Autonomic function and non-motor symptoms in primary chronic autonomic failure disorders

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Copyright Declaration

Declaration of originality

I declare that all studies presented in this thesis are my own work which were undertaken during my studies at Imperial College London and have never been submitted for any other degree or professional qualification.

Apart from described below, all data were prospectively collected and analysed by myself. Any information or details from previous works that were conducted by others have been explicitly acknowledged.

Cardiovascular autonomic function data were recorded by clinical autonomic scientists as part of routine clinical tests at the Autonomic Unit, National Hospital for Neurology and Neurosurgery (NHNN) or the Neurovascular Autonomic Unit, St Marys Hospital. These data was then reduced and used to analyse various quantitative cardiovascular autonomic function measures in Chapter 4.1.1 (Quantitative cardiovascular autonomic function in patients with chronic autonomic failure), Chapter 4.1.3 (Pressor tests in MSA, Parkinson's disease with and without autonomic failure), Chapter 4.1.4 24 hour-Ambulatory Blood Pressure Monitoring (24 hr-ABPM) in MSA, Parkinson's disease with and without autonomic failure and its efficacy to detect orthostatic hypotension using diary) and Chapter 6 (Initial symptoms and clinical characteristics in Pure Autonomic Failure).

Abstract

The autonomic nervous system innervates and influences every organ in the body through two major efferent pathways; the sympathetic and parasympathetic nervous systems. Autonomic dysfunction, especially orthostatic hypotension (OH) and olfactory dysfunction, are commonly present in a variety of neurological disorders, particularly, multiple system atrophy (MSA) and allied disorders, such as Parkinson's disease (PD) and Pure Autonomic Failure (PAF) and can significantly impact quality of life and cause significant morbidity. Similarly, non-motor symptoms have been increasingly recognized in PD. The overlapping autonomic features of PD, PAF and MSA, e.g., OH, can sometimes make it difficult to differentiate a diagnosis between these conditions. The further evaluation of autonomic function, e.g., cardiovascular, olfactory and gastrointestinal function, in patients with primary chronic autonomic failure disorders could offer better diagnostic accuracy, improve the understanding of disease progression and inform the development of treatments.

Cardiovascular autonomic function screening tests are commonly used to confirm a diagnosis of autonomic failure, e.g., orthostatic hypotension, but OH is not often reliable for distinguishing between PD and MSA. Novel indices of cardiovascular autonomic function in patients with chronic autonomic failure have therefore been evaluated as well as the severity of olfactory dysfunction and other non-motor (e.g., daily activities and depression) symptoms in MSA, PD and PAF. Results demonstrated that baroreflex sensitivity and blood pressure recovery time (BRPT) in response to the Valsalva Manoeuvre are useful for differentiating MSA from PD with autonomic failure (PD+AF). BPRT was also significantly prolonged in PD patients compared to healthy controls. In addition, an association of BPRT and disease duration in PD also suggests that this index may be useful for monitoring disease progression in PD. Other findings indicated that assessing olfactory function is also helpful for distinguishing between PD, MSA and PAF. A greater degree of depression and impairment of daily activities in MSA relative to PD and PAF were also evident.

In order to further investigate the presenting symptoms and features of PAF, a time when diagnosis is often still unclear, and other disorders, such as MSA, can be suspected, the clinical characteristics and laboratory investigations, in a large cohort of PAF patients were examined. Results indicated that abnormal white matter lesions are prevalent in PAF. Furthermore, gastrointestinal symptoms were also evident in PAF and can also occur in other autonomic disorders, e.g., MSA. Using electrogastrography, impaired indexes of gastric motility were also evident in PAF.

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Abbreviations

^{123}I-MIBG - ^{123}I-metaiodobenzylguanidine

24 hr-ABPM – 24 hour-Ambulatory Blood Pressure Monitoring

ACE - Angiotensin Converting Enzyme

Ach - Acetylcholine

AD - Alzheimer disease

ADH - Antidiuretic Hormone

AF - Autonomic Failure

AFT- Autonomic Function screening Tests

A2- Angiotensin II

ANCOVA- Analysis of Covariance

ANS - Autonomic Nervous System

AON - Anterior Olfactory Nucleus

ATP - Adenosine Triphosphate

BP - Blood Pressure

BPRT - Blood Pressure Recovery Time

BRS - Baroreflex sensitivity

B-SIT - Brief Smell Identification Test

CAF - Chronic Autonomic Failure

cAMP- cyclic Adenosine Monophosphate

CASS - Composite Autonomic Scoring Scale

CC-SIT - Cross-Cultural Smell Identification Test

CGRP - Calcitonin Gene-Related Peptide

CO - Cardiac Output

COMPASS - The Composite Autonomic Symptom Scale

CP - Cold Pressor

CV - Cardiovascular

CVL - Caudal Ventrolateral medulla

DBH - Dopamine-β-hydroxylase

DBHD - Dopamine β-hydroxylase Deficiency

DBP - Diastolic Blood Pressure

DF - Dominant Frequency

DLB - Dementia with Lewy bodies

DMV - Dorsal Motor nucleus of the Vagus

ED - Erectile dysfunction

EGG – Electrogastrography

EMSA-SG - European Multiple System Atrophy Study Group

ENS – Enteric Nervous System

FDG - Fluorodeoxyglucose

FFT - Fast Fourier Transform

GI - Gastrointestinal

GCI – Glial Cytoplasmic Inclusion

GH - Growth Hormone

GMA - Gastric Myoelectrical Activity

GMP - Guanosine monophosphate

HAM-D - Hamilton Depression Rating Scale

HR - Heart Rate

HR_{DB} - Heart Rate response to Deep Breathing

HUT - Head-Up Tilt

HY - Hoehn and Yahr

ICC- Interstitial Cells of Cajal

ICDF - Instability Coefficient of Dominant Frequency

IE - Isometric Exercise

IML - Intermediolateral

IPANs - Intrinsic Primary Afferent Neurons

IQR - Inter-Quartile Range

L-DOPA - L-dihydroxyphenylalanine

MA - Mental Arithmetic

MAP - Mean Arterial Blood Pressure

MMSE- Mini-Mental State Examination

MSA - Multiple System Atrophy

MSA-C - Multiple System Atrophy Cerebellar Subtype

MSA-P - Multiple System Atrophy Parkinsonian Subtype

MSA-QoL - the MSA Health-Related Quality of Life

NA - Noradrenaline

NAmb - Nucleus Ambiguous

NCI - Neuronal Cytoplasmic Inclusion

NO - Nitric Oxide

NPY - Neuropeptide Y

NTS - Nucleus Tractus Solitarii

OH - Orthostatic Hypotension

PAF - Pure Autonomic Failure

PAG - Periaqueductal Grey

PD - Parkinson’s Disease

PD+AF - Parkinson’s Disease with autonomic failure

PDQ-39 - The 39 item Parkinson's Disease Questionnaire

PET - Positron Emission Tomography

Phase II_E - Phase II Early

Phase II_L - Phase II Late

PSP - Progressive Supranuclear Palsy

QOL - Quality of Life

RAS - Renin-angiotensin-aldosterone

RBD - REM Sleep Behavior Disorder

ROC - Receiver operating characteristic

RSA - Respiratory Sinus Arrhythmia

RVLM - Rostral Ventrolateral Medulla

SCOPA-AUT- The Scale for Outcomes in Parkinson’s disease for Autonomic Symptoms

SD - Standard Deviation

SE - Schwab and England Activities of Daily Living Scale

SF-36 - The 36-item Short Form Health Survey

SHT - Supine Hypertension

SS-16 - Sniffin’ Sticks 16 items

SBP - Systolic Blood Pressure

SPECT - Single-Photon Emission Computed Tomography

TPR - Total Peripheral Resistance

TST -Thermoregulatory Sweat Test

UMSARS - Unified Multiple System Atrophy Rating Scale

UPDRS - Unified Parkinson's Disease Rating Scale

UPSIT - University of Pennsylvania Smell Identification Test

UK-PDSBB - United Kingdom Parkinson's Disease Society Brain Bank diagnostic criteria

VIP - Vasoactive Intestinal Polypeptide

VLM - Ventrolateral Medulla

VM - Valsalva manoeuvre

VR - Valsalva ratio

WML - Cerebral White Matter Lesions

Chapter 1

Introduction

Chapter 1

1. Introduction

The autonomic nervous system (ANS) innervates and influences every organ in the body. This complex system is mediated by two major efferent pathways, the sympathetic and parasympathetic nervous systems, which obtain afferent signals from different parts of the body, such as, neurons in the spinal cord and the cerebral autonomic centre; primarily the hypothalamus, midbrain and brainstem. The gastrointestinal tract is controlled by both the sympathetic and parasympathetic pathways and additionally the enteric nervous system (ENS). The ENS lies within two plexuses, Meissner's and Auerbach's plexuses, which mainly control the motility and secretion in the small and large intestines (Cersosimo & Benarroch, 2008).

Since the ANS innervates organs throughout the body, dysfunction of the autonomic nervous system can present with a variety of symptoms according to the major system that is impaired, such as, cardiovascular, sudomotor, gastrointestinal, urinary or sexual function. Cardiovascular autonomic dysfunction can present with abnormal blood pressure (BP) regulation such as postural hypotension (or orthostatic hypotension; OH) and/or lability of BP and attendant symptoms of hypoperfusion of various organs and/or vascular beds. Orthostatic hypotension (OH) is a cardinal feature of cardiovascular autonomic dysfunction and can significantly impact quality of life and cause significant morbidity (Schrag *et al.*, 2000b; Rahman *et al.*, 2008). OH is defined as a fall in systolic BP of $\geq$20 mm Hg or diastolic BP of $\geq$10 mmHg on standing or during head-up tilt (HUT) (Freeman *et al.*, 2011) can lead to various problems, e.g., falls or injuries due to collapse or syncope (fainting). The associated symptoms of OH range from dizziness, light-headedness, visual disturbances, cognitive dysfunction, chest pain, neck pain (in "coat hanger" area) to non-specific symptoms, like fatigue or tiredness (Mathias, 2003).

Autonomic dysfunction is commonly present in a variety of neurological disorders, particularly, Multiple System Atrophy (MSA) and allied disorders, such as Parkinson's disease (PD), Pure Autonomic Failure (PAF), Dementia with Lewy bodies (DLB) and less commonly in autosomal dominant spinocerebellar ataxia (ADCA), fragile X-associated tremor/ataxia syndrome (FXTAS), Wilson's disease, fatal familial insomnia and paraneoplastic syndromes. Autonomic dysfunction of the various organs/domains can commonly present in autonomic dysfunction disorders with overlapping features, often making it difficult to distinguish between different autonomic disorders.

Primary chronic autonomic failure disorders including MSA, PAF and Parkinson's disease with autonomic failure (PD+AF), form the majority of patients with autonomic disorders. These disorders have quite distinctive pathophysiology but often present with similar features of autonomic dysfunction.

Multiple System Atrophy (MSA)

MSA is a sporadic, progressive adult-onset disorder, which is characterized by orthostatic hypotension (OH) or urinary disturbances, with erectile dysfunction in males, in addition to parkinsonism with a poor response to levodopa, or a cerebellar syndrome (Gilman *et al.*, 2008).

Parkinson's disease (PD)

PD is characterized by extrapyramidal signs, including bradykinesia, tremor, rigidity and sometimes gait or postural disturbances (Hughes *et al.*, 1992). Some PD patients, who develop autonomic failure, including OH, are typically diagnosed as PD+AF.

Pure Autonomic Failure (PAF)

PAF is an idiopathic, sporadic, neurodegenerative disorder characterized by autonomic failure without other neurological symptoms and signs (Kaufmann, 1996).

Among these disorders, PD is by far the most common; the prevalence in the UK ranges from 105 to 168 per 100,000 (Schrag *et al.*, 2000a; Hobson *et al.*, 2005) and the incidence rises sharply with age, ranging from about 17 per 100,000 person-years between 50-59 years to 93 per 100,000 person-years between 70-79 years (de Rijk *et al.*, 1995; Bower *et al.*, 1999). It is slightly more prevalent in males than females with an average age of onset of 60. PD patients have a higher life expectancy than other forms of parkinsonian disorders, such as MSA or DLB. In contrast to PD, MSA is less common with a prevalence ranging from 1.9-4.9 per 100,000 (Schrag *et al.*, 1999; Chrysostome *et al.*, 2004). Symptom onset typically starts in the mid-fifties but never before the age of 30. Patients with MSA have a relatively poor prognosis with a median survival of 6-9.5 years from symptom onset (Wenning *et al.*, 1994a; Ben-Shlomo *et al.*, 1997; Watanabe *et al.*, 2002). There are two forms of MSA according to the prominent features; MSA with a parkinsonian subtype (MSA-P) and MSA with a cerebellar subtype (MSA-C). Large epidemiology studies have shown that MSA-P is more common than MSA-C in European countries (Wenning *et al.*, 2013). This is in contrast to a previous study in Japan, where MSA-C was reported to be more prevalent (Watanabe *et al.*, 2002). PAF is much rarer and usually presents with orthostatic

hypotension (OH). There is no cerebellar or parkinsonian signs, unlike MSA and PD. The information on other clinical features is scarce and usually extracted from case series or research studies with limited amounts of patients. Abnormal sweating (both hyper and hypohidrosis) and bladder symptoms are also often reported in PAF. The exact prevalence and disease onset of PAF are unknown because of its rarity but the prognosis is better than MSA (Mabuchi *et al.*, 2005).

Extrapyramidal features are hallmark signs of PD and are quite common in other disorders, such as MSA. Non-motor symptoms have recently also been considered as important features in PD and some of the non-motor symptoms that have been reported include, constipation, urinary dysfunction, olfactory dysfunction, orthostatic hypotension, erectile dysfunction and abnormal sweating. These non-motor symptoms in PD can occur not only in the advanced stages but also in the early stages of PD (Khoo *et al.*, 2013) or even in those without any medication (Mollenhauer *et al.*, 2013). Among a range of non-motor features, depression and autonomic dysfunction, including cardiovascular, gastrointestinal, urinary and thermoregulatory dysfunction, are considered to be key features which have an impact on patient's quality of life (Gallagher *et al.*, 2010).

The overlapping features between parkinsonian syndromes and the complex features of MSA sometimes results in difficulties in differentiating the diagnosis between MSA and PD. Furthermore, a variety of presenting symptoms of MSA may lead the patient to see specialists in different areas of expertise prior to a neurologist, consequently delaying the correct diagnosis and management. The autonomic symptoms at the early stage of disease in a patient with Parkinsonism can provide vital evidence to help distinguish MSA from other parkinsonian disorders. For example, a more rapid progression of autonomic dysfunction is suggestive of MSA compared to those with PD (Lipp *et al.*, 2009). Some MSA patients present with an isolated feature at the onset, such as orthostatic hypotension, urinary symptoms (including erectile dysfunction), stridor without parkinsonism or the combination of these symptoms. Consequently, autonomic dysfunction can predate the diagnosis of MSA for a while and patients can sometimes be misdiagnosed with another autonomic disorder, such as PAF, before the development of other hallmark MSA features.

Orthostatic hypotension is one of the most common non-motor symptoms of PD which can occur at any stage of PD and has frequently been reported in previous studies (Chaudhuri *et al.*, 2006b; Martinez-Martin *et al.*, 2007). It is also one of the diagnostic criteria for MSA (Gilman *et al.*, 2008). There are several causes of orthostatic hypotension in patients with parkinsonian features. It can be caused by the adverse side effects of anti-

parkinsonian treatment, such as L-DOPA (with or without COMT inhibitors). Dopamine agonists and MAO-B inhibitors (Selegiline and Rasagiline) are among other common causes of OH. Other drugs which are used to treat other conditions, for example anti-hypertensive agents, vasodilator agents and diuretics, can also contribute to OH and intensify the severity of OH in patients with autonomic failure. The complication of metabolic disorders, particularly Diabetes Mellitus, can result in OH as part of autonomic neuropathy. Primary autonomic failure disorders, such as MSA or PD+AF, are therefore often considered after the various above causes are excluded.

Cardiovascular autonomic screening tests are therefore used to confirm the diagnosis of autonomic failure but given that OH is common across a variety of autonomic/movement disorders and/or is a consequence of various medications, it may not ideally used as a single autonomic feature for distinguishing autonomic disorders, such as PD and MSA (Riley & Chelimsky, 2003). There are other autonomic function tests, for instance pressor tests (isometric exercise, mental arithmetic and cold pressor), 24 hour blood pressure (BP) and heart rate (HR) monitoring and other novel indices of quantitative cardiovascular autonomic function, such as, baroreflex sensitivity, blood pressure recovery time in response to a manoeuvre that reduces blood pressure and heart rate responses to alterations in blood pressure and respiration that have recently been developed and additionally been proposed to be useful in evaluating the autonomic function in chronic autonomic failure disorders (Vogel *et al.*, 2005; Schrezenmaier *et al.*, 2007). These indices are frequently abnormal in patients with autonomic failure relative to healthy controls (Schrezenmaier *et al.*, 2007). The efficacy of using these indices of cardiovascular autonomic function for distinguishing different chronic autonomic disorders, as well as their relationship with disease duration, is still uncertain and has not been thoroughly investigated. **Therefore, Aim #1 of this thesis is to examine cardiovascular autonomic function in primary chronic autonomic failure disorders using various parameters of cardiovascular autonomic function**. These findings might reveal which cardiovascular autonomic indices could be useful markers for differentiating PD+AF, MSA and PAF.

Impaired olfactory function is also a prominent non-motor feature in individuals with PD (Ansari & Johnson, 1975; Hawkes *et al.*, 1997). Hyposmia is considered to be an early marker which predates motor symptoms in PD (Ponsen *et al.*, 2004). Although impaired olfaction has been extensively studied and confirmed as a potential maker for PD, there are only a few studies investigating olfactory function in MSA and PAF (Silveira-Moriyama *et al.*, 2009b). In addition, results from previous studies were uncertain due to the relatively small

number of participants. In addition, it is still not clear whether there is a progression of impaired olfaction with disease duration and severity in PD and it is unknown if there are alterations in olfactory function with disease progression in MSA or PAF.

In addition to classical motor features, patients with PD are commonly affected with non-motor symptoms. A previous study showed that non-motor features can present in up to 88% of PD patients after 7-years of follow-up (Shulman *et al.*, 2001). These non-motor aspects of parkinsonian and autonomic disorders often have profound effects on overall features of life, such as capacity for daily life activities, quality of life and psychological health, e.g., depression, which have typically been investigated in PD, MSA and PAF in isolation but have not been simultaneously compared between these disorders or related to other indices of autonomic function. **Therefore, Aim #2 of this thesis will examine olfactory function, other non-motor aspects and quality of life in primary chronic autonomic failure disorders**.

PAF is characterized by autonomic failure without other neurological features. Cardiovascular autonomic dysfunction can present in PAF with a range of symptoms, such as dizziness, visual disturbances, lightheadedness, loss of consciousness, temporary cognitive impairment and neck muscle pain ("coat hanger" ache). These symptoms are caused by a reduction of organ perfusion which is well described in patients with orthostatic hypotension. There are several other clinical manifestations of PAF which suggest autonomic dysfunction, for example, urinary urgency and incontinence, dysphagia, constipation and erectile dysfunction. There are a number of reports demonstrating the presenting symptoms and clinical features of MSA but only a few studies that have specifically examined the presenting symptoms and clinical features of patients that have later obtained a confirmed diagnosis of PAF (Mathias *et al.*, 1999). This is important to investigate because at the time of presentation, and just after, in a PAF patient, the diagnosis is often unclear, and other disorders, such as MSA, can be suspected. **Therefore, Aim #3 of this thesis is to analyse the initial symptoms and clinical characteristics in patients with a confirmed PAF diagnosis.** This aim will be achieved by evaluating autonomic symptoms, autonomic function test results and intracranial imaging to further characterise initial and presenting features in PAF and identify possible associated factors which will facilitate a better definition of the disease progression in this cohort.

