
α LF Mean	2.537	-0.018	0.003	0.681
n LF Mean	1.789	0.040	0.003	0.698
mean value Modulus LF	2.452	-0.014	0.001	0.824
α HF Mean	2.336	0.000	0.000	0.996
n HF Mean	1.710	0.023	0.003	0.709
mean val Modulus HF	2.394	-0.005	0.000	0.885
mean modulus	2.534	-0.017	0.004	0.659
BRS0	2.018	0.025	0.009	0.501
NUM0	2.489	-0.003	0.002	0.746
BRS+0	1.990	0.029	0.011	0.470
NUM+0	2.504	-0.007	0.003	0.715
BRS-0	2.085	0.019	0.006	0.580
NUM-0	2.455	-0.005	0.001	0.793
BRS1	1.807	0.045	0.024	0.283
NUM1	2.240	0.003	0.001	0.804

BRS+1	2.017	0.027	0.010	0.491
NUM+1	2.395	-0.003	0.001	0.867
BRS-1	1.719	0.054	0.046	0.133
NUM-1	1.990	0.047	0.027	0.249
BRS2	2.027	0.026	0.012	0.451
NUM2	2.117	0.016	0.013	0.434
BRS+2	2.390	-0.005	0.001	0.862
NUM+2	2.226	0.010	0.003	0.682
BRS-2	2.036	0.030	0.017	0.355
NUM-2	1.817	0.197	0.085	0.038
BRS3	2.218	0.008	0.001	0.832
NUM3	1.706	0.038	0.050	0.114
BRS+3	2.198	0.009	0.002	0.760
NUM+3	1.836	0.048	0.054	0.100
BRS-3	2.605	-0.021	0.007	0.571
NUM -3	2.234	0.017	0.003	0.706
Total BRS gain	1.984	0.028	0.009	0.505
Total BRS plus	1.955	0.031	0.012	0.451
Total BRS minus	2.090	0.019	0.004	0.646
Avg RSA DB	2.287	0.003	0.000	0.928
Avg HRV DB	1.638	0.561	0.003	0.726
Vals Ratio	3.006	-0.468	0.007	0.560
MBP fall Vals(>20)	2.397	-0.015	0.013	0.447
MBP PH2 Vals	2.216	0.300	0.023	0.301
MBP PH4 Vals	1.384	0.934	0.019	0.348
RR30:15	3.718	-1.289	0.016	0.380
Fall SBP standing	2.238	0.016	0.008	0.547
HG	1.617	0.043	0.022	0.295
aTP_STND	2.310	0.000	0.015	0.399
aVLF_STND	2.386	0.000	0.016	0.385
aLF_STND	2.226	0.000	0.001	0.796
aHF_STND	2.249	0.000	0.018	0.352
lfhf_STND	2.153	0.004	0.000	0.970
LFnu_STND	1.896	0.004	0.002	0.772
HFnu_STND	2.312	-0.004	0.002	0.772
aSBPTP_STND	1.980	0.003	0.006	0.605
aSBPVLF_STND	1.794	0.018	0.029	0.245
aSBPLF_STND	1.750	0.017	0.025	0.282
aSBPHF_STND	2.282	-0.012	0.016	0.390
SBPLF/HF_STND	1.810	0.083	0.022	0.312
SBPHFnu_STND	2.667	-0.019	0.023	0.304
SBPLFnu_STND	0.735	0.019	0.023	0.304
PQ int(<200)	3.558	-0.008	0.019	0.343
QRS int(<120)	3.894	-0.015	0.036	0.193
QT int(<440)	1.977	0.001	0.000	0.940
QTcint(350-440,460))	3.195	-0.002	0.001	0.800

Calssification_localization(dwMS,gMS)

	dwMS				gMS				
	mean	std	min	max	mean	std	min	Max	P-value
Gender	0.364	0.481	0.000	1.000	0.333	0.471	0.000	1.000	0.840
AGE	44.682	11.663	28.00	68.000	50.714	14.55	19.00	69.00	0.150
Height (cm)	169.95	9.484	155.0	188.00	171.28	9.958	150.0	188.0	0.663
Weight(kg)	76.591	22.323	42.00	128.00	73.667	15.35	47.00	100.0	0.629
BMI	26.183	6.090	17.48	39.071	25.050	4.520	17.30	34.60	0.504
SBP	121.68	14.642	100.0	154.00	128.38	18.38	97.00	172.0	0.203
DBP	70.545	7.297	58.00	89.000	75.905	10.50	59.00	101.0	0.064
MBP	90.727	9.776	75.00	116.00	96.952	12.43	75.00	129.0	0.081
Heat Rate	68.227	19.477	1.000	116.00	68.381	12.91	49.00	98.00	0.977
mean RR	864.33	127.33	514.0	1128.0	911.94	155.5	615.6	1249.	0.289
SDNN (ms)	38.349	17.569	8.328	67.730	39.783	20.15	9.030	89.49	0.809
rMSSD	30.697	21.935	9.179	80.443	31.412	23.66	7.514	110.6	0.921
PNN50%	8.667	14.615	0.000	48.684	9.769	13.55	0.000	44.16	0.804
TP (ms2)	1287.5	1263.3	32.79	4675.0	1786.0	2345.	86.85	1128	0.399
VLF (ms2)	437.27	399.89	7.371	1628.9	740.04	731.6	31.90	2646.	0.106
LF (ms2)	509.95	633.50	2.636	2622.6	539.60	1082.	17.72	5256.	0.915
HF (ms2)	340.29	493.50	22.78	2106.2	506.44	740.9	14.06	3384.	0.401
LF/HF	2.227	2.420	0.116	10.378	1.622	1.270	0.148	4.784	0.325
LF (nu)	56.028	21.302	10.36	91.211	53.504	19.34	12.88	82.71	0.694
HF (nu)	43.972	21.302	8.789	89.632	46.496	19.34	17.28	87.11	0.694
TP SBP	20.318	11.391	3.106	48.300	61.681	144.5	6.284	688.7	0.199
VLF SBP	10.853	6.819	1.001	24.411	48.398	131.2	1.174	618.9	0.198
LF SBP	6.091	3.946	0.870	18.690	9.848	12.39	1.143	54.37	0.194
HF SBP	3.375	4.928	0.548	19.999	3.436	3.289	0.391	15.50	0.963
SBPLF/HF	4.153	3.557	0.215	12.850	3.910	3.853	0.468	15.95	0.835
SBPHFnu	31.608	22.126	7.220	82.303	31.228	17.57	5.897	68.11	0.952
SBPLFnu	68.392	22.126	17.69	92.780	68.772	17.57	31.88	94.10	0.952
α LF Coh	8.956	5.103	0.965	21.174	7.738	3.954	1.954	16.83	0.400
n LF Coh	21.773	7.440	2.000	28.000	21.619	8.062	5.000	28.00	0.950
α HF Coh	13.955	9.874	1.191	35.799	11.607	6.448	3.914	26.07	0.375
n HF Coh	54.318	8.615	36.00	64.000	49.429	14.71	14.00	64.00	0.199
α LF Mean	12.326	7.440	1.285	29.097	9.875	4.797	2.630	23.17	0.219
n LF Mean	12.727	3.003	6.000	18.000	13.857	2.396	9.000	19.00	0.192
mean value	9.270	5.223	0.971	21.174	7.781	3.665	1.961	15.53	0.299
α HF Mean	18.515	12.973	1.552	50.721	16.815	9.721	5.259	39.03	0.639
n HF Mean	27.864	4.288	18.00	36.000	26.857	5.339	17.00	39.00	0.509
mean val	13.962	9.835	1.252	36.606	12.281	7.009	3.945	29.36	0.534
mean	13.178	8.814	1.449	35.001	11.087	5.582	3.998	24.71	0.372
BRS0	11.694	6.797	1.346	28.399	13.009	8.430	4.230	39.29	0.585
NUM0	50.318	26.168	1.000	114.00	53.333	34.09	7.000	120.0	0.752
BRS+0	11.131	6.452	1.346	29.190	12.348	7.773	4.204	34.56	0.588
NUM+0	25.727	14.004	1.000	57.000	26.714	17.05	3.000	61.00	0.840
BRS-0	12.241	7.647	0.000	34.806	13.496	8.964	4.375	41.65	0.632
NUM-0	24.591	13.016	0.000	57.000	26.619	18.10	2.000	66.00	0.682
BRS1	11.192	7.500	1.164	29.694	11.744	6.269	5.921	32.72	0.800