Previous pathological studies of PAF showed abnormal Lewy bodies in the intermediolateral grey columns of the thoracolumbar level of spinal cord, supporting that PAF is a form of Lewy body disease (Johnson *et al.*, 1966; van Ingelghem *et al.*, 1994; Hague *et*

al., 1997). Cardiac MIBG uptake is markedly reduced in patients with PAF, which is consistent with PD, supporting a postganglionic sympathetic lesion in both disorders (Hirayama *et al.*, 1995). Gastrointestinal symptoms can sometimes be prominent in PAF. Gastric electrical dysrhythmia using electrogastography (EGG) has been detected in PD (Lu *et al.*, 2004), but not MSA patients (Suzuki *et al.*, 2005). Although no pathological study of the involvement of Interstitial Cells of Cajal (ICC), a key centre of the stomach pacemaker, has been previously reported, there is evidence of alpha-synuclein deposition in Auerbach's and Meissner's plexuses, which are closely linked to the ICC, in patients with PD (Braak *et al.*, 2006b). Given PD and PAF have a similar postganglionic sympathetic lesion, it may be that PAF patients have gastric dysrhythmia as a result of alpha-synuclein deposition in Auerbach's and Meissner's plexuses To date, there have not been any studies investigating EGG activity in patients with PAF. **Therefore, Aim #4 of this thesis will investigate gastric motility using Electrogastrography in primary chronic autonomic failure disorders with a particular focus on PAF.**

Chapter 2

Literature Review

Chapter 2

2. Literature review

2.1. Autonomic nervous system (ANS) function

The term “autonomic nervous system” was first introduced by Langley in 1898. It referred to neurons in ganglia outside the brain and spinal cord which can function “independently and autonomously” without central nervous system control. It has now become clear that the ANS not only involves the peripheral control of visceral organs via the sympathetic, parasympathetic and enteric nervous systems and the sensorimotor nerves but the system is also regulated by the central autonomic network, which includes various key structures, such as the insular cortex, anterior cingulate cortex, amygdala, hypothalamus, periaqueductal gray, nucleus tractus solitarius, ventrolateral reticular formation of the medulla and the medullary raphe.

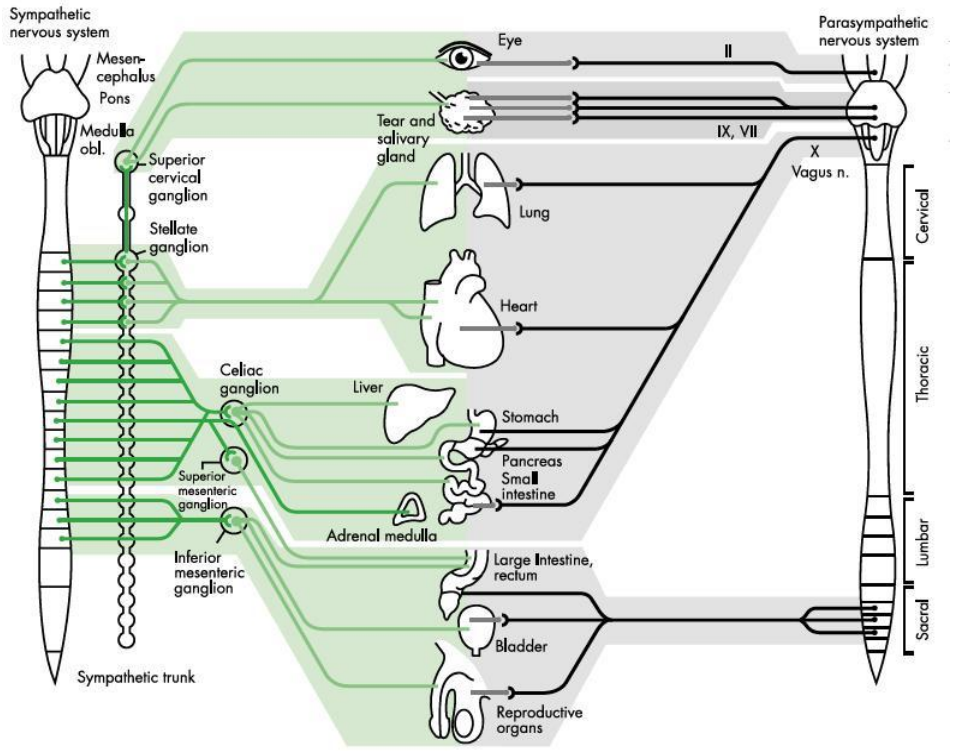


Figure 2.1. Organisation of the Autonomic Nervous System. ***Reproduced from Jänig (1995). In: Schmidt and Thews, eds. Physiologie des menschen, 26th ed.Heidelberg: Springer-Verlag, 340–369.***

The sympathetic and parasympathetic nervous systems share a similar characteristic with regard to their synaptic connection. Both systems consist of pre- and post-ganglionic neurons. Pre-ganglionic neurons start from their cell bodies in the spinal cord or brainstem

and send axons into the ganglia, where they synapse on the post-ganglionic neurons. The axons of post-ganglionic neurons then project to various tissues and organs in the body.

The obvious difference between the sympathetic and parasympathetic systems is the organisation within the ANS. The cell bodies of sympathetic pre-ganglionic neurons are located in the intermediolateral cell column of spinal cord from thoracic (T1 level) to lumbar (L3 level) regions. These neurons project axons to and synapse on post-ganglionic neurons either at the paravertebral or prevertebral sympathetic ganglia before innervating a range of organs, such as the eyes, salivary glands, as well as the adrenal medulla, intra-abdominal and pelvic organs and their blood vessels. More importantly, they also innervate and control the splanchnic circulation and blood vessels supplying muscle (Saper, 2002; Benarroch, 2008), which are mostly responsible for short-term blood pressure regulation. The sympathetic nervous system also controls skin vasomotor and sudomotor function, which are critical for thermoregulatory function and metabolic control during stress (Cersosimo & Benarroch, 2012).

In contrast, pre-ganglionic neurons of the parasympathetic system lie in the brainstem and sacral spinal cord (from levels S2 to S4). The cell bodies of the parasympathetic system are clustered as a number of autonomic nuclei in different areas within the midbrain, pons and medulla. The major autonomic nuclei in the brainstem include the dorsal motor nucleus of the vagus (DMV), the nucleus ambiguous (NAmb), salivary nuclei and the Edinger-Westphal nucleus. The DMV is crucial for gastrointestinal motility mediated by the enteric nervous system (Hopkins *et al.*, 1996; Chang *et al.*, 2003). The ventrolateral portion of the NAmb are essential for blood pressure and heart rate control via vagal output through the heart (Hopkins *et al.*, 1996). A group of neurons in the ventrolateral medulla is particularly important since these neurons give rise to the majority of pre-ganglionic innervation of the heart which are responsible for blood pressure and heart rate control (Spyer, 1994; Morrison, 2001; Dampney *et al.*, 2003). The sacral parasympathetic output starts from the pre-ganglionic neurons at the sacral level of the spinal cord and project to various organs, including the rectum, bladder and sexual organs (de Groat, 2006). In contrast to post-ganglionic sympathetic neurons, the parasympathetic post-ganglionic neurons are mainly located close to the innervated organs.

Gastrointestinal motility is uniquely different from other organs, as it is controlled by both the sympathetic and parasympathetic systems (extrinsic pathways), as well as by the enteric nervous system (intrinsic pathway). The enteric nervous system, containing the myenteric (Auerbach) and submucosal (Meissner) plexuses, is located throughout the gut

from the oesophagus to the anus.

Because the ANS innervates every organ in the body, abnormalities of the system can present with a wide range of symptoms, such as those that are a result of abnormal blood pressure and heart rate control, abnormal sweating and temperature regulation, bladder and sexual dysfunction and gastrointestinal dysfunction.

2.2 Neurotransmitters in the Autonomic Nervous System

Noradrenaline (NA) and acetylcholine (Ach) are the main neurotransmitters in the ANS. Ach is the primary neurotransmitter both in pre-ganglionic sympathetic, as well as in pre- and post-ganglionic parasympathetic neurons and the majority of ENS neurons, whereas NA is the primary neurotransmitter in post-ganglionic sympathetic neurons. Post-ganglionic sympathetic neurons innervating sweat glands and muscle are exceptions, where Ach is utilized as a neurotransmitter. In addition, there are a number of other neurotransmitters which are increasingly reported in the ANS and coexist with Ach and NA. These neurotransmitters include neuropeptides (e.g., neuropeptide Y, substance P, vasoactive intestinal polypeptide, calcitonin gene-related peptide, adenosine triphosphate (ATP), nitric oxide (NO) and others (GABA, glutamate, dopamine and 5-HT) and play an important part in regulating the normal function of the ANS. For simplicity, this review will only cover key neurotransmitters in the ANS.

Noradrenaline (NA)

NA is synthesized by converting tyrosine to L-dihydroxyphenylalanine (L-DOPA) via tyrosine hydroxylase, a rate limiting enzyme of catecholamine biosynthesis. L-DOPA is then converted to dopamine by DOPA decarboxylase. Noradrenaline and adrenaline are then synthesised via Dopamine-β-hydroxylase and phenylethnolamine N-methyltransferase, respectively. NA is co-released by exocytosis and then interacts with specific receptors, including, α_1, α_2 and β receptors, as well as their receptor subtypes. While the majority of α_1 receptors and its subtypes mediate vascular and visceral smooth muscle stimulation, the α_2 receptors are involved in inhibiting NA release and mediate smooth muscle contraction of blood vessels. The β receptors consist of β_1, β_2 and β_3 receptor subtypes (Guimaraes & Moura, 2001). The β_1 is responsible for sympathetic effects on heart rate, excitability and contractility, whereas the β_2 causes smooth muscle relaxation. β_3 receptor subtypes are in brown fat and its exact effect is still unclear. It has been found to be involved in

thermogenesis in animals.

Acetylcholine (Ach)

The synthesis of Ach involves the acetyl coenzyme A, choline and choline acetyl transferase. It is released and interacts with two types of receptors, nicotinic and muscarinic. The nicotinic receptors are ionotropic ligand-gated channels, whereas the muscarinic types are G protein-coupled receptors. While the former are mainly found in autonomic ganglia, the latter are extensively located in autonomic innervated organs, autonomic ganglia and smooth muscle. Nicotinic receptors mediate fast responses from the pre-ganglionic neurons to the autonomic ganglion cells and ENS. The muscarinic receptors can broadly be divided into M_1-like and M_2-like receptors. M_1-like receptors consist of M_1, M_3 and M_5, of which nerve depolarization is induced by K^+ channels inhibition and the release of Ca^{2+}. M_2-like receptors, including M_2 and M_4, are activated by the inhibition of adenylyl cyclise, K^+ channel activation and the inhibition of Ca^{2+} release. M_2 receptors inhibit the release of Ach in both cholinergic and sympathetic terminals. Examples of actions of this receptor include the reduction of excitability of the sinus node in the heart and counteracting the relaxation of muscle in the urinary bladder.

Neuropeptides

Neuropeptides (substance P, CGRP, neuropeptide Y and VIP) are extensively present in the ANS, especially in peripheral pathways and the ENS. These act through the activation of G protein-coupled receptors and modulate synaptic transmission and trophic and vasomotor actions. In addition, they frequently coexist with other neurotransmitters and generally require stronger stimuli than those from other neurotransmitters to activate exocytosis. Neuropeptides can generate multiple effects depending on the pattern of neuronal activity. Substance P is commonly found in the visceral organs and is involved in the control of smooth muscle contraction and endothelium nitric oxide-mediated vasodilatation. NPY, coexisting with NA in the sympathetic ganglia, mediate smooth muscle contraction and has inhibitory effects on NA release. VIP, which is located in the ENS, controls the relaxation of smooth muscle and also has a vasodilator effect within the intestines (Lundberg, 1996).

Purines

ATP and adenosine act via purinergic receptors, which are widespread in the sympathetic and parasympathetic nervous systems and the ENS. With regards to ATP, it

commonly co-releases with other neurotransmitters and interacts with two major receptor subtypes including P_{2x} and P_{2y}. P_{2x} receptors are ligand-gated channels widely present in nociceptive neurons in visceral organs and autonomic ganglia. These receptors mediate the fast response of smooth muscle contraction and initiate sensory inputs to activate pain and physiological reflexes (Burnstock, 2006). The P_{2y} receptors are G protein-coupled receptors involved in endothelium-mediated vasodilatation and control the release of neurotransmitters mediating slower responses. Adenosine also acts through G protein-coupled receptors that can be divided into 3 major subtypes; A_1, A_2 and A_3. The main action of A_1 is inhibiting the release of neurotransmitters including NA and Ach, whereas the A_2 can cause vasodilatation.

Nitric oxide (NO)

NO is formed from L-arginine and oxygen by nitric oxide synthetase and found in both pre- and post-ganglionic parasympathetic and some ENS neurons. It produces cyclic guanosine monophosphate (GMP) through the activation of cytoplasmic guanylate cyclase. This results in vasodilatation, penile erection and smooth muscle relaxation (Toda & Okamura, 2003).

2.3 Cardiovascular Autonomic Function

Maintaining blood pressure within safe limits is critical in order to sustain appropriate organ perfusion, particularly in those above the heart, such as the brain. A postural change from supine to standing results in a shift of ~500-700 ml of blood from central compartments to the lower body (Mathias, 2002). A variety of complex systems including the neural system (via baroreflexes), the humoral system (renin-angiotensin and vasopressin systems), the capillary-fluid-shift system and the renal-body-fluid pressure control system (aldosterone, antidiuretic hormone) work closely together to sustain normal blood pressure during gravitational stress. The function of these systems will be briefly described.

Neural Baroreflex Function

The baroreflex control system is responsible for the short-term blood pressure regulation (within seconds or minutes) via the regulation of total peripheral resistance (TPR; through vasoconstriction or vasodilation of vascular beds) and cardiac output (CO). The arterial and cardiopulmonary baroreceptors located in the carotid sinuses and aortic arch, and the heart and lungs are activated when there is a decrease or increase in BP and send

afferent signals to the cardiovascular control centre in the brainstem. An increase in BP results in increased vagal tone to the heart and decreased CO, as well as TPR, as a result. A reduction of BP causes the opposite effects, which increases sympathetic activity to the heart and inhibits vagal discharge, which results in a rise in TPR and CO in order to maintain BP. The latter phenomenon resembles the physiological changes during standing in humans.

The baroreflex system is divided into afferent and efferent pathways, the afferent pathway provides excitatory projections from the baroreceptors to the nucleus tractus solitarii (NTS) (Blessing, 2003). Efferent projection from the NTS plays an important role in sympathetic inhibition, which controls BP and HR by sending projections to the rostral ventrolateral medulla (RVLM) via interneurons in the caudal ventrolateral medulla (CVL). A group of neurons in the RVLM send a direct projection to pre-ganglionic neurons in the IML, which primarily generate vasoconstrictor effects in muscle, mesenteric and renal blood vessels. On the other hand, the NTS also sends output to a group of neurons in the ventrolateral portion of the nucleus ambiguous, which directly projects to cardiac ganglion neurons. This output is vital for controlling HR through the sinus node (Figure 2.2).

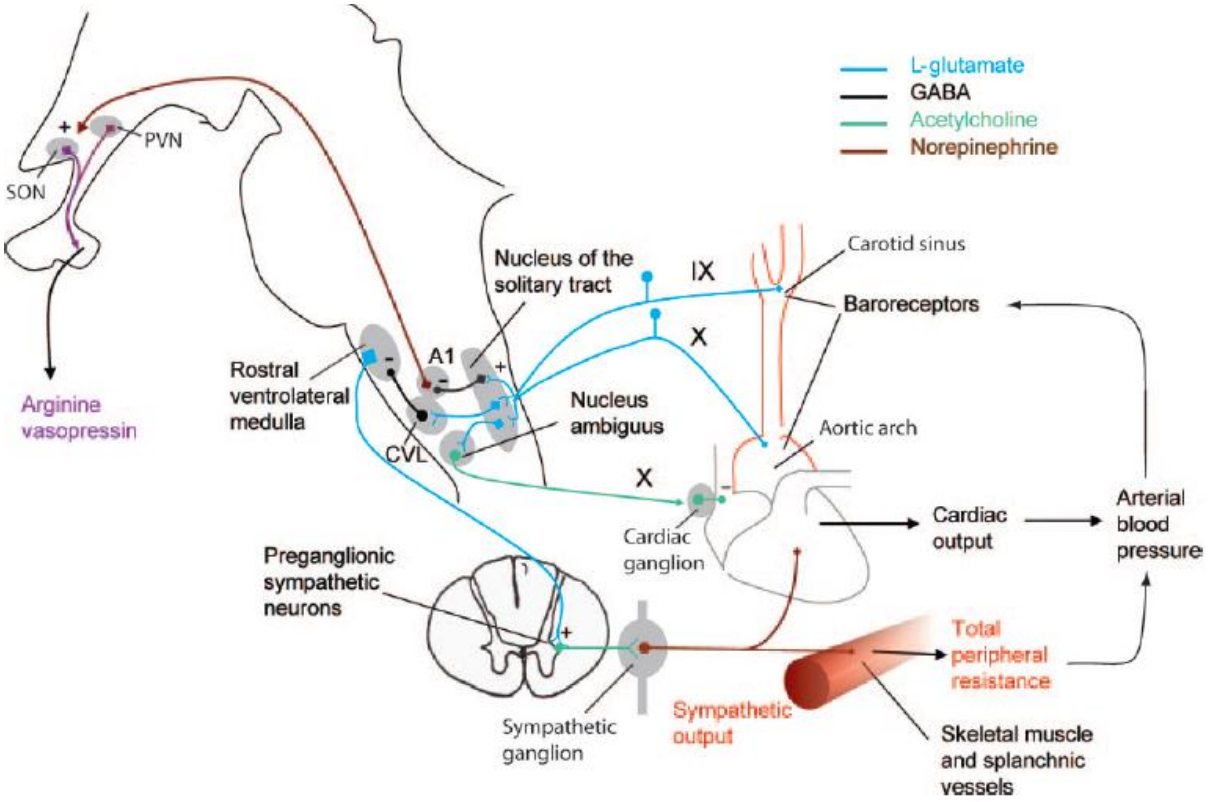


Figure 2.2. Neural Baroreflex pathways and their role in BP regulation. ***Adapted from (Benarroch, 2008).***

Humoral system (renin-angiotensin and vasopressin systems)

Renin-angiotensin-aldosterone (RAS) and vasopressin systems are two major humoral mechanisms, which are responsible for long-term (minutes to hours) BP control. These systems are minimally involved in the initial hemodynamic adjustments to alterations in BP if there is adequate blood volume in the circulation. By contrast, activation of these mechanisms is more prominent during hypovolemic states or prolonged orthostasis, such as orthostatic hypotension.

Renin-angiotensin-aldosterone system (RAS)

Renin is synthesized in juxtaglomerular cells located in the renal arterioles and secreted into the circulation in response to a reduction of renal blood flow and an increase in sympathetic discharge. Renin hydrolyses angiotensinogen, a peptide in the liver, to form angiotensin I. Angiotensin I is then converted into angiotensin II (A2) by angiotensin-converting-enzyme (ACE), mainly in the lungs and blood vessels. A2 is a strong vasopressor substance, which actively involves the vasoconstrictive function of renal arterioles and controls aldosterone secretion from the adrenal cortex. Aldosterone activates the Na^+/K^+ pump in the distal renal tubule, which is important for Na^+ and water reabsorption as well as K^+ excretion from urine. A2 is also involved in sympathetic activation, decreasing baroreflex sensitivity and closely interacts with neurohormonal and fluid-electrolyte changes.

Antidiuretic hormone (ADH) or Vasopressin system

ADH is synthesized in the supraoptic and paraventricular nuclei of the hypothalamus and stored in the posterior pituitary gland. ADH is released in response to an increase in osmolarity and a reduction of fluid volume. The latter is controlled by feedback from the stretch volume receptors, which are located in the left atrium, inferior or superior vena cavae, pulmonary veins, carotid sinus and aortic arch. It has an important role in water reabsorption via cyclic AMP on the renal distal tubules and collecting ducts. In normal subjects, there is a rise in ADH during the upright posture.

2.4 Classification of autonomic disorders

There are a number of ways to classify autonomic disorders. They can be divided by the onset and progression of disease (e.g. acute or chronic), regional involvement (e.g. localized or generalized), reversibility (fixed/chronic or intermittent) or the pathophysiological

mechanism (e.g. neurodegenerative, toxic or metabolic substances, autoimmune-mediated) or according to its onset and aetiology, for example, primary or secondary autonomic failure. Primary autonomic failure describes when the autonomic dysfunction occurs without a clear cause, while secondary autonomic failure is used when there are associated factors, disease or conditions that the autonomic dysfunction is secondary to, e.g., diabetes and amyloidosis. Autonomic failure can be a result of a lesion of central or peripheral origin. This thesis will focus on primary chronic autonomic failure.

2.5 Primary chronic autonomic failure (CAF)

Primary chronic autonomic failure is a group of neurological disorders without identifiable causes (for the autonomic failure). This consists of multiple system atrophy (MSA), Pure autonomic failure (PAF), Parkinson's disease with autonomic failure (PD+AF) and dementia with Lewy bodies (DLB). The focus of this thesis will be MSA, PD and PAF.