NUM1	28.545	21.458	1.000	81.000	25.714	21.93	3.000	74.00	0.678
BRS+1	11.277	8.027	1.164	32.743	11.292	6.618	0.000	31.15	0.995
NUM+1	22.136	16.529	1.000	55.000	18.714	16.31	0.000	60.00	0.509
BRS-1	11.687	8.497	0.000	37.500	11.417	7.208	0.000	33.78	0.913
NUM-1	6.409	6.372	0.000	26.000	7.000	6.422	0.000	24.00	0.769
BRS2	12.504	9.303	0.000	41.909	11.428	7.805	0.000	35.11	0.691
NUM2	15.364	14.971	0.000	61.000	10.952	12.99	0.000	51.00	0.320
BRS+2	11.561	10.114	0.000	41.909	11.274	9.815	0.000	41.78	0.927
NUM+2	12.773	12.442	0.000	52.000	8.952	11.69	0.000	44.00	0.318
BRS-2	10.302	9.900	0.000	35.595	9.492	7.190	0.000	22.47	0.767
NUM-2	2.591	3.085	0.000	11.000	2.000	2.160	0.000	7.000	0.483
BRS3	13.282	7.822	0.000	35.487	14.129	7.524	1.705	28.20	0.726
NUM3	18.364	13.819	0.000	46.000	14.952	8.904	1.000	33.00	0.355
BRS+3	13.419	8.817	0.000	42.575	14.400	10.52	1.705	47.06	0.748
NUM+3	11.773	10.698	0.000	44.000	9.952	8.392	1.000	31.00	0.549
BRS-3	12.365	7.692	0.000	28.034	12.285	7.781	0.000	26.43	0.974
NUM -3	6.591	8.026	0.000	37.000	5.000	4.650	0.000	18.00	0.445
Total BRS	11.694	6.667	1.255	27.262	12.732	6.951	4.813	30.45	0.628
Total BRS	11.446	6.843	1.255	30.607	12.358	6.981	4.359	29.17	0.675
TotalBRSm	12.144	6.974	0.000	33.175	13.331	6.847	6.108	31.19	0.586
Avg RSA DB	12.878	5.276	2.700	27.690	11.607	7.686	2.040	31.00	0.539
Avg HRV DB	1.274	0.208	1.020	1.920	1.182	0.129	1.030	1.540	0.100
Vals Ratio	1.551	0.373	1.040	2.780	1.597	0.387	1.130	3.000	0.708
MBP fall Vals	10.150	10.859	-	29.000	9.000	14.18	-	31.00	0.781
MBP PH2	0.000	1.000	-1.000	1.000	0.200	0.980	-	1.000	0.537
MBP PH4	0.900	0.436	-1.000	1.000	1.000	0.000	1.000	1.000	0.324
RR30:15	1.209	0.172	0.990	1.620	1.170	0.180	0.910	1.630	0.490
Fall SBP	-3.955	8.710	-	14.000	-5.105	11.88	-	15.00	0.730
HG	17.318	6.086	7.000	33.000	16.476	7.156	8.000	29.00	0.686
aTP_STND	2466.2	2333.8	60.75	8513.4	2022.9	1607.	131.0	7093.	0.500
aVLF_STND	1069.4	1039.3	17.94	3467.3	891.56	985.5	32.90	4643.	0.588
aLF_STND	889.52	917.39	14.85	3688.9	770.83	590.4	26.16	1924.	0.640
aHF_STND	507.30	757.20	27.95	3151.4	360.59	400.1	12.10	1637.	0.464
lfhf_STND	2.658	2.132	0.531	7.483	3.774	3.169	0.540	12.99	0.199
LFnu_STND	64.209	16.244	34.69	88.211	70.086	16.27	35.08	92.85	0.267
HFnu_STND	35.791	16.244	11.78	65.302	29.914	16.27	7.148	64.91	0.267
aSBPTP_STN	44.545	24.010	11.88	108.54	55.606	32.15	14.39	150.3	0.227
aSBPVLF_ST	19.687	13.875	3.804	53.618	21.546	20.83	3.079	87.02	0.742
aSBPLF_STN	17.647	11.987	3.341	53.995	27.085	17.03	3.674	64.17	0.050
aSBPHF_STN	7.211	6.619	1.071	28.148	6.975	4.598	1.469	20.69	0.899
SBPLF/HF_ST	3.933	3.542	0.727	12.535	4.481	3.055	1.263	13.54	0.610
SBPHFnu_ST	29.974	15.616	7.388	57.914	23.046	10.30	6.874	44.19	0.116
SBPLFnu_ST	70.026	15.616	42.08	92.612	76.954	10.30	55.80	93.12	0.116
PQ int(<200)	162.42	21.867	133.0	207.00	163.55	39.39	93.00	277.0	0.912
QRS	115.66	25.876	88.00	193.00	114.15	22.33	91.00	186.0	0.846
QT int	402.47	38.239	294.0	453.00	418.20	41.91	345.0	503.0	0.228
QTcint	433.95	24.267	399.0	485.00	439.75	34.73	402.0	532.0	0.548

Type of ms-progress

	RPMS				RRMS				SPMS				
	mea	std	min	max	mea	std	min	max	mea	std	min	max	P-
Gender	0.50	0.50	0.00	1.00	0.33	0.47	0.00	1.00	0.38	0.48	0.00	1.00	0.80
AGE	56.00	3.74	50.0	60.00	44.74	13.65	19.0	68.00	56.75	10.67	33.0	69.00	0.03
Height (cm)	174.2	8.17	165.	187.0	171.0	10.09	150.	188.00	169.7	9.74	156.	188.0	0.77
Weight(kg)	82.25	24.26	50.0	115.0	74.44	19.73	42.0	128.00	74.75	9.69	60.0	92.00	0.75
BMI	26.88	6.97	17.3	34.16	25.21	5.52	17.4	39.07	25.93	2.49	22.2	30.47	0.81
SBP	123.0	18.17	97.0	141.0	123.8	17.88	100.	172.00	139.2	17.48	110.	172.0	0.10
DBP	71.75	12.87	59.0	93.00	72.10	8.75	58.0	101.00	78.50	8.96	71.0	100.0	0.22
MBP	92.25	14.75	75.0	115.0	92.79	11.26	75.0	124.00	102.1	11.34	88.0	129.0	0.14
Heat Rate	59.00	33.93	1.00	87.00	71.56	12.89	49.0	116.00	64.50	9.31	52.0	83.00	0.20
mean RR	792.9	55.88	702.	856.4	866.2	137.5	514.	1249.5	938.5	122.2	723.	1123.	0.19
SDNN (ms)	29.75	15.11	16.8	55.35	40.60	18.83	8.33	89.49	36.85	20.07	11.3	67.34	0.53
rMSSD	20.69	15.54	10.7	47.54	33.33	24.49	7.51	110.69	27.78	19.94	7.81	71.45	0.54
PNN50%	3.04	5.27	0.00	12.18	11.09	16.04	0.00	48.68	5.22	7.41	0.00	22.85	0.40
TP (ms2)	769.4	752.7	230.	2068.	1720.	1991.	32.7	11287.	1135.	946.7	86.8	2914.	0.49
VLF (ms2)	282.6	127.8	147.	488.9	568.7	564.4	7.37	2646.4	574.6	596.7	55.0	1922.	0.62
LF (ms2)	193.2	211.0	56.0	558.3	651.8	963.3	2.64	5256.1	371.1	447.8	17.7	1465.	0.49
HF (ms2)	293.5	420.8	27.5	1021.	500.1	682.6	22.3	3384.8	189.5	143.1	14.0	395.7	0.41
LF/HF	1.49	0.85	0.55	2.60	2.02	2.37	0.12	10.38	1.92	1.06	0.66	3.98	0.90
LF (nu)	54.63	15.42	35.3	72.21	53.36	21.52	10.3	91.21	61.02	13.76	39.7	79.92	0.64
HF (nu)	45.37	15.42	27.7	64.65	46.64	21.52	8.79	89.63	38.98	13.76	20.0	60.21	0.64
TP SBP	30.49	11.28	18.7	48.30	48.21	111.3	6.28	688.78	18.52	10.72	3.11	41.01	0.73
VLF SBP	18.31	4.22	13.3	22.63	33.00	98.39	1.17	618.90	9.77	8.16	1.00	28.04	0.78
LF SBP	5.03	1.17	3.06	5.95	9.80	11.68	1.14	54.37	7.03	3.54	0.87	11.55	0.59
HF SBP	7.15	7.55	0.78	20.00	5.41	13.19	0.39	83.79	1.72	0.96	0.85	4.11	0.69
SBPLF/HF	1.81	1.35	0.30	3.94	3.87	3.46	0.22	15.96	5.34	3.78	0.70	12.85	0.26
SBPHFnu	44.85	20.91	20.2	77.07	31.34	19.60	5.90	82.30	23.69	20.51	0.14	58.69	0.25
SBPLFnu	55.15	20.91	22.9	79.74	68.66	19.60	17.7	94.10	74.60	18.99	41.3	92.78	0.30
α LF Coh	5.91	2.96	2.90	10.75	8.45	4.59	0.96	21.17	8.06	4.43	1.95	14.73	0.58
n LF Coh	24.00	3.08	19.0	27.00	20.87	8.35	2.00	28.00	25.88	2.62	20.0	28.00	0.22
α HF Coh	9.98	9.32	3.14	25.93	12.94	8.67	1.19	35.80	11.75	4.70	4.60	21.04	0.77
n HF Coh	60.50	1.50	59.0	63.00	50.46	13.42	14.0	64.00	52.38	8.73	38.0	62.00	0.32
α LF Mean	7.38	3.49	3.29	12.83	11.38	6.50	1.28	29.10	10.32	5.68	2.63	19.65	0.48
n LF Mean	13.75	2.68	11.0	18.00	13.28	2.74	6.00	18.00	13.63	3.08	8.00	19.00	0.92
mean value	5.84	2.71	2.95	10.23	8.67	4.58	0.97	21.17	8.12	4.41	1.96	14.73	0.50
α HF Mean	14.32	13.42	5.40	37.50	17.86	12.01	1.55	50.72	15.73	5.51	6.32	26.18	0.78
n HF Mean	26.50	5.17	18.0	32.00	27.51	4.40	19.0	39.00	27.50	5.55	17.0	36.00	0.92
mean val	10.22	9.68	3.18	26.81	13.34	8.95	1.25	36.61	11.71	4.76	4.49	20.61	0.74
mean	9.35	8.18	3.44	23.40	12.10	7.77	1.45	35.00	11.56	3.83	4.97	19.24	0.78
BRS0	8.66	5.09	2.46	16.07	12.75	8.04	1.35	39.29	10.75	3.93	5.38	19.13	0.51
NUM0	39.75	34.16	6.00	96.00	54.92	29.43	1.00	120.00	39.25	20.99	7.00	69.00	0.29
BRS+0	9.74	6.73	2.11	20.34	12.09	7.56	1.35	34.57	10.69	3.61	5.15	17.25	0.76
NUM+0	18.75	15.80	1.00	44.00	27.54	14.95	1.00	61.00	20.88	12.57	3.00	39.00	0.34
BRS-0	7.69	3.99	2.53	12.46	13.31	8.68	0.00	41.65	10.73	4.28	5.56	20.24	0.34
NUM-0	21.00	18.51	5.00	52.00	27.38	15.63	0.00	66.00	18.38	10.09	4.00	32.00	0.29
BRS1	10.55	5.73	2.86	18.94	11.64	7.22	1.16	32.73	10.15	3.23	6.42	17.49	0.83