MSA

MSA is a sporadic adult-onset neurodegenerative disorder. It is characterized by orthostatic hypotension or urinary disturbances with erectile dysfunction in males, in addition to parkinsonian, cerebellar and pyramidal signs. Autonomic dysfunction is an essential diagnostic criterion for possible and probable MSA (Gilman *et al.*, 2008). The overlapping features between parkinsonian syndromes and the complex features of MSA sometimes result in difficulties in a diagnosis of MSA. Some patients present with an isolated feature, such as orthostatic hypotension, urinary symptoms (including erectile dysfunction), stridor without parkinsonism or a combination of these symptoms at the onset. These features can predate the diagnosis of probable MSA for years. A variety of presenting symptoms of MSA may lead the patients to see specialists in different areas of expertise prior to a neurologist, which in turn delays the diagnosis and most appropriate management. Autonomic symptoms at the early stages of the disease in patients with parkinsonism provide vital evidence to distinguish MSA from other parkinsonian disorders. In addition, a more rapid progression of autonomic dysfunction is more likely in diagnosis of MSA than PD (Lipp *et al.*, 2009).

PD

PD is the second most common neurodegenerative disorder characterized by extrapyramidal signs, including bradykinesia, tremor, rigidity and sometimes gait or postural disturbances. These signs are classified as motor features of PD and have generally been

used as a diagnostic criteria for the disorder (Hughes *et al.*, 1992). Over the last decade, non-motor symptoms of PD have increasingly become of interest and studied. This is partly due to a neuropathological study by Braak *et al* (Braak *et al.*, 2003) which described a categorisation of the progress of PD into 6 stages based on the areas of the brain that are affected. Although support for this hypothesis is equivocal, it proposes that PD could start from impairments in the gastrointestinal tract, abnormal olfaction and autonomic disturbances which correspond to abnormal Lewy body pathology in the enteric plexus, anterior olfactory nuclei and dorsal motor nucleus of the vagus nerve, respectively. The locus ceruleus, raphe and lateral tegmental nuclei are proposed to be affected in stage 2. In stage 3, the substantia nigra is affected and it is in this stage when the hallmark motor symptoms of PD develop in accordance with the large dopaminergic neuron loss (Schwarz *et al.*, 2000; Marek *et al.*, 2001). Lewy body pathology then affects other parts of the brain, including the basal forebrain and neocortex in stages 4-6. PD patients are typically diagnosed in these stages (stages 3-6) because of the prominent motor signs which correspond to at least 50% of substantia nigra cells loss (Fearnley & Lees, 1991). Based on this hypothesis, abnormal olfaction, impaired cardiovascular autonomic control and gastrointestinal disturbance may predate the motor symptoms and cognitive dysfunction in PD.

PAF

PAF is a relatively rare neurodegenerative condition, characterized by orthostatic hypotension (OH) without other neurological features (Kaufmann, 1996). Although the prevalence of this disorder is unknown due to the limited number of patients, previous studies indicated that the prognosis of this condition is much better with a slow disease progression and patients generally live longer than other forms of primary chronic autonomic failure (Mabuchi *et al.*, 2005). PAF has received more research attention recently. This is due to common features with patients with PD. Both patient groups have hyposmia, reduced cardiac MIBG reuptake and lewy body deposition both in the skin and central nervous system (Hirayama *et al.*, 1995; Ikemura *et al.*, 2008; Silveira-Moriyama *et al.*, 2009b; Shishido *et al.*, 2010). These features raise a question of whether or not PAF is a limited form of PD without motor features. Some even hypothesized that PAF may have a protective factor/substance that prevent patients from developing PD (Kaufmann & Goldstein, 2010).

2.5.1 Non-motor symptoms in Primary CAF

2.5.1.1 Cardiovascular autonomic symptoms in Primary CAF

Cardiovascular autonomic dysfunction results in a range of symptoms such as dizziness, visual disturbances, lightheadedness, loss of consciousness and temporary cognitive impairment and neck muscle pain ("coathanger" ache), which improve after lying down. These symptoms are caused by a reduction in organ perfusion and are well described in patients with orthostatic hypotension (OH) which is a well-known feature of cardiovascular autonomic dysfunction. It is defined by an orthostatic decrease of blood pressure within 3 minutes of standing by at least 20 mmHg of systolic or 10 mmHg of diastolic blood pressure (Kaufmann, 1996). The prevalence of OH varies considerably depending on the study population. OH was reported in ~30% of the otherwise healthy population over 60 years of age (Cunha *et al.*, 1991; Raiha *et al.*, 1995) and this figure rises with increasing age (Lipsitz, 1989; Masaki *et al.*, 1998) . Nevertheless, many people may be asymptomatic even if they meet the criteria of OH (Forman & Lipsitz, 1997).

Pathophysiology of OH

To maintain arterial BP during standing and counteract the shift of blood to the lower part of the body, neural pathways, local reflexes and humoral systems are required to function appropriately. Cardiac output (CO; heart rate x stroke volume) and total peripheral resistance (TPR) are key determinants of arterial BP. A fall in BP alongside a reduction in CO (mediated by a decrease in stroke volume) during orthostatic stress will be compensated by increasing TPR via peripheral vasoconstriction, including constriction of the splanchnic, muscle, skin and renal vascular beds. Splanchnic vasoconstriction is particularly vital for maintaining BP during reductions in BP and subsequent sympathetic activation. The humoral system provides further stimulation for vasoconstriction as well as the balancing of body fluids. Both the RAS and Vasopressin systems play major roles in BP regulation. These mechanisms of BP control are usually somewhat impaired in patients with OH. Further details on the differences in clinical features and pathophysiological mechanisms of cardiovascular autonomic dysfunction according to specific primary CAF disorders will be discussed in the following section.

MSA

Orthostatic hypotension (OH) is one of the key features in the diagnostic criteria of MSA. Apart from the lesion in the ANS, OH can be caused by other factors such as

dehydration, high ambient temperature and the influence of drugs (e.g., L-dopa). Given this reason, the OH cut-off point was changed to an orthostatic decrease of blood pressure within 3 minutes of standing by at least 30 mmHg of systolic or 15 mmHg of diastolic blood pressure in order to improve the diagnosis accuracy in the latest OH guidelines (Gilman *et al.*, 2008). MSA pathology consists of widespread neuronal loss in the basal ganglia (Ozawa *et al.*, 2004), cerebellum (Sakakibara *et al.*, 2004b), brainstem (Benarroch & Schmeichel, 2001), locus coeruleus (Wenning *et al.*, 1994b), nucleus ambiguous (Benarroch *et al.*, 2003), dorsal motor nucleus of vagus nerve (Benarroch *et al.*, 2006b), ventrolateral medulla (Benarroch *et al.*, 1998), hypothalamus (Benarroch *et al.*, 2006a), as well as the intermediolateral (IML) column (Graham & Oppenheimer, 1969) and Onuf's nucleus (Mannen *et al.*, 1982) of the spinal cord. These neuronal losses accompany the glial cytoplasmic inclusions (GCIs) in neurons, particularly in the oligodendroglia.

Although orthostatic symptoms are common among MSA patients (Mathias, 2006) syncope was reported in less than half of patients in comparison with PAF patients (Mathias *et al.*, 1999) and the early development of autonomic dysfunction may also indicate a shorter survival in patients with MSA (Tada *et al.*, 2007). Head-up tilt testing is routinely used for assessing OH (Figure 2.3) in autonomic laboratories. In the clinic this can also be performed by lying down and then sitting or standing. Tilt testing alongside plasma noradrenaline concentrations is often used as investigations in patients with MSA. Noradrenaline levels in MSA patients are typically within the normal range in the supine position and there is a slight increase in the upright position relative to normal controls (Mathias & Bannister, 1999). These findings are consistent with preserved post-ganglionic sympathetic function in MSA.

Food ingestion can lower blood pressure in MSA (Figure 2.4) but not in healthy individuals which have normal compensatory cardiac and regional haemodynamic responses (Smith *et al.*, 1998). A standard liquid meal of mixed composition can be used to determine post-prandial hypotension. BP before and after the liquid meal are measured in the supine and head-up tilt (or standing) positions in order to ascertain if food unmasks or exacerbates orthostatic hypotension (Mathias *et al.*, 1991). Post-prandial hypotension is present in both MSA-P and MSA-C, but the severity in MSA-C is greater (Smith *et al.*, 1998). Exercise-induced hypotension has also been reported in MSA (Smith & Mathias, 1995) as well as an exacerbating of OH after exercise (Smith *et al.*, 1993). Exercise-induced hypotension in MSA-C was found to be greater than those with MSA-P (Smith & Mathias, 1996).

Mechanisms of OH in MSA

The fundamental pathology which contributes to cardiovascular autonomic dysfunction, particularly OH, in MSA is the intermediolateral (IML) neuronal loss in the spinal cord of patients with MSA (Oppenheimer, 1980). Additionally, other neuropathology abnormalities were reported in a number of areas which are responsible for cardiovascular autonomic control. These include neuronal loss in the ventrolateral medulla (VLM), which consists of the rostral C1 neurons and non-C1 cells (Benarroch *et al.*, 1998). The IML receives projections from these neurones and plays a crucial role in maintaining BP and regulating baroreflex function. A loss of neurons in the caudal ventrolateral medulla has also been reported in MSA (Benarroch *et al.*, 2006a). As this area consists of noradrenaline-producing A1 neurons which project to the neurons in supraoptic and paraventricular areas of the hypothalamus, noradrenaline-producing A1 neuronal loss results in impaired vasopressin secretion in response to hypotension. Neuronal loss in the ventrolateral portion of the Nucleus ambiguous was also described in MSA but not in PD (Benarroch, 2002). These neurons are linked to the impairment of vagal control of the heart which can be impaired in MSA. Clonidine stimulation has been proposed to be a useful investigation for distinguishing MSA from PD. While clonidine can stimulate a release of growth hormone in PD, this response is blunted in patient with MSA (Kimber *et al.*, 1997) as a result of central neurotransmitter and alpha 2-adrenoceptor-hypothalamic abnormalities in MSA.

PD

Autonomic dysfunction has been increasingly recognized in Parkinson's disease (PD) over the last decade. A number of autonomic nervous system disturbances, such as constipation, erectile dysfunction and orthostatic hypotension, have frequently been reported as common non-motor symptoms in PD patients (Tolosa *et al.*, 2009). These autonomic disturbances significantly impact quality of life and cause significant morbidity. Among different autonomic abnormalities, cardiovascular autonomic dysfunction, such as orthostatic hypotension (OH), has been considered as one of the most important non-motor symptoms in PD. Dizziness is commonly reported as a frequent symptom of OH compared to other non-motor symptoms in PD. Although OH is commonly present in healthy individuals who are aged over 50 (van Dijk *et al.*, 1993), the prevalence of OH seems to be higher in PD varying between 30 and 58% depending on the type of study, participants and methodology (Allcock *et al.*, 2004; Goldstein, 2006). It could be due to the fact that many PD patients do not have symptoms even if they have OH (Mihci *et al.*, 2006) and as a result it has not been recognized by clinicians (Senard *et al.*, 1997). Moreover, various medications, particularly

dopaminergic therapy which is a mainstay of PD treatment, can also contribute to OH. It has now become clear that OH can be seen in any stage of PD but it is more prevalent in later stages. Since several studies reported OH in early stages of PD without medications, this supported that autonomic failure is a part of the disease progression (Bouhaddi *et al.*, 2004). In addition, recent studies have also found cardiovascular dysautonomia even in untreated patients with early stages of PD (Oka *et al.*, 2006). Given these findings, OH may not be used as a single autonomic feature for distinguishing PD and MSA (Lipp *et al.*, 2009). Constipation, bladder symptoms and abnormal sweating can also occur in PD patients but with less severity compared to MSA (Lipp *et al.*, 2009; Yamamoto *et al.*, 2011).

Mechanisms of OH in PD

Proposed mechanisms of OH in PD include both pre- and post-ganglionic lesions, the former perhaps from autonomic lesions in the upper brainstem that affect blood pressure control during orthostasis through baroreflex dysfunction and the latter via cardiac denervation and/or impaired sympathetic mediated vascular responses (Asahina *et al.*, 2012). The alpha-synuclein related pathology in the intermediolateral column of the spinal cord, sympathetic ganglia and adrenal medulla (Braak *et al.*, 2004; Bloch *et al.*, 2006; Orimo *et al.*, 2007; Dickson *et al.*, 2009; Beach *et al.*, 2010) and post-ganglionic cardiac denervation (Orimo *et al.*, 2008) have been suggested as part of the pathogenesis of OH in PD. The autonomic centres in the brainstem that may also contribute to OH in PD, include the caudal raphe nuclei and the C1 group of the RVLM (Halliday *et al.*, 1990a; Gai *et al.*, 1995; Jellinger, 2011).

PAF

OH and its related symptoms, such as lightheadedness or dizziness, is the most common presenting feature in PAF (Mathias *et al.*, 1999). Although there are only a few case reports on the symptoms of PAF, sudomotor dysfunction was consistently reported in patients with PAF (Hague *et al.*, 1997; Kaufmann *et al.*, 2001). It can lead to the symptoms of heat intolerance or even fainting when patients stay in hot climates. Exercise and food can also worsen orthostatic symptoms in PAF. Bladder symptoms are also present in some PAF patients but these features are less severe than in MSA (Sakakibara *et al.*, 2000). While respiratory symptoms such as stridor or sighing are common in MSA, these symptoms were not reported in patients with PAF (Mabuchi *et al.*, 2005). There are only a few studies that have reported pathology in patients with PAF. Intracytoplasmic eosinophilic inclusions with Lewy bodies resembling those in PD patients were reported in the neurons in autonomic

ganglia and post-ganglionic nerves (Kaufmann *et al.*, 2001). Typical PAF patients have no parkinsonian features. Nevertheless, there was a report of Lewy body deposition in the substantia nigra, locus coeruleus, thoracolumbar and sacral spinal cord with limited neuronal loss (Hague *et al.*, 1997). These findings raise the possibility by some that PAF may be a part of a "Lewy body disease" spectrum representing the peripheral form, alongside with PD and DLB (Kaufmann & Goldstein, 2010).

Mechanisms of OH in PAF

The pathogenesis of OH in PAF is thought to be mainly from post-ganglionic autonomic dysfunction. This hypothesis is supported by alpha-synuclein accumulation in cytoplasmic inclusions with accompanying neuronal loss in the IML column and sympathetic ganglia (Hague *et al.*, 1997). Interestingly, these findings were also evident in the substantia nigra pars compacta, locus ceruleus and thoracolumbar/sacral spinal cord, which resemble findings in PD patients (Kaufmann *et al.*, 2001). A recent study also reported a diminished reuptake of cardiac MIBG in patients with PAF resembling those with PD, which supports a post-ganglionic autonomic pathology (Kashihara *et al.*, 2006). Vasopressin secretion appears intact during episodes of hypotension in patients with PAF (Kaufmann *et al.*, 1992).

While patients with PD and MSA can present with difficulty/abnormal movement or a wide range of non-motor symptoms, patients with PAF usually present with orthostatic hypotension. OH can sometimes make the diagnosis more difficult. For example, OH can be a presenting feature in PD, but also the hallmark of patients with MSA. This overlapping feature makes this more difficult to distinguish between these disorders. On the other hand, some patients with MSA can present with isolated OH without other features for several years before developing MSA features. Thus, it is recommended to follow-up these patients before diagnosing someone with PAF to ensure the correct diagnosis. As a consequence, this could also lead to the difficulty in diagnosis and uncertainty in predicting prognosis for the clinician, as these two disorders have a significant difference in life expectancy.

2.5.1.2 Cardiovascular autonomic screening tests

Cardiovascular autonomic function tests are essential for the diagnosis of cardiovascular autonomic dysfunction and/or autonomic failure and the purposes of these can be divided into 3 reasons; 1) to evaluate whether autonomic function is normal or abnormal. 2) If any abnormalities present, the severity of autonomic dysfunction would be assessed with an

emphasis on the site of lesion and the functional deficits and 3) to establish whether the autonomic dysfunction is a part of a primary or secondary autonomic disorder which would be useful to determine the extent of further investigations, prognosis and treatments (Mathias & Bannister, 2002). Autonomic screening tests have generally been used to evaluate the extent of cardiovascular autonomic dysfunction.

OH/HUT

OH is ideally assessed by head-up tilting in an autonomic laboratory because it can be used in patients who are less able to stand upright due to severe hypotension or neurological deficits. Furthermore, continuous measurement of heart rate (HR) and blood pressure (BP) by non-invasive techniques can be useful for early detection and safe monitoring when hemodynamics become unstable. Nonetheless, OH can also be performed by lying down and then sitting or standing in the clinic when an autonomic laboratory is not available.

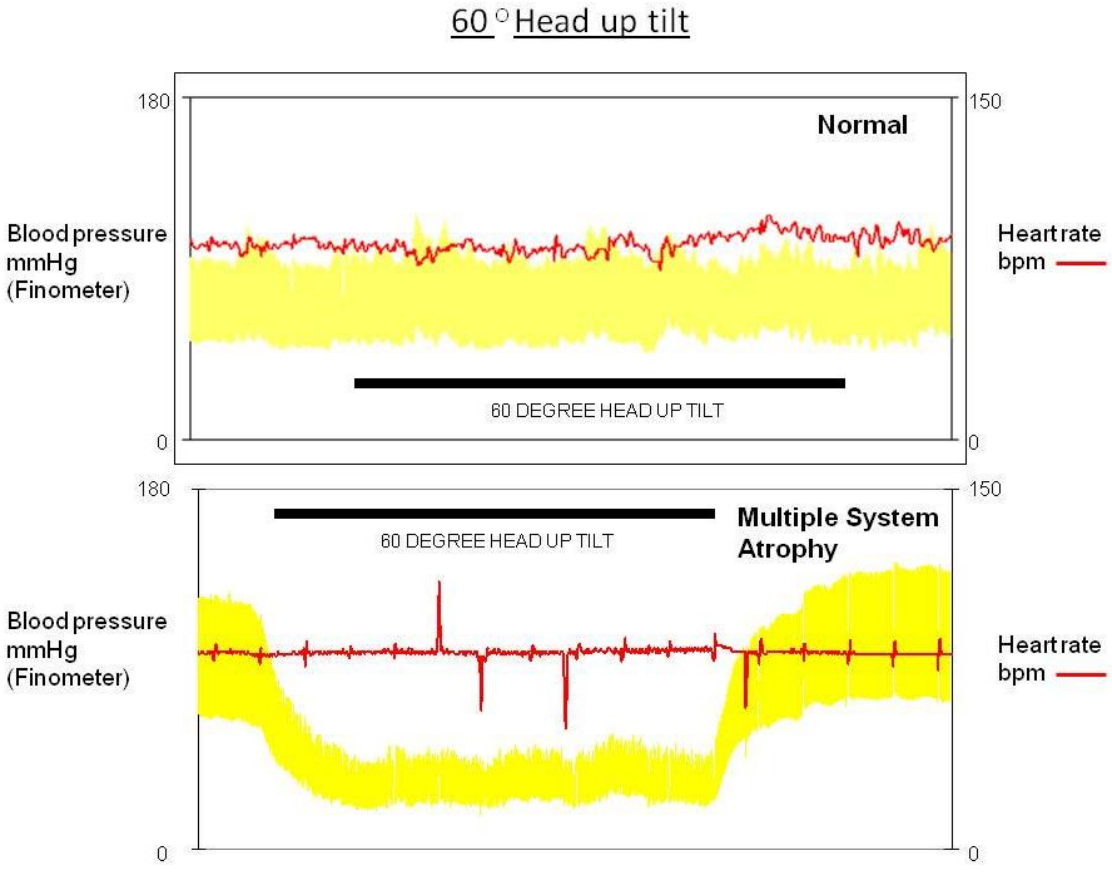


Figure 2.3. Blood pressure and heart rate before and during 60 degrees head up tilt (HUT) in (upper panel) a normal subject and (lower panel) a patient with MSA. In the normal subject, there is no fall in BP during HUT, unlike in a patient with autonomic

failure in whom BP falls promptly and remains low until return to horizontal *(Adapted from Mathias 2006).*

MIBG

Cardiac ^{123}I-metaiodobenzylguanidine (MIBG) imaging has been helpful for making the diagnosis of chronic autonomic failure disorders. A number of previous studies demonstrated that cardiac MIBG uptake is markedly reduced in PAF and Parkinson's disease, even in the early stage of disease (Spiegel *et al.*, 2005), which results from post-ganglionic sympathetic dysfunction. The uptake is typically normal in MSA which can be explained by the pre-ganglionic lesion of MSA. Previous studies using MIBG to identify patients with PD from normal subjects showed that the sensitivity varies from 84.3-89.7% and specificity ranges from 89.5-94.6% when using MIBG to discriminate PD from MSA (Braune, 2001; Sawada *et al.*, 2009). According to these results, cardiac MIBG may provide an additional benefit for separating PD and MSA when using it as a concomitant investigation. However the considerable overlap in the reduction of cardiac reuptake between PD, MSA, DLB and PSP indicated that MIBG cannot entirely discriminate PD from these disorders (Rascol & Schelosky, 2009). Moreover, the MIBG scan is a relatively invasive, costly and time-consuming technique. The correlation between a reduction of cardiac MIBG uptake and several clinical features of PD has been extensively studied. It is unclear whether the severity of PD and autonomic function are correlated with MIBG uptake in previous studies (Orimo *et al.*, 1999; Nagayama *et al.*, 2005; Matsui *et al.*, 2006; Kim *et al.*, 2008) but no correlation between rigidity or postural instability and MIBG uptake were found in advanced PD (Suzuki *et al.*, 2007). Furthermore, MIBG uptake does not correlate with the severity of motor dysfunction and orthostatic hypotension in PD (Matsui *et al.*, 2006).