NUM1	36.50	24.74	10.0	73.00	28.72	21.16	1.00	81.00	19.75	14.13	5.00	45.00	0.39
BRS+1	11.16	6.72	2.52	21.31	11.61	7.53	1.16	32.74	9.42	4.45	0.00	16.43	0.74
NUM+1	28.25	18.05	7.00	49.00	21.15	15.78	1.00	60.00	15.50	13.53	0.00	44.00	0.43
BRS-1	10.92	1.95	8.84	14.11	11.35	8.45	0.00	37.50	9.59	5.39	4.44	22.79	0.85
NUM-1	8.25	9.12	2.00	24.00	7.56	6.95	0.00	26.00	4.25	3.27	1.00	9.00	0.45
BRS2	10.49	9.22	3.25	26.32	12.02	8.76	0.00	41.91	10.77	2.84	6.05	15.90	0.89
NUM2	24.25	17.88	7.00	51.00	12.77	13.22	0.00	61.00	12.75	14.80	1.00	39.00	0.31
BRS+2	10.66	9.81	2.95	27.50	11.81	11.14	0.00	43.04	9.73	4.59	0.00	14.89	0.87
NUM+2	21.25	15.16	7.00	44.00	10.10	11.36	0.00	52.00	10.88	13.49	0.00	39.00	0.24
BRS-2	8.07	6.90	0.00	18.88	10.39	9.14	0.00	35.60	7.00	6.35	0.00	18.93	0.58
NUM-2	3.00	2.74	0.00	7.00	2.67	2.94	0.00	11.00	1.88	2.85	0.00	9.00	0.76
BRS3	11.42	9.61	2.88	27.64	13.97	6.84	0.00	28.21	11.37	3.66	4.46	15.54	0.53
NUM3	25.50	11.01	10.0	39.00	16.03	10.87	0.00	46.00	15.63	11.38	2.00	34.00	0.28
BRS+3	12.53	9.41	6.06	28.74	14.24	8.75	0.00	47.07	9.93	3.09	4.46	14.64	0.42
NUM+3	14.00	10.49	2.00	30.00	10.33	9.43	0.00	44.00	10.63	8.94	1.00	26.00	0.77
BRS-3	7.50	3.21	2.68	11.14	13.79	7.32	0.00	28.03	10.69	6.79	0.00	20.00	0.18
NUM -3	11.50	14.77	2.00	37.00	5.69	4.52	0.00	19.00	5.00	5.63	0.00	18.00	0.20
Total BRS	9.80	6.53	2.94	20.45	12.53	7.02	1.26	30.45	10.50	3.29	6.85	17.68	0.59
Total BRS	10.67	8.02	2.78	24.02	12.14	7.15	1.26	30.61	10.52	2.68	6.81	15.70	0.79
TotalBRSm	8.14	3.86	3.19	13.43	13.23	7.21	0.00	33.18	10.58	3.69	6.40	19.06	0.26
Avg RSA DB	11.34	5.13	6.12	17.89	13.90	7.96	2.04	38.00	10.50	4.57	4.15	17.30	0.45
Avg HRV DB	1.16	0.07	1.09	1.23	1.26	0.19	1.02	1.92	1.17	0.07	1.06	1.27	0.30
Vals Ratio	1.38	0.21	1.18	1.68	1.56	0.39	1.04	3.00	1.59	0.18	1.33	1.82	0.61
MBP fall Vals	9.50	7.02	1.00	20.00	8.92	14.97	-	31.00	7.00	10.34	-	19.00	0.94
MBP PH2	0.00	1.00	-1.00	1.00	0.17	0.99	-1.00	1.00	0.25	0.97	-1.00	1.00	0.92
MBP PH4	1.00	0.00	1.00	1.00	0.94	0.33	-1.00	1.00	1.00	0.00	1.00	1.00	0.85
RR30:15	1.09	0.10	0.97	1.25	1.22	0.19	0.91	1.63	1.13	0.06	1.05	1.23	0.23
Fall SBP	1.50	7.70	-	11.00	-5.44	10.43	-	15.00	-5.33	11.23	-	7.00	0.47
HG	19.75	7.22	8.00	27.00	16.51	6.96	3.00	33.00	18.63	7.86	8.00	34.00	0.58
aTP_STND	1595.	1748.	319.	4599.	3462.	5523.	60.7	33689.	2309.	2284.	131.	7126.	0.72
aVLF_STND	507.2	411.2	120.	1198.	1096.	1145.	17.9	5125.5	1107.	1144.	92.7	3467.	0.61
aLF_STND	612.4	673.5	128.	1763.	1140.	1332.	14.8	6782.0	603.2	512.8	26.1	1547.	0.49
aHF_STND	476.1	671.2	61.1	1637.	1225.	3523.	27.9	21781.	598.9	735.2	12.1	2112.	0.84
lfhf_STND	2.10	0.72	1.08	3.00	3.02	2.83	0.31	12.99	1.92	1.55	0.54	5.03	0.55
LFnu_STND	65.68	8.81	51.8	74.97	64.02	18.77	23.7	92.85	57.18	17.42	35.0	83.42	0.68
HFnu_STND	34.32	8.81	25.0	48.15	35.98	18.77	7.15	76.26	42.82	17.42	16.5	64.92	0.68
aSBPTP_STN	57.07	29.25	22.7	103.6	54.06	40.88	11.8	257.83	57.92	44.70	15.1	150.3	0.97
aSBPVLF_ST	15.28	11.91	3.08	33.74	19.95	14.54	3.14	57.31	31.07	26.11	10.1	87.02	0.26
aSBPLF_STN	31.56	16.93	10.8	57.91	23.58	17.04	3.34	75.52	22.03	16.47	2.99	49.28	0.66
aSBPHF_STN	10.23	6.89	3.00	20.69	10.52	21.09	1.07	135.46	4.82	4.53	1.47	14.06	0.80
SBPLF/HF_ST	4.58	3.45	1.31	10.11	4.01	3.23	0.56	13.55	5.29	2.97	1.48	11.17	0.66
SBPHFnu_ST	25.51	13.29	9.00	43.21	28.03	15.01	6.87	64.21	19.90	10.03	8.22	40.34	0.46
SBPLFnu_ST	74.49	13.29	56.7	91.00	71.97	15.01	35.7	93.13	80.10	10.03	59.6	91.78	0.46
PQ int(<200)	168.2	24.30	140.	207.0	155.3	24.36	93.0	220.00	186.1	44.34	132.	277.0	0.04
QRS	98.00	4.30	92.0	104.0	113.8	22.72	88.0	193.00	120.0	22.05	88.0	149.0	0.29
QT int	427.5	36.98	385.	486.0	403.9	37.42	294.	469.00	426.5	39.51	380.	503.0	0.24
QTcint	476.2	43.81	420.	532.0	436.6	24.30	399.	486.00	442.1	29.83	405.	505.0	0.04

Appendix C
Ethics Approval Letters

The study of control (healthy normal) participants was approved by the Macquarie University Human Ethics Committee (reference number 5201300055). Written informed consent was obtained from all Multiple Sclerosis and healthy normal participants.

MACQUARIE UNIVERSITY

Mark Butlin <mark.butlin@mq.edu.au>

Ethics application ref: 5201300055 – Approved

1 message

Ethics Secretariat <ethics.secretariat@mq.edu.au>

Dear Dr Butlin

RE: "Blood pressure variability: its association with aortic pressure, aortic stiffness, baroreceptor function, and dietary intake of sodium and potassium." (REF: 5201300055)

Thank you for submitting the above application to the Macquarie University Human Research Ethics Committee (Medical Sciences) (HREC (Medical Sciences)) for ethical and scientific review. Your application has been reviewed by the HREC (Medical Sciences) and the Scientific Sub-Committee.

The HREC (Medical Sciences) is fully constituted and operates in accordance with the National Health and Medical Research Council's National Statement on Ethical Conduct in Human Research (2007) (the National Statement) and the CPMP/ICH Note for Guidance on Good Clinical Practice.

I am pleased to advise you that the HREC (Medical Sciences) has granted ethical approval of the above project to be conducted at Macquarie University/Macquarie University Hospital (MQ/MUH).