Pressor tests **(Handgrip Isometric exercise, Mental arithmetic and Cold pressor test)**

Blood pressure (BP) and heart rate (HR) normally increase during sustained isometric exercise, mental arithmetic and cold pressor tests. These first 2 tests are indexes for sympathetic efferent function while the latter mainly measures adrenergic function (Low & Benarroch, 2008). Reduced responses are commonly seen in patients with cardiovascular sympathetic dysfunction.

24 hour-Ambulatory BP and heart rate monitoring (24 hr-ABPM)

24 hour-ambulatory blood pressure monitoring (24 hr-ABPM) has traditionally been used as an investigation for arterial hypertension (Mancia, 1990) but is also routinely used in some autonomic laboratories for the assessment of cardiovascular autonomic dysfunction. Blood pressure (BP) in healthy individuals normally fluctuates with increased BP in the daytime while falling at night-time. This circadian rhythm is frequently found to be abnormal with a loss of nocturnal BP fall in MSA and PD patients compared to controls (Figure 2.4) (Schmidt *et al.*, 2009a). Nevertheless, only a few studies have used 24 hr-ABPM to compare between parkinsonian disorders. In fact, there has been no study specifically investigating the difference between 24 hr-ABPM profiles in patients with MSA and PD with autonomic failure.

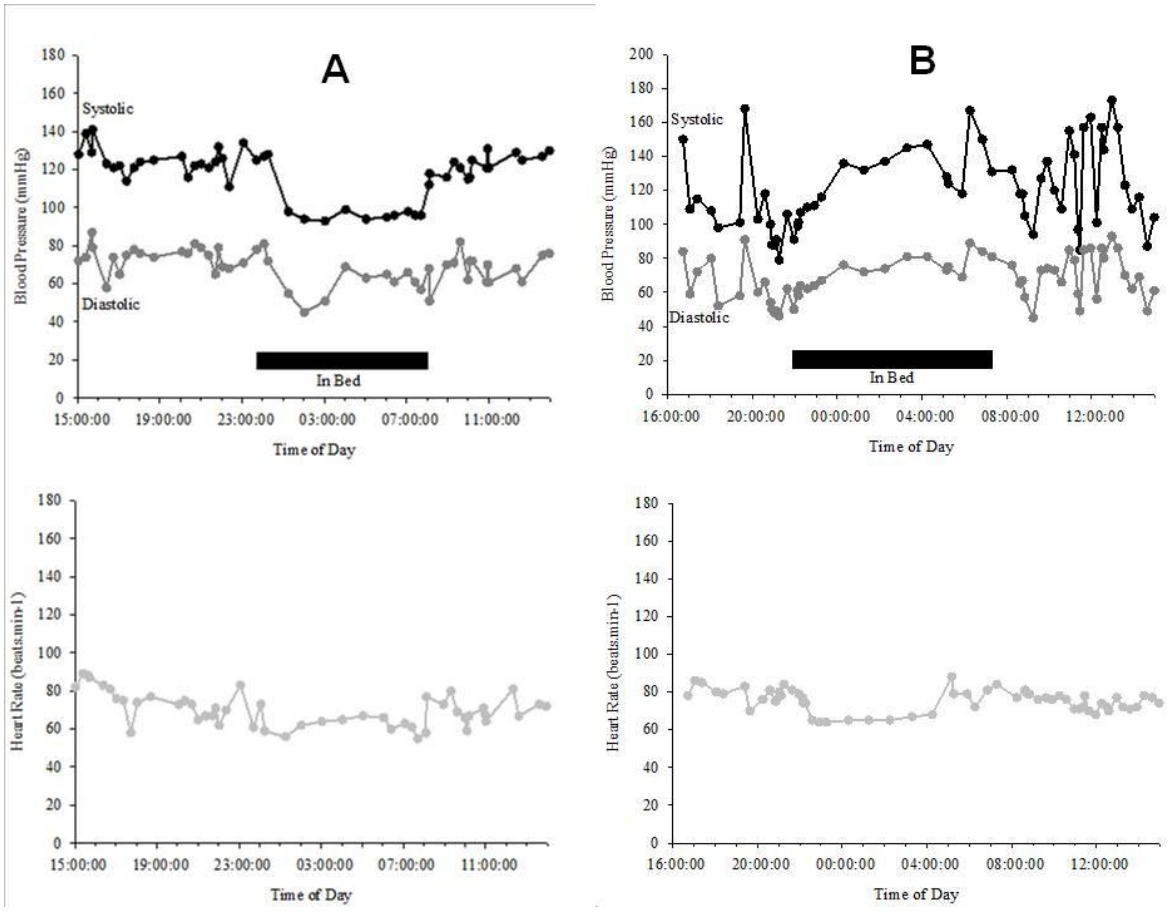


Figure 2.4. Blood Pressure (top panel; upper line systolic, lower line diastolic) and heart rate profiles (bottom panel) during 24 hr-ABPM of patients with a dipper (A) showing more than a 10% drop in BP at night time and (B) a reversed BP profile where BP increases at night time. Black bar indicates sleep. *Adapted from (Stuebner et al., 2013).*

2.5.1.3 Novel quantitative cardiovascular autonomic function tests

Cardiovascular autonomic screening tests have been used to confirm the diagnosis of autonomic failure but the use of OH as a marker of cardiovascular autonomic function may not be able to differentiate autonomic failure disorders (Riley & Chelimsky, 2003). Other indices of quantitative cardiovascular autonomic function, such as baroreflex sensitivity and blood pressure recovery time (BPRT), have recently been developed and additionally been proposed to be useful in evaluating autonomic function in chronic autonomic failure disorders (Vogel *et al.*, 2005; Schrezenmaier *et al.*, 2007). Beat-to-beat blood pressure and heart rate responses to the Valsalva manoeuvre (VM) depends on the integrity of the baroreflex pathways and has been proven to be useful for evaluating sympathetic and parasympathetic function. This has frequently been reported to be abnormal in patients with autonomic failure and have routinely been used as part of autonomic function tests. PRT derived from the VM was recently proved to be a valuable index of sympathetic function (Vogel *et al.*, 2005). Since blood pressure is regulated by changing heart rate and total peripheral resistance, via baroreceptors, the measurement of baroreflex sensitivity (BRS) has been used as a quantitative index of cardiovascular autonomic function and can be assessed from the VM (Low & Benarroch, 2008) (Figure 2.5). These indices are frequently abnormal in patients with autonomic failure (Schrezenmaier *et al.*, 2007).

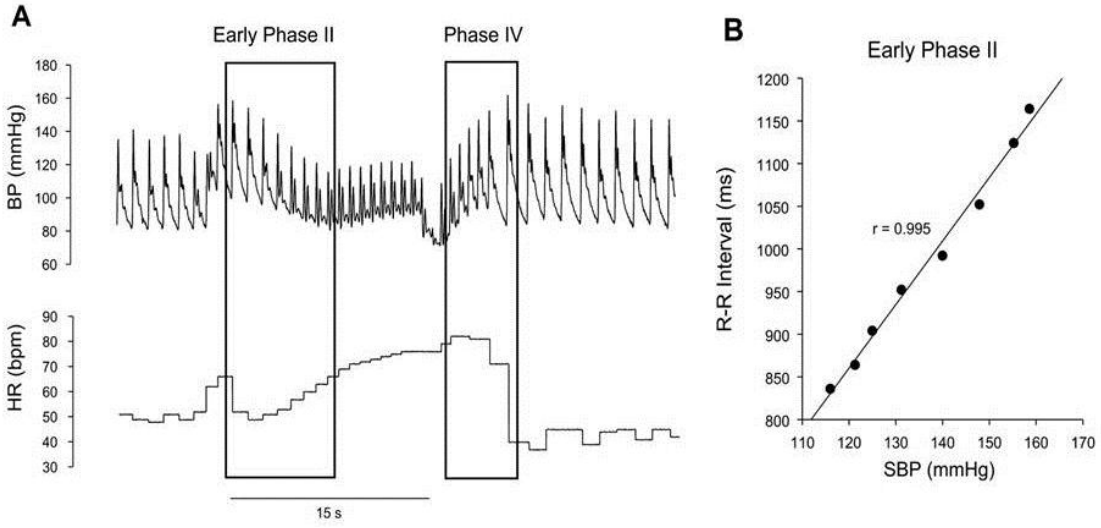


Figure 2.5. Baroreflex sensitivity (B) derived from the Valsalva manoeuvre (A). Blood pressure (BP; upper A) and heart rate (HR; lower A) during VM in a normal subject. Baroreflex sensitivity is obtained from the slope of the regression between R-R interval and systolic blood pressure (SBP) during phase II early of VM (B). *Adapted from (Fu et al., 2007).*

The Valsalva ratio (VR), derived from the VM by the ratio between the maximum and minimum heart rate during the manoeuvre are also helpful for evaluating parasympathetic function (Low *et al.*, 1997) (Figure 2.6). The heart rate responses to deep breathing (sinus arrhythmia), which mainly assess cardiac parasympathetic function, are frequently abnormal in patients with autonomic failure (Gurevich *et al.*, 2004; Deguchi *et al.*, 2006). the efficacy of these tests for distinguishing between primary chronic autonomic failure disorders are still uncertain however (Riley & Chelimsky, 2003).

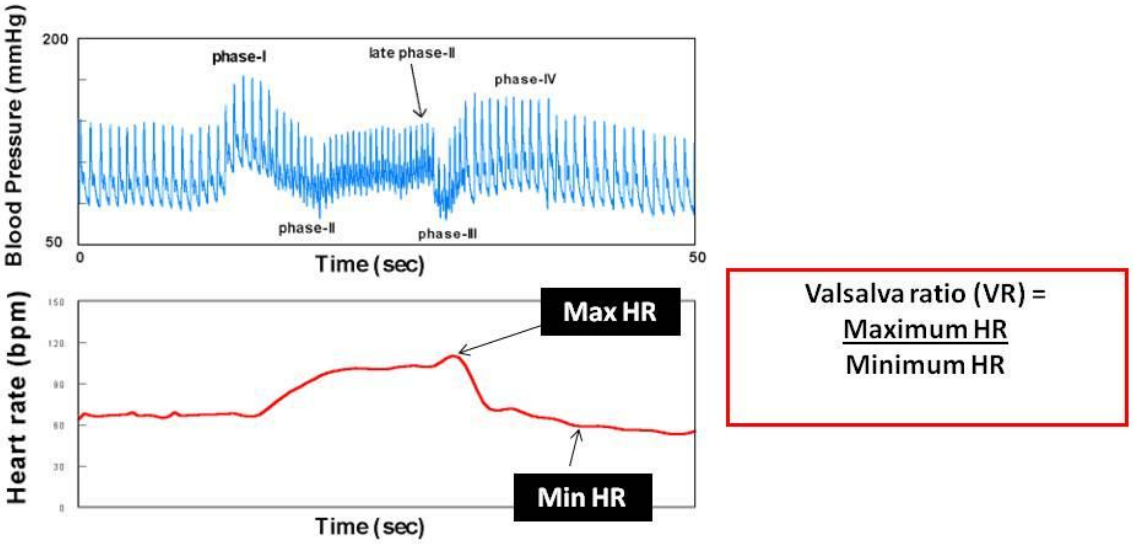


Figure 2.6. Blood pressure (BP, upper panel) and heart rate (HR, lower panel) during a Valsalva Manoeuvre (VM) in a normal subject. The Valsalva Ratio is calculated by the ratio between maximum HR and minimum HR during and just after the VM.

2.5.2 Other non-motor features in patients with Primary CAF

2.5.2.1 The olfactory system and pathology in Primary CAF

The olfactory system consists of the olfactory epithelium, the olfactory bulb, the olfactory cortex and the orbitofrontal cortex. The olfactory signal starts from the activation of the bipolar olfactory sensory receptors, G-protein-coupled receptors which are located in the olfactory epithelium, by odorants. Adenylyl cyclise III subsequently synthesizes cyclic adenosine monophosphate (cAMP) (Kaupp, 2010), which causes depolarization by increasing intracellular calcium concentrations. The axons of olfactory sensory receptors enter through the cribriform plate and synapse with the dendrite of Mitral and Tufted cells within the glomerulus in the olfactory bulb. These cells project its axons to the anterior olfactory nucleus and different parts of the primary olfactory cortex, including, the olfactory

tubercle, piriform cortex and entorhinal cortex (Mouret *et al.*, 2009). The primary olfactory cortex also connects to the amygdala, hippocampus and hypothalamus (Figure 2.7).

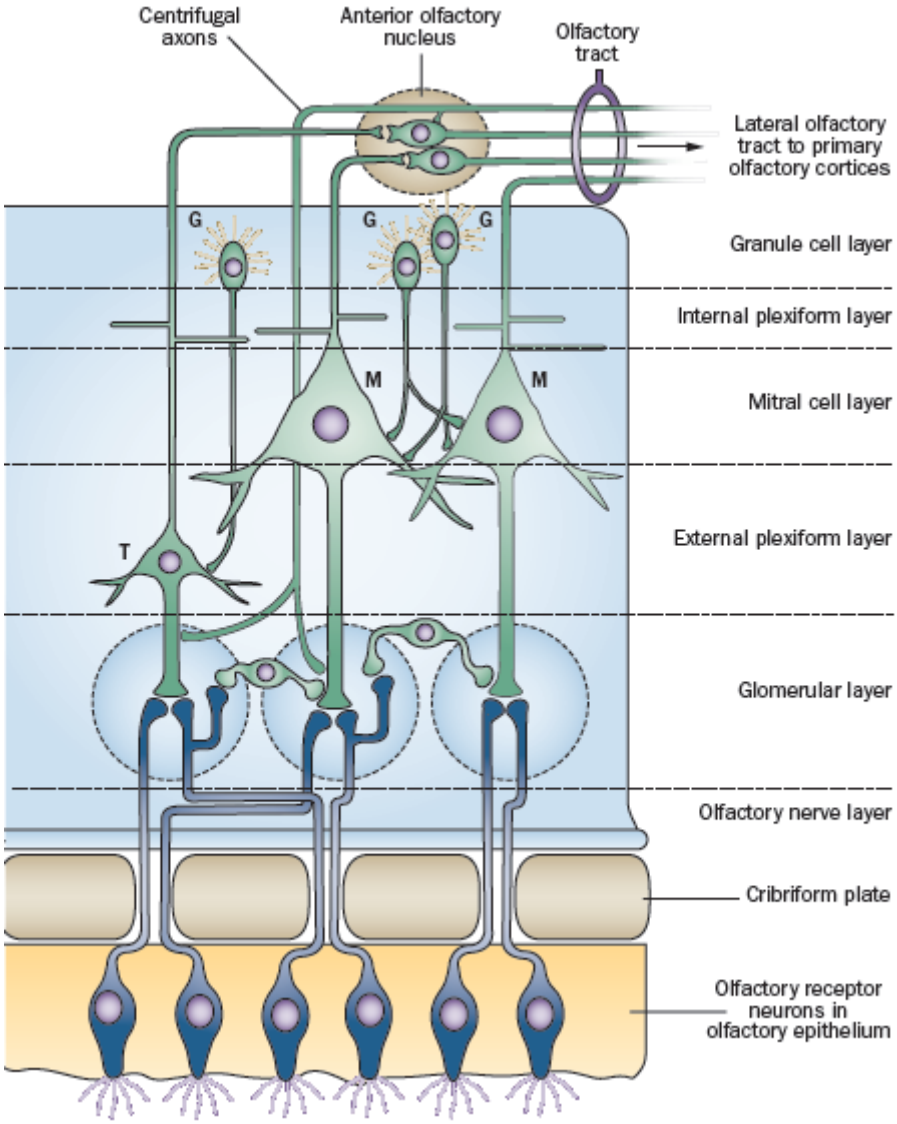


Figure 2.7. Major olfactory structures, including the Olfactory epithelium (orange), Olfactory bulb (blue) and the Anterior olfactory nucleus (brown circle). The Olfactory bulb consists of 5 layers (from lower to upper): Glomerular layer, External plexiform layer, Mitral cell layer, Internal plexiform layer and the Ganule cell layer. Abbreviation: G, granule cell; M, Mitral cell; T, tufted cell. *Adapted from (Duda, 2010).*

Despite frequent olfactory dysfunction in various neurodegenerative conditions, the olfactory pathology can vary. In PD, previous studies have consistently demonstrated abnormal olfactory pathology, including alpha-synuclein-related pathology, similar to that in the substantia nigra (Beach *et al.*, 2009). Lewy bodies and Lewy neurites have been reported in the olfactory bulbs, anterior olfactory nucleus and several areas in the primary olfactory cortex and occasionally in the neocortex (orbitofrontal/dorsolateral cortices and

insular cortex) (Harding *et al.*, 2002; Braak *et al.*, 2003; Silveira-Moriyama *et al.*, 2009a). This alpha-synuclein-related pathology is regularly present in the olfactory bulb, particularly in the mitral and tufted cells and the granule and periglomerular cells along with olfactory neuronal loss (Pearce *et al.*, 1995). The degree of neuronal loss in the anterior olfactory nucleus was negatively correlated with PD disease duration (Pearce *et al.*, 1995). It is important to note that the severity of alpha-synuclein-related pathology seemed to correlate with the number of projections from the olfactory bulb (Sengoku *et al.*, 2008). The distribution of alpha-synuclein-related pathology also progresses from the anterior olfactory nucleus centrally towards the olfactory primary cortex without the involvement of non-olfactory cortical structures (Braak *et al.*, 2003). Interestingly, tau-pathalogy in the form of neurofibrillary tangles is also commonly present alongside alpha-synuclein pathology in the olfactory system in PD patients. This abnormal pathology was found in the olfactory bulb and the anterior olfactory nucleus with the associated olfactory neuron loss and is consistently reported not only in PD but also in those with Alzheimer's disease (AD) and dementia with Lewy bodies (LBD) (Tsuboi *et al.*, 2003; Mundinano *et al.*, 2011).

In contrast to PD, there are only few studies that have reported olfactory pathology in MSA. Glial cytoplasmic inclusions in oligodendrocytes were found in the olfactory bulb with olfactory neuronal loss in the anterior olfactory nucleus (Daniel & Hawkes, 1992; Kovacs *et al.*, 2003). There has been no report of olfactory pathology in patients with PAF.

Impaired olfaction is a common symptom among the general population. Complaints of abnormal taste can also sometimes be caused by smell loss because of the ability to appreciate the flavours of food depends upon olfaction (Deems *et al.*, 1991). Upper respiratory tract infections, head injury and nasal/paranasal sinus were reported as frequent causes of impaired olfaction and account for almost two-thirds of patients with chronic anosmia/hyposmia (Deems *et al.*, 1991). Age is the most important confounder for olfactory dysfunction. More than 60% aged over 80 have impaired olfaction (Murphy *et al.*, 2002). This figure was even higher (more than three-quarters) in a study from a specialist clinic (Doty *et al.*, 1984a). Gender is another factor which affects olfaction. Previous studies consistently showed that women usually performed smell tests better compared to men in the same age groups but there is no difference in the rate of deterioration with age (Doty *et al.*, 1988; Hawkes *et al.*, 1997). Olfactory tests are necessary because previous studies demonstrated that self-reporting of abnormal olfaction is often unreliable (Hawkes *et al.*, 1997).

2.5.2.1.1 Assessment of olfactory function

Smell function has been tested using various methods mainly divided into three main categories, including smell detection thresholds, smell discrimination and smell identification. Smell detection thresholds is the ability to recognize the perception of smell at the lowest concentration of an olfactory stimulus while smell discrimination is the ability to distinguish between different olfactory stimuli which are not dependent on the recognition of the smell. Smell identification is the most widely used method because it is more feasible and the easiest way to assess subjects both in clinical settings and research studies. Olfactory dysfunction is usually graded from normal to severe as normosmia, microsmia (which is often subdivided into mild, moderate and severe microsmia) and anosmia. There have been several validated tools which can be used for smell testing. The University of Pennsylvania Smell Identification Test (UPSIT) is a 40-item scratch and sniff test which has been developed since 1984 in the US (Doty *et al.*, 1984b). Each odour is contained in a microencapsulated paper strip and is released by scratching with a pencil. Patients select an answer which is the most likely from four choices. UPSIT has been most frequently used compared with other smell tests and normal values in different age distributions and genders are available. Given that culture greatly influences the result of smell tests, a 12-item Cross-Cultural Smell Identification Test (CC-SIT), which is also known as the Brief Smell Identification Test (B-SIT), was later created from selected items from the UPSIT (Doty *et al.*, 1996). The B-SIT has also been validated with the UPSIT and has been adapted for use in other countries (Double *et al.*, 2003). Sniffin' Sticks is another smell test which was originally developed in Germany (Hummel *et al.*, 1997).