This research meets the requirements of the National Statement which is available at the following

website: http://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/e72.pdf.

This letter constitutes ethical and scientific approval only. Please ensure that your research conforms to any governance or institutional requirements set out by MQ/MUH.

The following documentation has been reviewed and approved by the HREC (Medical Sciences):

1. Macquarie University ethics application form.
2. Participant information and consent form (Version 1, 20 March 2013.

The following personnel are authorised to conduct this research at MQ/MUH:

1. Dr Mark Butlin
2. Professor Albert Aviolio
3. Dr Martin Turner
4. Dr Edward Barin
5. Abhishek Madras

Please note the following standard requirements of approval:

1. The approval of this project is conditional upon your continuing compliance with the National Statement. It is the responsibility of the Principal Investigator to ensure that the protocol complies with the HREC-approval and that a copy of this letter is forwarded to all project personnel.
2. The National Statement sets out that researchers have a "significant responsibility in monitoring, as they are in the best position to observe any adverse events or unexpected outcomes. They should report such events or outcomes promptly to the relevant institution/s and ethical review body/ies, and take prompt steps to deal with any unexpected risks" (5.5.3). Please notify the Committee within 72 hours of any serious adverse events or Suspected Unexpected Serious Adverse Reactions or of any unforeseen events that affect the continued ethical acceptability of the project.
3. Approval will be for a period of five (5) years subject to the provision of annual reports. Your first progress report will be due on: 10 April 2014.

NB. If you complete the work earlier than you had planned you must submit a Final Report as soon as the work is completed. If the project has been discontinued or not commenced for any reason, you are also required to submit a Final Report for the project.

Progress reports and Final Reports are available at the following website:

http://www.research.mq.edu.au/for/researchers/how_to_obtain_ethics_approval/human_research_ethics/forms

1. If the project has run for more than five (5) years you cannot renew approval for the project. You will need to complete and submit a Final Report and submit a new application for the project. (The five year limit on renewal of approvals allows the Committee to fully re-review research in an environment where legislation, guidelines and requirements are continually changing, for example, new child protection and privacy laws).

2. All amendments to the project must be reviewed and approved by the Committee before implementation. Please complete and submit a Request for Amendment Form available at the following website:

http://www.research.mq.edu.au/for/researchers/how_to_obtain_ethics_approval/human_research_ethics/forms

3. At all times you are responsible for the ethical conduct of your research in accordance with the guidelines established by the Hospital and University. This information is available at the following websites:

http://www.mq.edu.au/policy/

http://www.research.mq.edu.au/for/researchers/how_to_obtain_ethics_approval/human_research_ethics/policy

If you will be applying for or have applied for internal or external funding for the above project it is your responsibility to provide the Macquarie University's Research Grants Management Assistant with a copy of this email as soon as possible. Internal and External funding agencies will not be informed that you have ethics approval for your project and funds will not be released until the Research Grants Management Assistant has received a copy of this email.
If you need to provide a hard copy letter of ethics approval to an external organisation as evidence that you have approval please do not hesitate to contact the Ethics Secretariat at the address below.

Please retain a copy of this email as this is your official notification of ethics approval.

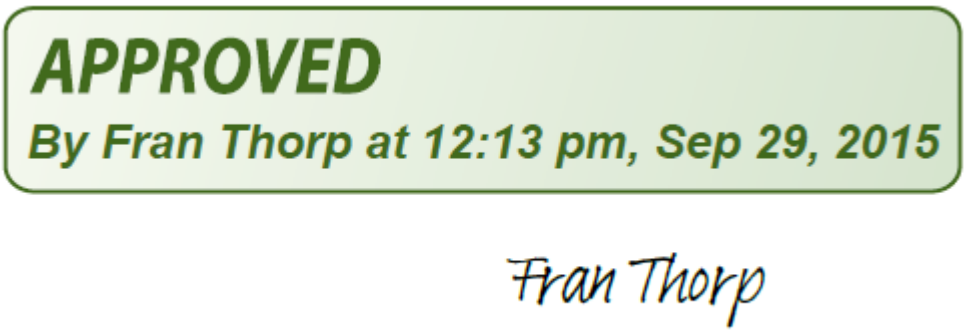
Yours sincerely
Dr Karolyn White
Director of Research Ethics

Chair, Human Research Ethics Committee (Medical Sciences)
--
Office of the Deputy Vice Chancellor

(Research) Ethics Secretariat

Research Office
Level 3, Research HUB,
Building C5C Macquarie
University
NSW 2109

The request for adding Fatemeh Shirbani to the above ethics application and project was approved by:



Ethics approval of study of Multiple Sclerosis patients was approved by the Northern Sydney Local Health District (Ethics Committee reference number: HREC/12/HAWKE/397) with acceptance of this external ethics approval by the Macquarie University Human Ethics Committee (Reference number 5201600002).