Sniffin' Sticks were originally used for testing smell thresholds, smell discrimination and smell identification. The combination of the scores of the different methods in Sniffin' Sticks are summarized as a TDI (Threshold, Discrimination, Identification) index which was validated and normative data are also available (Kobal *et al.*, 2000). Since using TDI scores can be time-consuming, performing only part of this test, particularly smell identification testing, can provide useful information regarding a patient's sense of smell. The smell identification part of the Sniffin' Sticks test is performed by using scented felt tipped pens which have 16 different odours. Patients are asked to sniff the tip of pens at an interval of 30 seconds each. Participants need to identify one of 4 choices by selecting the one which best describes the presented odour. Previous studies demonstrated that the 16-item smell identification test has been useful as a screening tool for detecting abnormal olfaction and

has been validated previously in several countries and is a good non-invasive instrument for distinguishing PD from control subjects (Muller *et al.*, 2002).

2.5.2.1.2 Olfactory function in Primary CAF

Abnormal olfaction in PD has long been known since the first report in the 1970s (Ansari & Johnson, 1975). Nevertheless, it has become increasingly recognized among clinicians over the last decade. An increased recognition of this condition reflects the expansion of research relating to non-motor symptoms of PD in order to find a reliable biomarker for PD. Olfactory dysfunction in PD is prevalent ranging from 45-90% (Ansari & Johnson, 1975; Ward *et al.*, 1983; Doty *et al.*, 1988; Hawkes *et al.*, 1997). The variation in studies might be explained by several factors, including, the dissimilarity of smell tests and methods used, age of participants, sample size, normative data and particularly cultural differences. It has also been suggested that olfactory dysfunction can predate motor symptoms of PD. This evidence came from the Honolulu Heart Program population-based prospective study (Ross *et al.*, 2008). Olfactory function was assessed by B-SIT. 35 out of 2,267 male participants aged between 71-95 years developed PD at the end of an 8 year-follow-up period. The results showed that there was approximately a 5 times higher risk of developing PD within 4 years in participants who were in the lowest quartile of B-SIT scores compared to those in the highest quartile. This evidence was present even after adjustment for age and other potential confounders.

Combining olfactory tests and functional imaging techniques were used for predicting PD by Ponsen et al., (2004). 361 asymptomatic relatives of PD patients were evaluated by smell tests (odor detection, discrimination and identification). 38 normosmic and 40 hyposmic relatives were assessed by single-photon emission computed tomography (SPECT), using [^{123}I] β-CIT for evaluating nigrostriatal dopaminergic function at baseline and after a 2 year follow-up. The results showed that 10% of hyposmic relatives subsequently developed clinical features of PD whilst none in the normosmic relatives had parkinsonian features (Ponsen *et al.*, 2004). Previous studies have also showed that impaired olfaction was not associated with motor disability, tremor or cognitive function in PD however (Doty *et al.*, 1988; Doty *et al.*, 1989). Subsequent studies found that olfactory dysfunction was not correlated with dopaminergic medication (Doty *et al.*, 1992) and there was no change during “on” and “off” states in PD patients with motor fluctuations (Quinn & Spraguer, 1986). It is still debatable whether there is a progression of impaired olfaction along with PD disease

duration and severity. Most studies suggest that there is no significant deterioration of smell deficit in PD patients with increasing disease duration (Doty *et al.*, 1988; Double *et al.*, 2003; Kim *et al.*, 2007) while only a few studies showed that olfactory dysfunction was increased with the severity of disease (Tissingh *et al.*, 2001). Interestingly, there was no correlation between striatal dopaminergic terminal loss in functional brain imaging and olfactory dysfunction (Siderowf *et al.*, 2005; Goldstein *et al.*, 2008).

Olfactory dysfunction has also been reported in other neurodegenerative disorders but the severity is more prominent in PD, Alzheimer disease (AD) and Dementia with Lewy bodies compared to those with multiple system atrophy (MSA) and pure autonomic failure (PAF). It is important to note that Progressive supranuclear palsy (PSP), Cortico-basal degeneration and Vascular Parkinson's disease are more likely to have relatively normal or only minimal impairment smell function (Hawkes, 2003), therefore smell tests are particularly useful for distinguishing PD from certain parkinsonian disorders. The sensitivity of smell tests for PD diagnosis were reasonably high; 80-90% among different methods and studies (Hawkes *et al.*, 1999; Becker *et al.*, 2002; Berg, 2006). Olfactory dysfunction has also recently been linked with other disorders such as REM sleep behaviour disorder (RBD) which is considered to a pre-motor symptom of PD (Postuma *et al.*, 2006). To date, there have been only a few studies that have formally assessed sense of smell in a wide range of autonomic disorder patient groups such as MSA, PAF and PD (Silveira-Moriyama *et al.*, 2009b; Garland *et al.*, 2011). Although an impaired olfaction in PD was consistently found in many studies, there are conflicting results in patients with MSA and PAF. While one study reported intact olfactory function in MSA (Garland *et al.*, 2011), the other demonstrated mildly impaired olfaction in PAF (Silveira-Moriyama *et al.*, 2009b). These contradictory results raise a possibility that there may be other factors contributing to olfactory dysfunction in these disorders.

2.5.2.2 Gastrointestinal dysfunction

2.5.2.2.1 Neural control of the gastrointestinal function

Appropriate autonomic nervous system function is critical for effective gastrointestinal motility and secretion. The gastrointestinal tract is controlled by both intrinsic and extrinsic pathways. The intrinsic pathway is the enteric nervous system (ENS), consisting of the myenteric and submucosal plexus. The myenteric plexus is located in between the longitudinal and the circular smooth muscle layers, whereas the submucosal plexus is within

the submucosa. The extrinsic pathway consists of the pre-ganglionic sympathetic and parasympathetic output. The former sends output from the prevertebral ganglia (Szurszewski, 1981; Furness, 2006), whereas the latter mainly projects from two nuclei of the medulla, the dorsal motor nucleus of vagus nerve (DMV) and the nucleus ambiguous (Hopkins *et al.*, 1996; Chang *et al.*, 2003) and the sacral parasympathetic nucleus (Brading & Ramalingam, 2006).

The pre-ganglionic sympathetic fibers arise from the thoracic level of spinal cord and synapse at the cell bodies of post-ganglionic neurons in three prevertebral ganglia, including celiac, superior and inferior mesenteric and hypogastric plexuses. Some of these sympathetic fibers are involved in vasoconstrictive function whereas others influence secretory function. Moreover, a number of sympathetic fibers innervate and terminate within the myenteric and submucosal plexuses. These projections inhibit gastrointestinal motility by controlling circular smooth muscle contraction.

The extrinsic pathway projecting from neurons in the DMV predominantly control the smooth muscle contraction and relaxation of the oesophagus and stomach, which are important for swallowing and gastric emptying (Travagli *et al.*, 2006). Striated muscle in the upper esophagus and pharynx receive projections from the nucleus ambiguous and work together with neurons in the NTS for the sequential motor pattern of swallowing and its coordination. While primary peristalsis is under the control of the DMV (extrinsic), which is influenced by the NTS, secondary peristalsis (intrinsic) is thought to involve local reflexes, which are interceded by the myenteric neurons.

In the lower esophageal sphincter, there are both vagal excitatory and inhibitory pathways. These not only control tonic contractions during the resting state but also selectively activate esophageal sphincter relaxation. The NTS also has a major role in controlling gastric motility (Travagli *et al.*, 2006). The vagal inhibitory pathway is responsible for stomach relaxation, which mainly occurs in the fundus (proximal). This is contradictory to the excitatory pathway, where it activates contraction of the corpus and the antrum (distal). The latter plays an important role in the gastric emptying process. While all extrinsic efferents utilize acetylcholine (Ach) as a primary neurotransmitter, the inhibitory pathway mainly uses nitric oxide (NO), vasoactive intestinal polypeptide (VIP) or adenosine triphosphate (ATP) (Chang *et al.*, 2003).

The extrinsic pathway also supplies the anorectal organs and pelvic floor. These structures receive output from the sacral parasympathetic nucleus in the spinal cord and

sympathetic and somatic nerves. The Onuf nucleus, located in the sacral spinal cord, sends somatic projections to the external anal sphincter and puborectalis muscle, whereas the internal anal sphincter receives tonic excitatory sympathetic input. These structures are vital for faecal continence (Brading & Ramalingam, 2006; Bharucha & Fletcher, 2007). Both the excitatory and inhibitory components of the parasympathetic output are key parts in controlling defecation. The excitatory pathway is for the colonic propulsive activity, whereas the inhibitory pathway elicits colonic relaxation. The relaxation of the puborectalis muscle, the internal anal sphincter and the external anal sphincter are required for defecation. Furthermore, the Onuf nucleus also receives direct inputs from several brainstem nuclei and projects to the external anal sphincter and pelvic floor for contributing to the control of defecation.

The role of the extrinsic pathway is significantly less prominent in the small intestine and colon. Instead, gastrointestinal motility and secretion within these organs are mainly controlled by the intrinsic pathway within the ENS (Costa *et al.*, 2000; Furness, 2000). While smooth muscle contraction of the intestines largely depends on the myenteric plexus, the submucosal plexus is primarily responsible for controlling mucosal blood flow and secretion. There are different types of neurons in the ENS, including intrinsic primary afferent neurons (IPANs), motor neurons, interneurons, the interstitial cells of Cajal, secretomotor, vasomotor neurons and intestinofugal neurons. The IPANs, located in submucosal and myenteric plexus, receive signals from local reflexes (e.g. changes in chemical substances in the intestinal lumen and/or mechanical stretch of the intestinal wall) and further synapse with motor neurons and interneurons. The interneurons are closely involved with excitatory and inhibitory motor neurons, which are responsible for normal peristaltic reflex. The majority of excitatory neurons in the ENS are mainly cholinergic neurons, whereas the inhibitory interneurons and motor neurons mainly express VIP, NO, substance P, neuropeptide Y or ATP, in various combinations. The interstitial cells of Cajal, the specialized pacemaker cells utilizing NO, generate gastric slow waves by itself which are responsible for relaying the signals between excitatory and inhibitory function of myenteric motor neurons to the smooth muscle cells (Huizinga *et al.*, 2004; Ward & Sanders, 2006) and control smooth muscle membrane potentials (Hirst & Edwards, 2004). Intestinal blood flow and secretion are under control of the secretomotor and vasomotor neurons, which lie in the submucosal plexus. These neurons innervate both the gut epithelium and blood vessels and control the balance between blood flow and epithelial absorption within intestines. The intestinofugal neurons send signals from the gut to the prevertebral ganglia. These neurons are responsive to mechanical stimuli and are thought to be directly activated by the stretch of circular muscles,

which also contribute to the inhibitory reflex during active intestinal contractions (Miller & Szurszewski, 2002, 2003) (Figure 2.8).

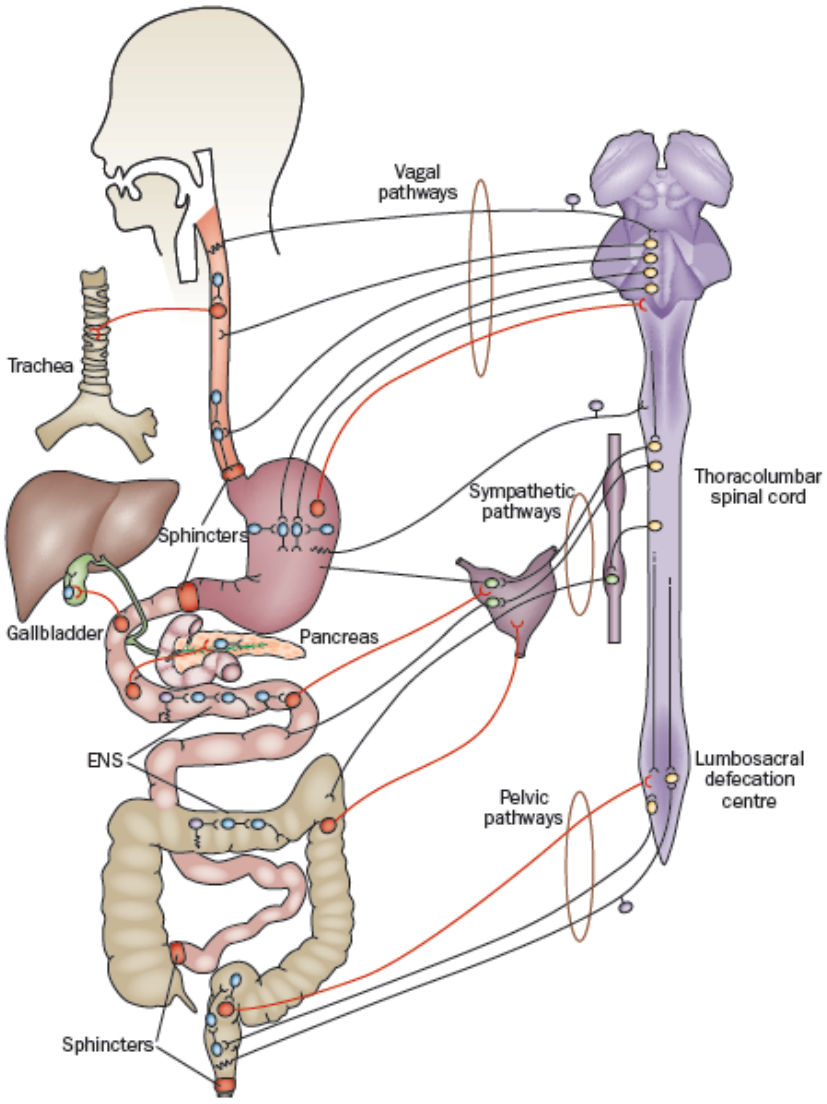


Figure 2.8. The innervation of the gastrointestinal tract. The outputs from CNS (Vagal and Pelvic pathways) mainly control esophageal, stomach and anorectal function. The intrinsic pathway within the ENS is primarily responsible for gastrointestinal motility and blood flow and secretion in the small intestine and colon. Motor neurons and interneurons (blue circles) and sensory neurons (purple circles) control local reflexes. Intestinofugal neurons (red circles) project from the gastrointestinal tract to the CNS (yellow circles in spinal cord). Sympathetic prevertebral ganglia (green circles) work closely with the ENS by receiving inputs from the CNS and ENS. Sensory information projects to both the ENS via intrinsic primary afferent neurons (purple lines) and the CNS via extrinsic primary afferent neurons (also purple lines). Abbreviations: CNS, central nervous system; ENS, enteric nervous system. *Adapted from (Furness, 2012).*

2.5.2.2.2 Gastrointestinal function in Primary CAF

Gastrointestinal (GI) symptoms can sometimes be prominent in PD, MSA and PAF patients. Some GI symptoms, in particular constipation, may predate the onset of typical motor symptoms in patients with PD. These features are in line with the pathological studies reporting the involvement of the enteric nervous system in PD (Wakabayashi & Takahashi, 1997a; Braak *et al.*, 2006a; Braak *et al.*, 2006b), as well as the dorsal motor nucleus of the vagus (Halliday *et al.*, 1990b; Gai *et al.*, 1995), which are vital for maintaining appropriate GI function.

A variety of GI features have been reported in PD, ranging from sialorrhea, dysphagia, delayed gastric emptying, small intestine motility dysfunction, colonic dysmotility and anorectal dysfunction. Previous studies showed that there was no increase of saliva in PD. In fact, saliva production is actually decreased and sialorrhea could be explained by the reduction of and inefficient swallowing in PD (Bagheri *et al.*, 1999; Proulx *et al.*, 2005). Oropharyngeal dysphagia is not uncommon among PD patients but unlike MSA it is usually asymptomatic and present in advanced stages. These findings came from both survey and objective studies. The results from large survey studies showed that the proportion of PD patients who reported difficulty of swallowing was between 30-82% (Kurihara *et al.*, 1993; Leopold & Kagel, 1996; Clarke *et al.*, 1998). Previous studies confirmed that at least more than 63% of PD patients show some abnormality in the modified barium swallow test (Fuh *et al.*, 1997; Leopold & Kagel, 1997). Dysphagia was found in both oral and pharyngeal phases in PD patients even in asymptomatic patients (Robbins *et al.*, 1986; Bushmann *et al.*, 1989; Stroudley & Walsh, 1991; Nagaya *et al.*, 1998). Esophageal dysphagia is also prevalent in patients with PD. This was demonstrated by abnormalities in studies using videofluoroscopy (Eadie & Tyrer, 1965; Gibberd *et al.*, 1974), which were consistent with manometric esophageal studies (Bassotti *et al.*, 1998; Castell *et al.*, 2001). Dysphagia variably responded to dopaminergic treatment in PD patients, which indicates that dopaminergic cell loss might play a part in this symptom but is not entirely responsible for this problem (Hunter *et al.*, 1997).

Delayed gastric emptying has often been reported in PD patients. These patients may present with nausea, early satiety and abdominal distension. Impaired gastric motility was found to be longer even in untreated PD cases compared to controls (Hardoff *et al.*, 2001). A recent study reported different patterns of electrogastrography (EGG) between PD, multiple system atrophy and controls. This study found that PD patients had irregular gastric slow waves which suggested abnormalities of gastric pacemaker cells (Interstitial cells of

Cajal), whereas regular slow waves with low variability were found in MSA which may be explained by parasympathetic dysfunction. Small intestine dysmotility can be present in PD. This was confirmed by abnormal small intestine motor patterns in manometry (Bozeman *et al.*, 1990). In colonic dysmotility, constipation is the most frequent lower GI symptom (Sakakibara *et al.*, 2008) and has widely been reported ranging from 20-79% in PD (Siddiqui *et al.*, 2002; Jost & Eckardt, 2003). Recent studies proposed that it resulted from delayed transport of the bowel with or without abnormal anorectal evacuation (Sakakibara *et al.*, 2008). A epidemiological study has also shown that there is approximately a 4 fold higher risk of PD in male participants who have bowel movements less than once a day compared to those with two or more times a day (Abbott *et al.*, 2001). Therefore, constipation was suggested to be one of the potential markers for diagnosing PD before patients develop motor signs. With regards to this, colon transit time was also found to be longer in PD patients compared with age-matched controls (Sakakibara *et al.*, 2003). Delayed colonic transit time was thought to be due to the degeneration of parasympathetic nuclei. It was unclear whether colon transit time correlated with the severity of disease but the severe constipation was found to be associated with the disease duration and severity of PD (Krogh *et al.*, 2008). A number of colon abnormalities, including megacolon, intestinal pseudo obstruction and colonic obstruction have often been reported in PD (Kupsky *et al.*, 1987; Shimada *et al.*, 2006). Difficulty and incomplete evacuation are also common in PD(Edwards *et al.*, 1991). Incoordination of external and internal anal sphincter contractions was considered to be the contributing factor of this symptom (Stocchi *et al.*, 2000). Anorectal manometric abnormalities, including lower basal sphincter pressure, abnormal phasic contractions during voluntary contractions were observed in previous studies (Ashraf *et al.*, 1995; Normand *et al.*, 1996; Stocchi *et al.*, 2000). Pelvic floor dyssynergia and the lack of puborectalis relaxation might contribute to constipation (Mathers *et al.*, 1988; Mathers *et al.*, 1989).

A wide range of gastrointestinal (GI) symptoms are frequently reported in patients with MSA. These include, swallowing dysfunction, gastric dysfunction and anorectal dysfunction. Dysphagia is a common feature in MSA patients. It was a significant feature for differentiating atypical parkinsonian syndromes from PD if symptoms developed within 1 year after the disease onset. The latency of dysphagia and dysarthria were correlated with shorter survival rate (Muller *et al.*, 2001). Choking and coughing, particularly with liquid intake, were commonly reported in MSA (Mathias, 1996). Videofluoroscopy and manometry are valuable tools for investigating dysphagia in MSA. Swallowing dysfunction in MSA is frequently due to abnormalities in the oropharyngeal phase with a delay in bolus transport

from the oral cavity to the pharynx which is consistent with PD (Higo *et al.*, 2003). Swallowing pressures were found to be lower compared to controls. Interestingly, a history of aspiration pneumonia was increased with the severity of disease (Higo *et al.*, 2003) but not the duration of disease (Higo *et al.*, 2005) and up to one-third of patients with MSA had confirmed aspiration by Videofluoroscopy even in patients who never reported swallowing problems (Smith, 1992). Gastrointestinal symptoms, such as abdominal pain, bloating and nausea/vomiting, are often reported in both PD and MSA which may sometimes be due to delayed gastric emptying. Comparing PD, MSA and healthy age-matched controls, there was a significant decreased gastric emptying time in MSA and PD compared to controls but there was no difference between these two disorders (Thomaides *et al.*, 2005). Constipation is a well-known feature in MSA and may occur even during the early stages of disease (Mathias, 1996). Approximately one third of MSA patients reported the symptom of constipation in the EMSA-SG (The European MSA Study Group) registry (Stefanova *et al.*, 2009). This may be explained by the prolonged colonic transit time in line with the reduction of phasic rectal contraction and abdominal strain in patients with MSA (Sakakibara *et al.*, 2004a). The involvement of Onuf's nucleus results in abnormalities of the anal sphincter EMG, which are in parallel with those obtained by urethral sphincter EMG (Yamamoto *et al.*, 2005).

There are a number of previous studies that have reported gastrointestinal abnormalities in patients with PD. These investigations are more likely to be relatively invasive and require special gastrointestinal motility laboratory tests. Electrogastrography (EGG) is an ambulatory technique that has been used for recording gastric myoelectrical activity (GMA) using 5-lead cutaneous electrodes placed on the abdomen. GMA was thought to originate from the Interstitial cells of Cajal (ICC), located on the major curvature of the stomach. ICC are considered as gastric pacemaker cells because they generate rhythmic depolarization with the same frequency as slow waves of gastric myoelectrical activity (Camborova *et al.*, 2003). The frequency of gastric myoelectrical activity slow waves is approximately 3 cycles per minute in healthy subjects (Chang, 2005) and can be easily detected by cutaneous EGG. A number of studies have used EGG in various gastric disorders, for instance diabetic gastropathy (Koch, 2001), functional dyspepsia (van der Voort *et al.*, 2003) and recently in Parkinson's disease and Multiple system atrophy (Sakakibara *et al.*, 2009). Gastric electrical dysrhythmia was detected in patients with PD (Lu *et al.*, 2004) but not in MSA (Suzuki *et al.*, 2005). Although no pathological study of the involvement of ICC has been previously reported, there was evidence of alpha-synuclein deposition in Auerbach's and Meissner's plexuses, which are closely linked to the ICC, in

patients with PD (Braak *et al.*, 2006b). These abnormal gastric slow waves might reflect gastrointestinal involvement which is considered as a potential early marker of the pre-motor stage of PD and consistent with the neuropathological studies by Braak *et al* (Braak *et al.*, 2003). Gastrointestinal symptoms, such as abdominal bloating, nausea/vomiting, are also common in patients with PAF. It is clear that PAF and PD share a number of characteristics, such as autonomic dysfunction, hyposmia, reduced cardiac MIBG reuptake and a presence of Lewy bodies, both in skin and the central nervous system (Hirayama *et al.*, 1995; Ikemura *et al.*, 2008; Silveira-Moriyama *et al.*, 2009b; Shishido *et al.*, 2010). However, EGG has not been used to investigate GI function in patients with PAF, thus it is unknown if PAF patients have gastric electrical dysrhythmia similar to PD patients. Similarly, MSA represents a pre-ganglionic autonomic disorder whilst PAF has mostly post-ganglionic autonomic pathology (Hague *et al.*, 1997). Comparing between these disorders using EGG would thus be an ideal model to evaluate autonomic disorders with different pathological lesions that present with similar clinical characteristics.

2.5.3 Quality of life in patients with Primary CAF

Quality of life (QOL) is recognised as one of the most important health outcomes in clinical research. As the purpose of QOL assessment is to measure a patient's perceptions of the effects of their illness on their life, the instruments (e.g. QOL questionnaires) are usually scored by patients, rather than clinicians, in order to more closely reflect the actual impact of the disease towards a patient's daily life. This may or may not correlate with objective measures of the patient's health status. There are different types of QOL questionnaires but they can broadly be divided into 2 types: generic and disease-specific QOL questionnaires. Examples of generic QOL questionnaires are the 36-item Short Form Health Survey (SF-36) and the EuroQoL (EQ-5D). The benefit of generic questionnaires is an ability to measure QOL and compare various medical conditions at the same time. On the other hand, this type of questionnaire has the limitation of an absence of disease-specific questions; this could potentially lead to an inability to detect an impact from general features of a generic questionnaire and/or the lack of specific features which are important for specific conditions. This can be overcome by using disease-specific QOL questionnaires, which contain questions relevant to the disease of the patient. For example, the disease specific QOL questionnaire for PD, contains items focussing on the impact of parkinsonian features on daily life while the QOL questionnaire for Rheumatoid Arthritis will more likely cover the impact of pain on the patient's function. The limitation of disease-specific QOL

questionnaires is that data cannot be readily compared between different, albeit overlapping, disorders, e.g., PD vs. PAF. This section will cover only brief details on selected QOL questionnaires, which are included in this thesis, namely the SF-36, PDQ-39 and MSA-QoL.

PDQ-39

The PDQ-39, a disease- specific QOL questionnaire for PD, covers 8 domains which impact a PD patient's quality of life: mobility, activities of daily living (ADL), emotional wellbeing, stigma, social support, cognitive, communication and bodily discomfort, in 39 questions. It also has a summary index score (PDQ-SI), which could be used to quantify an overall QOL score in PD (Peto *et al.*, 1995). Several factors, such as motor symptoms (Gomez-Esteban *et al.*, 2007), PD subtype (Hariz & Forsgren, 2011) and most importantly non-motor symptoms, have been reported to influence a PD patient's quality of life using the PDQ-39 as an outcome measure. These non-motor symptoms, have ranged from depression (Schrag *et al.*, 2000b), cognitive impairment (Marras *et al.*, 2008), fatigue (Havlikova *et al.*, 2008), sleep disorders (Forsaa *et al.*, 2008) and pain (Rahman *et al.*, 2008; Santos-Garcia & de la Fuente-Fernandez, 2013). These non-motor symptoms seem likely to have a greater impact on quality of life than motor symptoms (Martinez-Martin *et al.*, 2011).

MSA-QoL

A previous study using the SF-36 or EQ-5D consistently showed a significantly impaired QOL in patients with MSA compared to PD (Schrag *et al.*, 2006). As both generic questionnaires do not contain specific items for autonomic symptoms, which are very common among patients with MSA, the MSA-QOL has recently been developed for dealing with this issue (Schrag *et al.*, 2007). It consists of 40-item questions divided into 3 domains: motor, non-motor and emotional/social domains. A recent study showed that MSA-QoL was severely impaired in patients with MSA and significantly worse after approximately 1-year of follow-up (Meissner *et al.*, 2012).

SF-36

The SF-36 is a self-reported generic QOL questionnaire which consists of 36 items that define 8 category scales: physical functioning (PF), role limitation caused by physical problems (RP), bodily pain (BP), general health (GH), vitality (VIT), social functioning (SF), role limitation caused by emotional problems (RE) and mental health (MH). Scales range from 0 to 100 and a higher score indicates a better quality of life. The 8 category scales are often aggregated in 2 summary scales: the physical component

summary scale (PCS) and the mental component summary scale (MCS) (Manocchia *et al.*, 1998). It was designed to be used in a wide range of health conditions including PD, Multiple sclerosis (Riazi *et al.*, 2003) and other medical conditions, such as chronic fatigue syndrome (Komaroff *et al.*, 1996). The SF-36 has recently been challenged by disease-specific questionnaires in some conditions, such as PD and MSA, due to its lack of specific questions for those conditions, such as cognitive dysfunction in PD and autonomic features in MSA. Nevertheless, the SF-36 still provides a generic measure and can be used to compare the quality of life across different disorders.

Despite an increasing trend of QOL research, little work has been done on this measure in patients with chronic autonomic failure. There are a number of studies that have examined quality of life in patients with PD but only a few have assessed quality of life in patients with MSA and PAF. Validated questionnaires, such as the PDQ-39 and the PD-QoL, are widely used in PD research while little is known about quality of life in MSA and PAF. The MSA-QoL has been recently introduced and validated but this questionnaire has never been widely used in MSA research and there is no previous report on quality of life in patients with PAF. Some non-motor aspects of parkinsonian and autonomic disorders related to overall features of life, such as capacity for daily life activities, quality of life and depression have also been investigated in PD and MSA patients but have not been directly compared between these disorders or related to other indices of autonomic function.

Chapter 3
General Methods

Chapter 3

3. General Methods

3.1 Participant recruitment

All participants and patients for prospective studies were recruited from the Autonomic and Movement Disorder clinics at the National Hospital for Neurology and Neurosurgery (NHNN) and the Autonomic and Neurovascular Medicine Unit (St Mary's Hospital) in the UK. For retrospective studies, all records of patients referred to the Autonomic Units between 2001 and 2011 for testing with a diagnosis or clinical information containing the key words "parkinsonism", "parkinson's disease", "pure autonomic failure", "autonomic failure", "multiple system atrophy", "atypical Parkinson's Disease" and "Parkinson-plus syndrome" were identified. All patients' diagnoses had also been confirmed by autonomic and movement disorders specialists.

3.2. Experimental group design

Patients were divided into 3 groups:

1. Parkinson's disease

Idiopathic PD patients were diagnosed by neurologists using the UK Parkinson's Disease Society Brain Bank diagnostic criteria [UK-PDSBB] with an age at onset of more than 40 years and the presence of at least two of the three cardinal features of the disease (resting tremor, bradycardia or rigidity). PD severity ranged from mild to severe (Hoehn & Yahr stage I-IV). PD with autonomic failure (PD+AF) was defined as idiopathic PD with orthostatic hypotension (Orthostatic Systolic/Diastolic blood pressure falls $\geq$ 20/10 mmHg). For all PD+AF patients, records were checked to ensure that after autonomic failure had been documented rapid clinical deterioration or emerging additional features of MSA did not occur.

2. Multiple System Atrophy

MSA patients were diagnosed using Gilman's criteria (Gilman *et al.*, 1999; Gilman *et al.*, 2008) for probable MSA. Only probable MSA patients were included in studies. These patients were clinically defined criteria of autonomic failure/urinary dysfunction with additional features of either poor response to levodopa medication or cerebellar dysfunction, defined as:

A: Autonomic and urinary features.

Orthostatic fall in blood pressure (by 20 mmHg systolic or 10 mmHg diastolic) or urinary continence (persistent, voluntary partial or total bladder emptying, accompanied by erectile dysfunction in men) or both

B: Parkinsonian features.

Bradykinesia (slowness of voluntary movement with progressive reduction in speed and amplitude during repetitive actions) plus at least one of the following items:

1. Rigidity
2. Postural instability (not caused by primary visual, vestibular, cerebellar or proprioceptive dysfunction)
3. Tremor (postural, resting or both)

C: Cerebellar dysfunction.

Gait ataxia (wide based stance with steps of irregular length and direction) plus at least one of the following items:

1. Ataxic dysarthria
2. Limb ataxia
3. Sustained gaze-evoked nystagmus

3. Pure Autonomic Failure

PAF was diagnosed using the 1996 international consensus statement which consists of gradually progressive autonomic dysfunction with prominent orthostatic hypotension in the absence of cognitive, motor or sensory impairment (Neurology, 1996).

Healthy Participants

Healthy individuals were recruited if they did not fulfill any of the diagnostic criteria for PD, MSA and PAF and any other known cardiac, cardiovascular, neurological and metabolic disease.

<u>Exclusion criteria</u>

Participants were excluded if there was a chronic illnesses or the presence of conditions that potentially involved the autonomic nervous system. These included, congestive heart failure, recent (<6 months) myocardial infarction, severe anemia, diabetes mellitus, alcoholism,

malignant neoplasms, amyloidosis, hypothyroidism and history of the following diseases or treatments; sympathectomy, cerebrovascular disease and neurotoxins or neuroactive drug exposure. Individuals with a history of dementia or cognitive impairment and Schizophrenia, a history of severe head trauma leading to loss of consciousness or an active upper respiratory tract infection within 2 weeks prior to testing were also excluded. Patients were excluded if taking vasopressor, anti-hypertensive or other medications that could interfere with autonomic function.

3.3. Study preparation

All studies were performed in autonomic laboratories either at the National Hospital Neurology and Neurosurgery, Queen Square or at St Mary's Hospital, London. Similar autonomic function assessment protocols were used at both Units with controlled ambient temperatures.

Before each laboratory visit participants were asked to refrain from:

1. heavy exercise for 24 hours
2. alcohol ingestion for 12 hours
3. caffeine intake for at least 12 hours
4. eating for 1 hour
5. Depending on the patient's condition and medication, stop taking medication the night before each visit

If participants were involved in any other study at the time that would affect the autonomic nervous system they were excluded.

3.4. Cardiovascular autonomic function tests

A battery of cardiovascular autonomic function tests were performed in the supine position unless otherwise stated and included:

3.4.1 Head-up Tilt test

Participants rested in the supine position on a horizontal tilt table, with their feet flat against the foot rest. At the end of 10-minute rest, straps were placed around the lower limbs and

torso and the tilt table was adjusted to 60° head-up (Figure 3.1). Head-up tilting was continued for 10 minutes or until participants developed symptoms of imminent syncope or the patient was no longer to tolerate the tilt. The participant was then returned to the horizontal position. Blood pressure (BP) and heart rate (HR) were recorded every 3 minutes during supine rest and during head-up tilting.

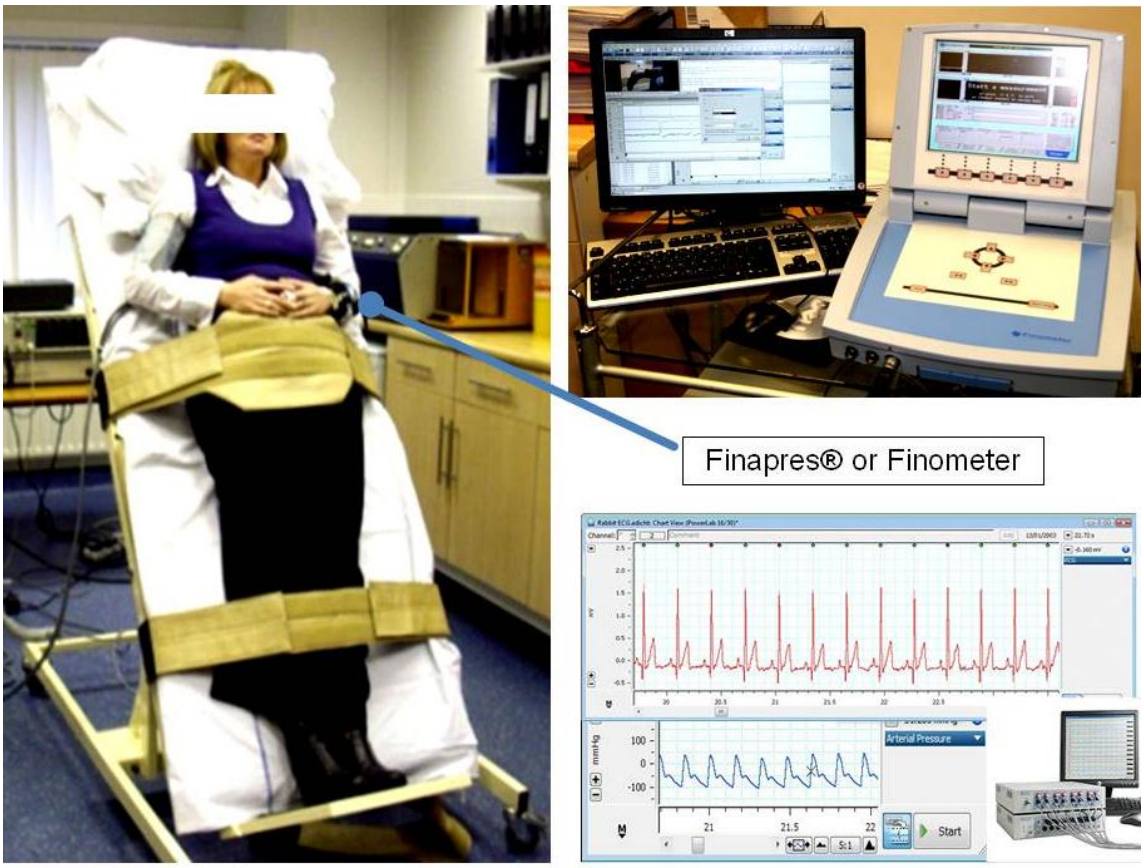


Figure 3.1. Head-up tilt table and a participant performing a 10-minute HUT (left) and examples of a beat-to-beat blood pressure recording (Finometer) and a computer with the data acquisition software (Right).

3.4.2 Noradrenaline plasma concentrations

Blood samples were collected during supine and head-up tilt tests to assess catecholamine (noradrenaline) concentrations as a biochemical marker of sympathetic neural activity. The plasma level of noradrenaline (NA) was measured using high-performance liquid chromatography (Mathias *et al.*, 1989).

3.4.3 Pressor tests

Handgrip isometric exercise Test

Participants were asked to perform a maximum voluntary handgrip contraction on 3-4 occasions in order to establish a maximum value. Participants then rested in the supine position and were then asked to conduct a handgrip contraction at 33% of their maximum contraction for 3 minutes (Figure 3.2). BP and HR were measured at baseline and during the last 30 seconds of isometric exercise.

Cold pressor Test

An ice pack was applied to their hand/wrist for 90 seconds. BP and HR were recorded prior to cold pack application and during the last 30 seconds of the cold stimulus (Figure 3.2). The ice pack was applied to the forearm of individuals with Raynaud's phenomenon.

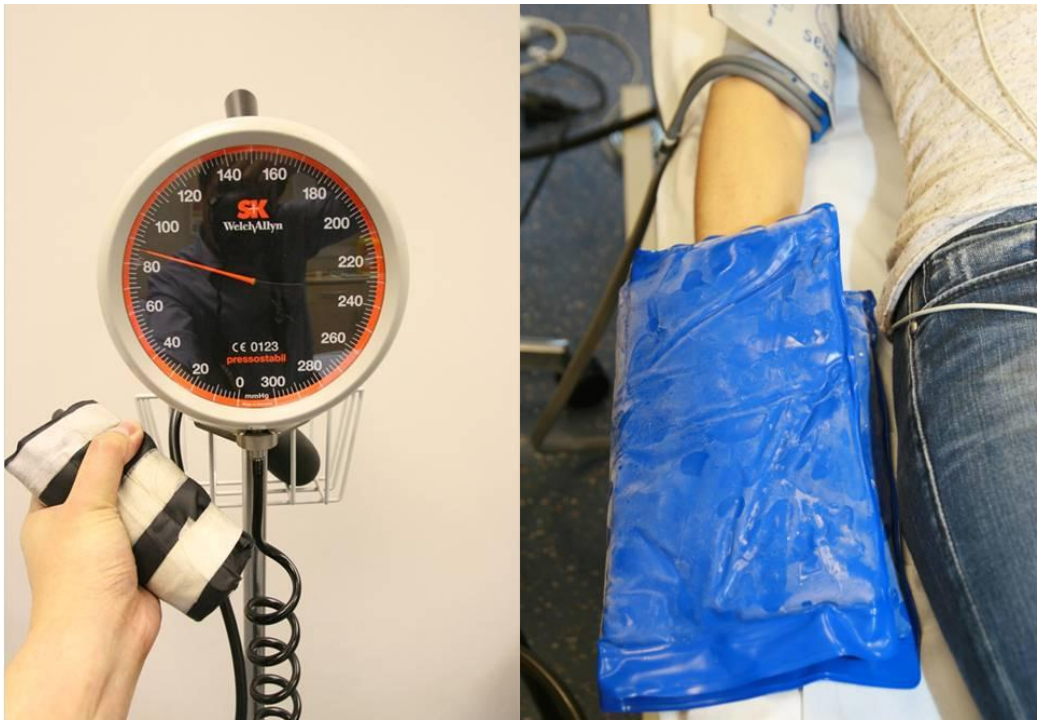


Figure 3.2. Manometer and a participant performing a handgrip contraction (left) and a participant with an ice pack on right hand and forearm during a cold pressor test (Right).

Mental Arithmetic Test

Participants were asked to serially subtract 7 or 17 from a suitable starting number (e.g. 400 or 1000), speaking the answers aloud, for a period of 2 minutes. BP and HR were recorded prior to and during the last 30 seconds of mental arithmetic.