5/3/2016 Macquarie University Student Email and Calendar Mail - Re: 5201600002_ Phenotypic Analysis of Multiple Sclerosis- EEA Approved

MACQUARIE University

FATEMEH SHIRBANI <fatemeh.shirbani@students.mq.edu.au>

Re: 5201600002_ Phenotypic Analysis of Multiple Sclerosis- EEA Approved

2 messages

From: Nitya Phillipson on behalf of Ethics Secretariat
Sent: 13 January 2016 15:35

To: Alberto Avolio

Subject: 5201600002_ Phenotypic Analysis of Multiple Sclerosis- EEA Approved

Dear Alberto

Re: Phenotypic Analysis of Multiple Sclerosis (MQ ethics ref. no. 5201600002) Thanks for sending these documents through.

Please take this email as confirmation that the project has been noted by the Macquarie University Research Office.

This project has received ethics approval from the Northern Sydney Local Health District

Many thanks for providing this information for our records. No further action is required. Any amendments must be submitted to the approving HREC.

Please do not hesitate to contact the Ethics Secretariat if you have any questions.

Ethics Secretariat

Research Office|Level ,C5C Building

MacquarieUniversity,NSW2109,Australia

T:+61 2 9850 7850 | mq.edu.au

From: Alberto Avolio

Sent: Wednesday, 13 January 2016 1:32 AM
To: Ethics Secretariat

Subject: Re: Human Research Ethics _ externally Approved Application

Hi Nitya,

I can confirm that no procedures will take place at Macquarie University. All data will be collected at Royal North Shore Hospital and only data analysis will take place at Macquarie Univerity.

Regards,

Alberto Avolio

Professor

Faculty of Medicine and Health Sciences | Ground Floor, F10A
Building 2 Technology Place

Macquarie
University,
Sydney, NSW
2109, Australia
T: +61 2 9850 2747 | F: +61 2 9812 3600

M: + 61 408 657616 | mq.edu.au

alberto.avolio@mq.edu.au Google Scholar Citations

Health
Northern Sydney
Local Health District

11 October 2013

Dr Yi-Ching Lee
3E Clinical Administration
Royal North Shore Hospital
St Leonards
2065

NSLHD Local Project Number: 1309-323M
Project Title: *Phenotypic Analysis of Multiple Sclerosis*
(HREC reference: *HREC/12/HAWKE/397*)
(SSA reference: *SSA/13/HAWKE/330*)

Dear Dr Lee,

Thank you for submitting an application for authorisation of this project. I am pleased to advise that the delegate of the Chief Executive for Northern Sydney Local Health District on **04 October 2013** has granted authorisation for the above project to commence at **Royal North Shore Hospital.**

The version of the SSA reviewed by NSLHD RGO was: **AU/2/A95415**.

The documentation authorised to be used at this site are:

- Participant Information Sheet – main, version 2, 5 July 2013
- Consent Form – main, version 2, dated 5 July 2013
- Revocation of Consent Form, version 1, dated November 2012
- Participant Information Sheet and Consent Form – Blood Sample Storage Information, version 3, dated 11 July 2013
- CogState Task Descriptions Template, TPRO 003, version 8, dated April 2011

Site authorisation will cease on the date of HREC expiry **20 September 2018.**

At this time, we also remind you that, in order to comply with the Guidelines for Good Clinical Research Practice (GCRP) in Australia, and in line with additional requirement of NSLHD, the Chief Investigator is responsible to ensure that:

1. The HREC is notified of anything that might warrant review of the ethical approval of the project, including unforeseen events that might affect the ethical acceptability of the project.
2. The HREC is notified of all Serious Adverse Events (SAEs) or Serious Unexpected Suspected Adverse Reactions (SUSARs) in accordance with the Serious Adverse Event Reporting Guidelines.
3. Proposed amendments to the research protocol or conduct of the research which may affect the ethical acceptability of the project, and are submitted to the lead HREC for review, are copied to the Research Governance Officer.
4. Proposed amendments to the research protocol or conduct of the research which may affect the ongoing site acceptability of the project are to be submitted to the Research Governance Officer.

The annual report acknowledgment from the Lead HREC should be submitted to the Research Governance Officer.

Standard forms and additional guidance documents are available on the Research Office Website:
http://www.nslhd.health.nsw.gov.au/research.html

Yours sincerely,

Kylie Becker
Research Governance Officer
RESEARCH OFFICE
NORTHERN SYDNEY LOCAL HEALTH DISTRICT

Research Office
Kolling Building, Level 13
Royal North Shore Hospital
St Leonards NSW 2065
Tel (02) 9926 4590 Fax (02) 9926 6179