3.4.4 Heart rate response to Deep breathing (HR_{DB})

Participants were asked to breath at a rate of 6 breaths per minute for one minute. Participants were coached on how long each breath should take (Figure 3.3). The change in heart rate between inspiration and expiration was calculated. The heart rate response to deep breathing (or respiratory sinus arrhythmia; RSA) was derived from the average of the differences between the minimum and maximum heart rates of the 6 breaths.

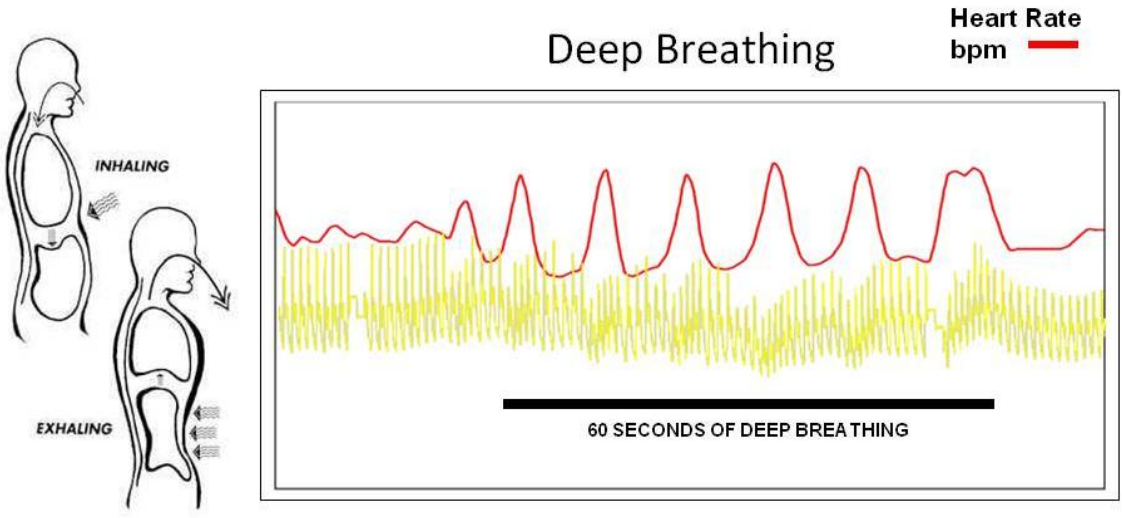


Figure 3.3. BP from Finometer (in yellow) and the average heart rate (in red). In the normal subject, heart rate fluctuates during deep breathing.

3.4.5 Valsalva Manoeuvre

Participants performed a forced expiration for 15 seconds against a fixed expiratory pressure of 40 mmHg for 10-15 seconds. A 2 ml syringe was used as a mouthpiece and was connected to a manometer (Figure 3.4). Participants inspired deeply, placed the tube in their mouth and sealed their lips before performing the expiration. The beat-to-beat BP responses during Valsalva manoeuvre were recorded (Figure 3.5). Three repetitions were performed with adequate time for hemodynamic recovery in between.

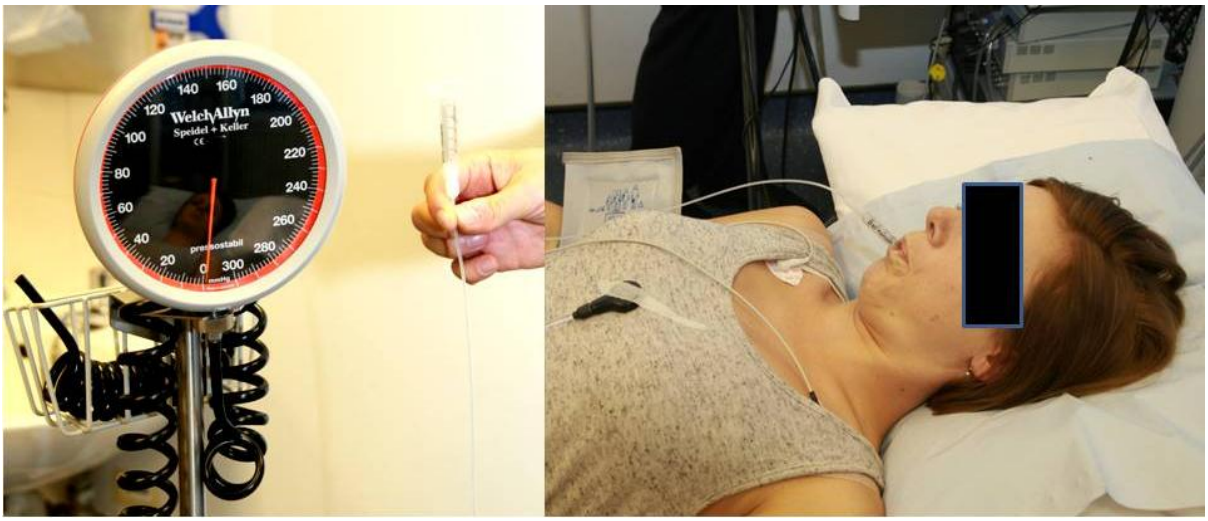
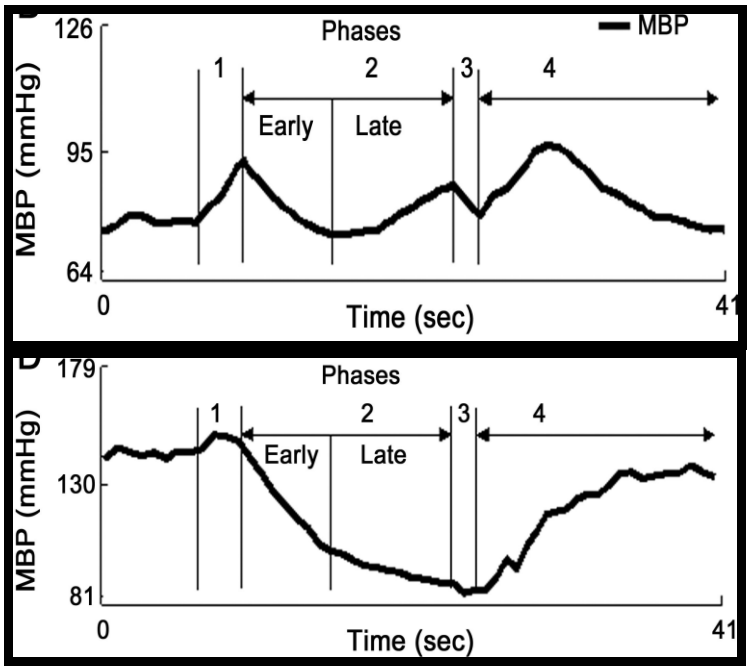


Figure 3.4. Manometer (left) and a participant blowing into the syringe connected to the Manometer for 15 seconds during the Valsalva Manoeuvre (Right).



Blood pressure profile during a Valsalva Manoeuvre in a normal subject. The BP response can be divided into 4 phases; Phase 1, Phase 2 early and late, Phase 3 and Phase 4

Blood pressure profile during the Valsalva Manoeuvre in a patient with autonomic failure. Note the absence of BP recovery during phase 2 late and a Prolonged blood pressure recovery time (BPRT) in phase 4

Figure 3.5. Blood pressure response during Valsalva Manoeuvre in a normal subject (upper panel) and a patient with autonomic failure (lower panel). *Adapted from (Novak, 2011).*

3.4.6 Quantitative cardiovascular autonomic function indices

Baroreflex sensitivity (BRS) and the blood pressure recovery time (BPRT) were derived from the Valsalva manoeuvre using on-line data acquisition software (Chart version 7.1, ADInstruments, Oxford, UK). The BP response to the VM occurs in different phases including phase 2 early (phase II_E), phase 2 late (phase II_L) and phase 4 (phase IV) were determined as previously described (Vogel *et al.*, 2005). BPRT was calculated as the time

interval starting from the lowest BP in phase 3 to the time with BP back to baseline (Vogel *et al.*, 2005) (Figure 3.6). BRS was calculated from the slope of the linear regression between the length of the RR interval and preceding SBP during phase 2 of the VM (Goldstein *et al.*, 1982). The Valsalva ratio (VR) was measured by the maximum HR generated by the VM divided by the lowest HR occurring within 30 seconds of the peak heart rate.

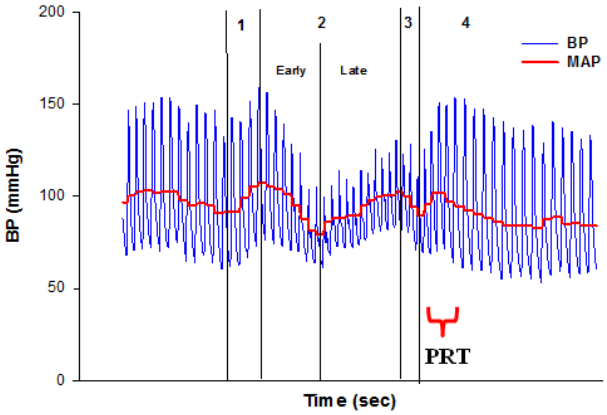


Figure 3.6. Representative Valsalva manoeuvre from a healthy individual. The amplitude of phase I is measured from baseline to peak of phase I. Phase II Early is measured from baseline to the trough of phase II. Phase II Late is determined from the end of phase II Early to the beginning of phase III. Phase III is measured from the end of phase II Late to the trough of phase III. Phase IV was determined as its height above baseline.

3.4.7 Liquid Meal test

After a baseline period of 15 min supine rest, participants consumed a 300 ml standard balanced liquid meal (66 g carbohydrate, 22 g fat and 18 g protein made up to 300 ml with full fat milk) through a straw whilst lying supine and then rested for ~45 min. A 10 min 60° HUT was performed before and 45 min after the liquid meal protocol. Blood pressure and heart rate were measured every 5 min before and after the meal.

3.4.8 Instrumentation

For all of the above experiments arterial blood pressure and heart rate were intermittently assessed using upper arm sphygmomanometry (GE Medical Systems, Tampa, FL, USA). Beat-to-beat heart rate and arterial blood pressure were assessed continuously using 5-lead chest electrode electrocardiography and digital photoplethysmography (Portapres® or Finometer®, TNO-TPD, Biomedical Instrumentation). The Finometer is a non-invasive technique to measure finger arterial blood pressure, using an inflatable finger cuff with a built in photoelectric plethysmograph. Each cuff has an infrared sensor running parallel to the digital artery and a pressure transducer, controlled by a valve, allowing rapid inflation and deflation of the cuff. The volume of the cuff is rapidly adjusted, under feedback control, to

keep the infrared signal amplitude constant. This in turn keeps the finger volume constant throughout the arterial pulse cycle. The signal output is displayed on screen, showing a real time continuous waveform, which is usually indistinguishable from that of a peripheral arterial, e.g., brachial, waveform. The Finometer technique has been validated by comparison with intra-arterial blood pressure during a range of manoeuvres, including orthostasis, and accurately reflects intra-brachial or radial blood pressure (Langewouters *et al.*, 1998; Hirschl *et al.*, 1999; van der Does *et al.*, 2013). It is also reliable for tracking changes in arterial blood pressure (Langewouters *et al.*, 1998; van der Does *et al.*, 2013). Participants are asked to keep the finger movements to a minimum during testing and a height correction unit is applied to the device and/or the hand is positioned at heart level to avoid unwanted hydrostatic effects of any changes in the arm. Blood pressure and heart rate were recorded continuously online (Powerlab systems, ADInstruments, Australia).

3.5 24 hour-Ambulatory BP and heart rate monitoring (24 hr-ABPM)

Participants that conducted a 24 hr-ABPM with a custom autonomic protocol diary (see Appendix I) wore a standard upper arm blood pressure cuff (90207 Ambulatory Blood Pressure monitors, Spacelabs Healthcare, Hertford, U.K, Figure 3.7).

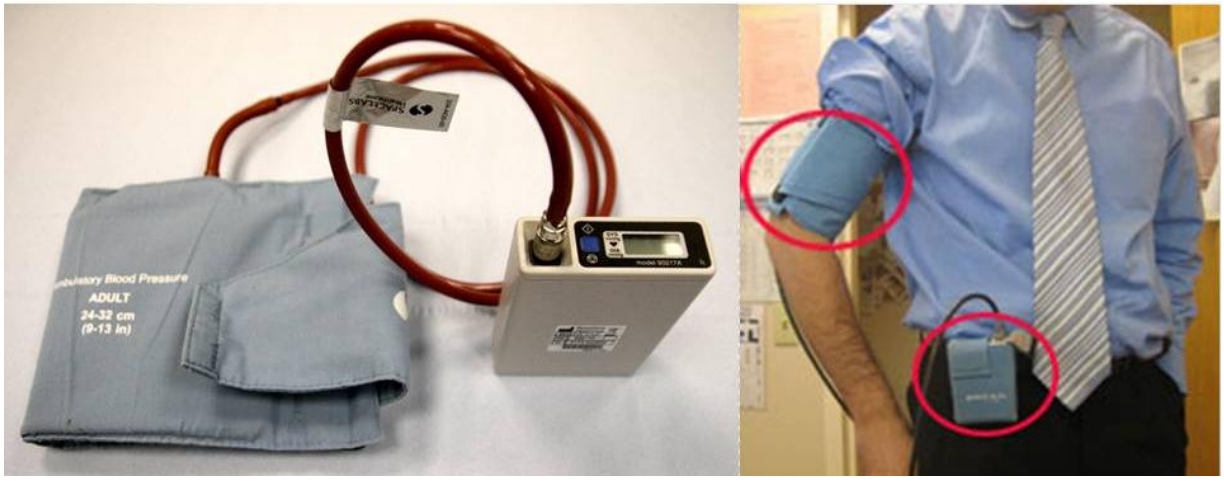


Figure 3.7. 24 hr-ABPM (left) and a participant with the 24 hr-ABPM during monitoring (Right).

3.6 Smell identification tests

Olfactory function was assessed using the Sniffin' Sticks (SS-16) Smell identification Test. Participants were asked to smell 16 different odored pens for up to 30 s (Figure 3.8) and then select 1 of 4 options that best describes the presented odour. Function was assessed as a score out of 16 (Hummel *et al.*, 1997).

Figure 3.8. Sniffin' Sticks (SS-16) smell test.

3.7 Questionnaires

Participants completed some or all of the following questionnaires:

Motor Symptoms

Unified Multiple System Atrophy Rating Scale (UMSARS) and Unified Parkinson's Disease Rating Scale (UPDRS) are validated disease-specific scales that represent the diverse signs and symptoms of MSA and PD, respectively, such as daily living activities and mobility. The scales comprise the following components: Part I, historical, 12 items; Part II, motor examination, 14 items; Part III, autonomic examination; and Part IV, global disability scale (Wenning *et al.*, 2004).

Hoehn and Yahr Staging (HY) was used to rate the degree of functional ability in patients. The same investigator conducted these assessments in all patients.

Non-motor Symptoms

The Mini-Mental State Examination (MMSE) rating scale was used to rate all participants' cognitive function. The MMSE score ranges from 0-30, with a lower score indicating greater impairment of cognitive function. Given that cognitive impairment is a confounder for the

validity of quality of life questionnaires, all patients with an MMSE score of less than 24 were not included in studies.

The Hamilton Depression Rating Scale (HAM-D) was used to assess symptoms of depression and its severity in both patient and control groups. The scale consists of 17-item questions. The score ranges from 0-53 and the higher score correlates with more severe depression. A score between 0-7 is generally accepted as a normal range but a score of 20 or higher suggests depression (Hamilton, 1960).

The Schwab and England Activities of Daily Living Scale (SE) was used by the participants to rate how much their condition affects their functional capability. The SE score ranges from 100 (Completely independent) to 0 (bedridden).

Quality of life

Patient population specific scales, as well as a generic scale in PAF, were used to assess Quality of Life.

The 39 item Parkinson's Disease Questionnaire (PDQ-39) was used in PD patients. It was completed by patients and it covers 8 domains which impact a PD patient's quality of life: mobility, activities of daily living (ADL), emotional wellbeing, stigma, social support, cognitive, communication and bodily discomfort in 39 questions. It has also a summary index score (PDQ-SI), which can be used to quantify an overall QOL score in PD. The higher score represents poorer quality of life.

The MSA Health-Related Quality of Life Scale (MSA-QoL) was used in MSA patients. It was completed by patients and consists of 40-item questions and is divided into 3 domains: motor, non-motor and emotional/social domains. A higher score indicates poorer quality of life.

The 36 item Short Form Health Survey (SF-36) was used in PAF patients due to the lack of a disease-specific QOL questionnaire for PAF. The SF-36 is a self-reported questionnaire that has been used to assess quality of life in various medical populations, and consists of 36 items that define 8 category scales: physical functioning (PF), role limitation caused by physical problems (RP), bodily pain (BP), general health (GH), vitality (VIT), social functioning (SF), role limitation caused by emotional problems (RE), and mental health (MH).

The 8 category scales are often aggregated in 2 summary scales: the physical component summary scale (PCS) and the mental component summary scale (MCS) (Manocchia *et al.*, 1998). Scales range from 0 to 100 and a higher score indicates better quality of life.

Healthy control participants only completed the Short Form Health Survey (SF-36), the Mini-Mental State Examination (MMSE) and the Hamilton Depression Rating Scale (HAM-D).

All questionnaires include in the studies are listed in the Appendix.

3.8 Electrogastrography (EGG)

For the liquid meal test, a four-channel cutaneous EGG recorder (Nipro EG; Nipro, Japan), which consists of four surface recording electrodes and one reference electrode, was used to record gastric myoelectrical activity (GMA). All electrodes were placed on abdominal skin as shown in Figure 3.9.

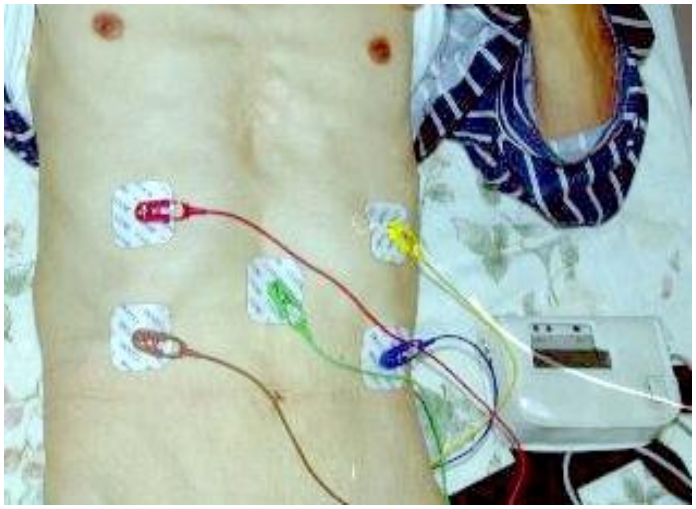


Figure 3.9. EGG recording device with 4 surface electrodes (Red, Yellow, Brown and Blue) with one reference electrode (Green). *Images courtesy of Dr Masato Asahina, Chiba University, Japan*.

GMA was recorded during the 15 minutes before and 45 minutes after the ingestion of the liquid meal and data were analysed using EGG software (EGS2 Ver 3.1 software, EG, Ram Co., Japan). Movement artefacts were excluded from the raw EGG data before obtaining EGG power spectra using Fast Fourier Transformation (FFT). The main outcome measurements were the dominant frequency (DF) of the GMA slow waves, which was defined as the frequency at which the overall power spectrum showed peak power in normal frequency ranges (NFR) and the instability coefficient of DF (ICDF), which is a marker of the

variability of the DF and is derived from the proportion of the standard deviation and the mean of the DF.

3.9 Statistical Analyses

Data are presented as mean (± 1 SD). Normally distributed data between patient groups were compared using ANOVA (3 or more groups) or by t-tests (2 groups). Non-parametric tests, including Kruskal-Wallis and Mann-Whitney U tests, were used for non-normally distributed data, where appropriate. Chi-square analyses were used for analysis of categorical variables. Repeated measures ANOVA was used for comparisons of data from more than two different time points. Receiver operating characteristic (ROC) analyses were used to determine the sensitivity and specificity for detecting and distinguishing the presence of disorders. Multiple logistic regression analysis was used to determine what factors (binary variables) were associated with outcome variables. The relationships between cardiovascular autonomic function indices and other factors, such as disease duration and disease severity (continuous variables), were assessed by correlation analysis (Pearson's correlation for normally distributed data and Spearman's correlation for non-normally distributed data) or multiple linear regression analysis. Statistical analyses were carried out using STATA 11.0 (STATA Corporation, College station, Texas, USA). All tests were 2-sided and a p value of ≤ 0.05 was considered significant.

Chapter 4

Evaluation of cardiovascular autonomic function in chronic autonomic failure

Chapter 4

4.1 Evaluation of cardiovascular autonomic function in chronic autonomic failure

4.1.1 Quantitative cardiovascular autonomic function in patients with chronic autonomic failure (PD+AF, MSA and PAF)

4.1.1.1 Introduction

The overlapping features of cardiovascular autonomic dysfunction, particularly OH, in parkinsonian syndromes and MSA sometimes results in difficulties in differentiating the diagnosis between MSA and PD and suggests that OH may not be a suitable single autonomic feature for distinguishing PD and MSA (Lipp *et al.*, 2009). Furthermore, some MSA patients present with an isolated feature at the onset, such as orthostatic hypotension, and consequently, autonomic dysfunction can predate the diagnosis of MSA for a while, or patients can sometimes be misdiagnosed with idiopathic PD before the development of other hallmark MSA features. It is a critical and quite common question in clinical practice whether a patient presenting with parkinsonism and autonomic dysfunction has MSA or PD because of their vast difference in terms of disease prognosis, and as a result, is a vital differentiation to make judiciously with regards to instigating appropriate treatment strategies.

Cardiac ^{123}I-metaiodobenzylguanidine (MIBG) imaging has been proposed to be helpful for distinguishing between MSA and PD. Cardiac MIBG uptake was reported to be markedly reduced in Parkinson's disease (Braune, 2001) which was consistent with a reduction of tyrosine hydroxylase immunoreactivity in myocardial sympathetic nerves (Amino *et al.*, 2005). In contrast, cardiac uptake was mostly normal in MSA (Spiegel *et al.*, 2005) Nevertheless, the reliability of this test has been reported as inconsistent in several studies and results have often showed no differences between these disorders (Orimo *et al.*, 1999; Druschky *et al.*, 2000; Kimpinski *et al.*, 2012). In addition, the MIBG scan is a relatively invasive, costly and time-consuming technique.

Cardiovascular autonomic screening tests are typically used to confirm the diagnosis of autonomic failure, including the use of head-up tilting for the assessment of OH. In order to overcome the limitation of the use of OH as index for differentiating autonomic disorders other indices of quantitative cardiovascular autonomic function, such as, baroreflex sensitivity (BRS) and blood pressure recovery time (BPRT) to perturbations in blood pressure have recently been developed and been proposed to be useful in evaluating autonomic function in chronic autonomic failure disorders (Vogel *et al.*, 2005; Schrezenmaier

et al., 2007). BRS was previously reported to be abnormal in a few studies in PD+OH when compared with PD without OH (PD-OH) (Barbic *et al.*, 2007) and MSA compared with MSA+OH (Goldstein *et al.*, 2003b). More recently, BRS calculated from trigonometric spectral analysis was reported to be significantly lower in patients with MSA compared to PD (Friedrich *et al.*, 2008). Beat-to-beat blood pressure and heart rate responses to the Valsalva manoeuvre (VM) are also useful for evaluating sympathetic and parasympathetic function, as well as baroreflex sensitivity (Vogel *et al.*, 2005). The heart rate responses to deep breathing (sinus arrhythmia) can also be used to assess cardiac parasympathetic function and are frequently abnormal in patients with autonomic failure (Gurevich *et al.*, 2004; Deguchi *et al.*, 2006). Both BRS and BPRT have however never been specifically compared between MSA with autonomic failure and PD with autonomic failure. The relationship between these novel cardiovascular autonomic function indices and disease duration in MSA and PD+AF has also not been thoroughly examined either. The aim of this study was therefore to determine cardiovascular autonomic function in MSA and PD+AF using a standard orthostatic challenge test (Head-up Tilt test) and alternative indices of quantitative cardiovascular autonomic function, including baroreflex sensitivity (BRS), blood pressure recovery time (BPRT), Valsalva ratio (VR) and the heart rate responses to deep breathing (HR_{DB}).

4.1.1.2 Methods

Subjects

Patients with a confirmed diagnosis of Parkinson's disease with autonomic failure (PD+AF), Probable Multiple System Atrophy (MSA) with orthostatic hypotension and Pure Autonomic Failure (PAF) and healthy controls were included (Chapter 3). All patients with MSA were confirmed to have autonomic failure in addition to other classical features such as severe urinary disturbances, sexual dysfunction, sudomotor dysfunction, sleep dysfunction (REM sleep behavioural disorders) and respiratory involvement (sigh or stridor). Patients were excluded if they were unable to perform the VM or had a flat top VM response.

Clinical history

History and relevant information of all patients and participants were retrospectively evaluated from medical records. These included age, gender, duration of disease (time from first symptom to date of testing), dopaminergic medications and presenting symptoms.

Presenting symptoms were divided into; cardiovascular autonomic symptoms (e.g. dizziness, lightheadness, visual disturbances, syncope and coat-hanger ache) (Mathias *et al.*, 1999), bladder symptoms, parkinsonism and cerebellar symptoms.

Cardiovascular autonomic screening tests (AFT)

Cardiovascular autonomic function was assessed using Autonomic Unit protocols (Mathias *et al.*, 2013a). This included all pressor tests, Valsalva manoeuvre (VM), deep breathing, Hyperventilation, 10-minute 60 degrees HUT as described in Chapter 3. Beat-to-beat blood pressure and heart rate were recorded continuously on-line using digital photoplethysmography and electrocardiography, respectively. These variables were also recorded intermittently using upper arm sphygmomanometry. Beat-to-beat blood pressure and heart rate data were used to analyse quantitative cardiovascular autonomic function indexes including BP responses to VM, blood pressure recovery time (BPRT), Valsalva ratio (VR) and HR responses to deep breathing (HR_{DB}) off-line.

Quantitative cardiovascular autonomic function indexes

Phase 2 early (phase II_E), phase 2 late (phase II_L), phase 4 (phase IV), the Valsalva ratio (VR) and BPRT were determined from the VM as previously described (Vogel *et al.*, 2005). BRS was calculated from the slope of the linear regression between the length of the RR interval and preceding SBP during phase 2 of the VM (Goldstein *et al.*, 1982). The heart rate response during deep breathing (HR_{DB}) was measured by the mean of the heart rate change (maximum-minimum) during 6 breath cycles.

Statistical methods

Data are presented as mean (± 1 SD) or median (inter-quartile range), where appropriate. Analysis of covariance (ANCOVA) was used for comparisons of 3 or more groups normally distributed data while the Kruskal-Wallis test was used if data were non-normally distributed. If there was a significant difference, Mann-Whitney U tests were then used to compare between 2 groups for non-normally distributed data and unpaired t-tests for normally distributed data with Bonferroni corrections. Chi-square analyses were used for analysis of categorical variables. The relationships between quantitative autonomic measures and disease duration were assessed by regression analysis. Quantitative autonomic measures were set as the outcome variables while age, gender, disease duration and the use of dopaminergic medications were set as covariates. Statistical analyses were

carried out using STATA 11.0 (STATA Corporation, College station, Texas, USA). All tests were 2-sided and a p value of $\leq$0.05 was considered significant.

4.1.1.3 Results

74 patients (45 MSA, 18 PD+AF, 11 PAF) and 15 controls were included in the analyses (see Table 1). Patients with MSA were younger than the PD+AF patients ($p<0.05$). There was no difference in disease duration between disorders. Orthostatic symptoms were reported in 35 out of 45 (77.8%) MSA patients and in all PD+AF and PAF patients. Within the group of MSA patients, MSA-P was more common (26/45, 57.8%) compared to MSA-C. Age, disease duration and gender were not different between MSA sub-groups.

Table 4.1. Patient demographic data

Variable	MSA	PD+AF	PAF	Control
Number	45	18	11	15
% Male	56	67	91	47
Age at testing Mean (SD), yrs	62 (8)*	70 (9)*#	66 (7)	58 (8)
Disease duration Median (range), yrs	5 (2-11)	5 (2-18)	6 (1-16)	-
% Dopaminergic treatment	47	89#	-	-
*p<0.05 vs. Control, #p<0.05 vs. MSA				

In PD+AF, 94% (17/18) had parkinsonian symptoms. In contrast, 40% (18/45) of MSA patients reported this as an initial symptom. Symptoms from cerebellar dysfunction were the second most common presenting symptom in MSA (29%, 13/45) followed by bladder symptoms (22%, 10/45) and orthostatic symptoms (9%, 4/45). OH presented in patients with MSA with a wide variation with respect to time ranging from 2-11 years after the onset of their first symptom. This figure was also similar in patients with PD+AF (2-18 years)

and PAF (1-16 years). Approximately 58% (26/45) of patients with MSA had OH within the first 5 years of reporting their first symptom.

Table 4.2. Systolic and Diastolic blood pressure (SBP, DBP;mmHg) during supine and head-up tilt and orthostatic SBP changes in MSA, PD+AF, PAF and control groups.

Variable	MSA	PD+AF	PAF	Control
Supine SBP	135±21	134±24	134±21	124±18
Supine DBP	81±12	74±13	80±10	66±9
Tilt SBP	89±21*	91±24*	78±14*	128±16
Tilt DBP	59±13*	58±18*	51±9*	74±9
Orthostatic ΔSBP	-46±20*	-44±18*	-59±21*$^{\$}$	4±11
Values are mean±SD, * p<0.05 vs. Control $^{\$}$p<0.05 vs. PD+AF				

There was no difference in the group mean supine SBP among controls and each group of patients. The absolute SBP during tilt was significantly lower and the orthostatic SBP change was significantly greater in patients compared to controls ($p<0.05$). Systolic blood pressure during tilting in patients with MSA was not different when compared with PD+AF ($p=0.39$) and PAF patients ($p=0.1$). There was also no difference in the degree of orthostatic SBP change in MSA compared with PD+AF ($p=0.61$) and PAF ($p=0.9$). Orthostatic BP change was significantly larger in PAF compared to PD+AF ($p=0.04$) (Table 4.2).

The BP changes during phase 2 early and phase 2 late of the VM were significantly blunted in the patient groups when compared to healthy controls but there was no difference between disorders except in the BP change during Phase IV. The BP overshoot was significantly smaller in PAF compared with PD+AF ($p=0.01$) (Table 4.3).

Table 4.3. Systolic Blood pressure changes during the various phases of the Valsalva manoeuvre in MSA, PD+AF, PAF and control groups.

Variable	MSA	PD+AF	PAF	Control
Baseline SBP	136±29*	134±27*	131±29*	115±16
∆SBP Phase II_E	57±29*	54±21*	47±18*	20±9
∆SBP Phase II_L	2±4*	2±3*	3±4*	30±16
∆SBP Phase IV	4±7*	8±7*	2±5*$	45±16

Values are mean±SD, SBP=Systolic BP, Phase II_E= Phase 2 early of VM, Phase II_L= Phase 2 late of VM, Phase IV=Phase 4 of VM

*p<0.05 vs. Control $p<0.05 vs. PD+AF

Baroreflex sensitivity (BRS) was significantly lower in all patient groups compared to controls. Within the patient groups, BRS was significantly lower in MSA compared with PD+AF (p=0.024). PD+AF had a significantly higher BRS compared to patients with PAF (p=0.05). Blood pressure recovery time (BPRT) was significantly longer in all patient groups compared to controls but was significantly faster in patients with PD+AF patients relative to MSA (p<0.01) and PAF (p<0.01). After controlling for age, disease duration and gender, BRS remained significantly lower and BPRT was longer in patients with MSA compared to PD+AF (p=0.012 and p<0.01, respectively). BPRT in PAF was also significantly longer when compared with PD+AF after correcting for confounders.

Table 4.4. Cardiovascular autonomic parameters during the Valsalva manoeuvre; Baroreflex sensitivity, Blood pressure recovery time (BPRT); in MSA, PD+AF, PAF and control groups.

Variable	MSA	PD+AF	PAF	Control
BRS, ms/mmHg	1.4±1.1*	2.1±1.2*#	1.6±2.3*	4.3±1.8
BPRT, sec	26.6±16.3*	13.9±7.6*#	29.0±11.8*$	0.8±0.5
VR	1.2±0.1*	1.3±0.3*	1.2±0.2*	1.6±0.3
HR_{DB} (bpm)	6±5*	5±3*	4±2*	14±6

Values are mean±SD, BRS= baroreflex sensitivity, BPRT=Blood pressure recovery time, VR=Valsalva Ratio, HR_{DB} = Heart rate response during Deep Breathing

* p<0.05 vs. Control $p<0.05 vs. PD+AF #p<0.05 vs. MSA

HR_{DB} in controls was significantly higher when compared with those in MSA, PD+AF and PAF ($p<0.05$) but there was no difference between patient groups (MSA vs. PD+AF, $p=0.43$ and PAF vs. PD+AF, $p=0.27$). Similarly, there was a significantly lower Valsalva Ratio (VR) in patients compared to controls ($p<0.05$) but no difference among patient groups (MSA vs. PD+AF, $p=0.74$ and PAF vs. PD+AF, $p=0.7$). There were no differences in age, disease duration, and orthostatic BP changes during HUT, BRS, BPRT, VR and HR_{DB} between MSA-P and MSA-C sub-groups.

Autonomic function parameters, including orthostatic SBP changes during HUT, BRS, VR and HR_{DB}, were not correlated with disease duration in both MSA and PD+AF patients after adjusting for age and gender but there was a trend of association ($P = 0.07$) between BPRT and disease duration only in patients with PD+AF (see Table 4.5).

Table 4.5. Multiple regression analysis of the correlation between BPRT and disease duration adjusting for age and gender in PD+AF

Variable	Parameter estimate	Standard error	P-value
Disease duration	0.78	0.40	0.07
Age	0.03	0.19	0.87
Gender	0.72	3.81	0.85

4.1.1.4 Discussion

The aim of this study was to compare novel cardiovascular autonomic function indices in MSA and PD with autonomic failure using a variety of cardiovascular autonomic function markers, including blood pressure changes during HUT, BRS, BPRT, VR, HR_{DB}, and to examine whether any of these measures were correlated with disease duration. The main findings were that the MSA patients, relative to PD+AF, displayed no difference in orthostatic SBP changes but exhibited a longer blood pressure recovery time (BPRT) after the Valsalva Manoeuvre (VM) and a significantly lower BRS. Greater BP changes during HUT, lower BRS and prolonged BPRT in PAF compared with PD+AF indicate more severe adrenergic and cardiovagal dysfunction in PAF vs. PD+AF but these responses in PAF were comparable with MSA.

Previous studies have reported equivocal results of cardiovascular autonomic function in differentiating between MSA and PD (Riley & Chelimsky, 2003; Reimann *et al.*,

2010), which could be explained by differences in study populations (e.g. PD versus the combination of MSA with and without autonomic failure or PD without autonomic failure), data analyses (e.g. proportion of normal/abnormal tests vs. quantitative measures) and/or study protocols. Our study specifically compared PD with AF and MSA with AF using both standard autonomic testing (such as HUT, HR_{DB}) and other potential autonomic measures, such as BRS and BPRT, which have never been directly compared between these groups and could be derived from a non-invasive procedure, the Valsalva manoeuvre, which is commonly used in standard autonomic test protocols.

The similar orthostatic SBP responses, as well as Valsalva ratio (VR) and heart rate responses during deep breathing (HR_{DB}) in patients with PD+AF and MSA are consistent with a previous study showing that these tests could not differentiate between MSA and PD (Riley & Chelimsky, 2003), however, baroreflex sensitivity (BRS) and BPRT were not evaluated in that study. Our findings show that both these measures were abnormal in MSA patients compared to PD+AF. Our findings confirmed that some autonomic function tests, such as BP response to HUT, HR_{DB} and VR have limited effectiveness in detecting differences between MSA and PD (Riley & Chelimsky, 2003). In contrast, our study suggested that quantitative measures from the VM, BRS and BPRT, may provide a better diagnostic yield for this purpose compared to reporting only 'normal' or 'abnormal' grading of the VM, which has been used in a prior study and revealed no differences between MSA and PD (Schmidt *et al.*, 2009b).

A lower BRS in patients with MSA compared to those in PD+AF indicated a greater impairment of baroreflex dysfunction in MSA relative to PD+AF. BPRT is a useful measure of the adrenergic component, e.g. vasoconstrictor responsiveness, of baroreflex function (Vogel *et al.*, 2005). Prolonged BPRT in MSA may indicate greater impairment of adrenergic function in MSA compared to PD+AF. A sub-group analysis of the parameters between MSA and PD+AF patients with orthostatic symptoms was also performed. The results did not show any difference from our main findings. In accordance with a previous study (Schmidt *et al.*, 2008), we found no difference in any cardiovascular autonomic parameters between MSA-C and MSA-P.

Although there were some overlap in BPRT and BRS values between MSA and PD+AF patients in our study, suggesting that these measures may not be able to be used for distinguishing PD+AF from MSA with autonomic failure, our results demonstrated that MSA generally had lower BRS and longer BPRT than PD+AF. Given that these are non-invasive

and relatively simple tests to perform, both measures should be included as part of autonomic investigations in suspected MSA/PD+AF workups.

MSA patients were significantly younger and used less dopaminergic medications compared to PD+AF patients, These differences were not likely to have an impact on our results because both dopaminergic medications and increasing age were associated with reduced BRS (Oka *et al.*, 2003) and prolonged BPRT (Huang *et al.*, 2007). Thus, the lower BRS and longer BPRT in MSA relative to PD+AF could have been attenuated by the older age and greater use of dopaminergic medications in the PD group. In addition, differences in BPRT and BRS between MSA and PD+AF were still significant after adjusting for age, gender and disease duration.

Our findings cannot be used as epidemiological data showing the prevalence of autonomic dysfunction in MSA and PD because all patients were selected from autonomic laboratory investigations but it is important to note that cardiovascular autonomic symptoms were not present in more than 20% of MSA patients in our study even though they all met the criteria of Orthostatic Hypotension (OH). This symptomatic figure is close to a previous study (Ha *et al.*, 2011) and confirmed that OH and orthostatic symptoms are not always correlated and patients who have any suggestive features of MSA should undergo autonomic function testing. The evidence of autonomic dysfunction would improve diagnostic accuracy and provide better information on disease prognosis, as well as treatment plans, in these patient groups. Our study has also revealed that the presentation of OH can occur in the later stages of the disease progression in MSA and OH is not necessarily a presenting feature in patients with MSA as OH occurred after 5 years of disease onset in more than 40% of patients.

Pathological studies have confirmed widespread neuronal loss in key areas within the central autonomic network including the periventricular nucleus (Benarroch *et al.*, 2006a), ventrolateral medulla (VLM) (Benarroch *et al.*, 1998), dorsal motor nucleus of the vagus nerve (Benarroch *et al.*, 2006b) and intermediolateral cell column (Kennedy & Duchen, 1985) with the presence of glial cytoplasmic inclusions (GCIs) in MSA patients suggesting a pre-ganglionic lesion in origin while autonomic dysfunction in PD has been proposed to be caused by the combination of pre- and post-ganglionic abnormalities. These include abnormal Lewy bodies pathology in the hypothalamus, lower brainstem autonomic nuclei, insular cortex, anterior cingulate, intermediolateral column and evidence of a reduced uptake of cardiac MIBG and Lewy body deposition in peripheral autonomic ganglia, respectively (Wakabayashi & Takahashi, 1997b). A lower BRS in MSA might be explained

by greater neuronal loss in the ventrolateral nucleus ambiguous (NAmb), compared with PD (Benarroch *et al.*, 2003). The majority of neurons from this area consist of pre-ganglionic parasympathetic neurons projecting to the heart (Hopkins *et al.*, 1996) which contribute to cardiovagal control and may be responsible for greater impairment of baroreflex function. Furthermore, a significant reduction of C1 catecholaminergic neurons in the rostral ventrolateral medulla have also been reported in MSA (Kato *et al.*, 1995) and to a greater extent than in patients with PD+OH (Benarroch *et al.*, 2000). This area involves the control of sympathetic vasomotor outflow and may explain the different degrees of prolonged BPRT in MSA and PD+AF.

Pathological studies of PAF are quite limited because of the condition's rarity. While the majority of pathological studies in PAF reported some abnormal lewy body deposition and neuronal loss in pre-ganglionic sympathetic areas (intermediolateral cell column of thoracic spinal cord) and severe involvement of post-ganglionic sympathetic lesions (sympathetic ganglia, stellate ganglion and celiac ganglion) (van Ingelghem *et al.*, 1994; Arai *et al.*, 2000), other case studies have also reported lewy body deposition in the substantia nigra and brainstem structures, which is similar to patients with PD (Hague *et al.*, 1997). Nevertheless, greater BP changes during HUT, lower BRS and prolonged BPRT in PAF suggest more widespread impairment of both sympathetic and parasympathetic function in PAF compared with PD+AF. Comparing pathological involvement of the autonomic nervous system between PAF and PD+AF may provide further insight into whether there are differential neuronal losses to explain a greater autonomic dysfunction in PAF relative to PD+AF.

There was no clear association between any cardiovascular autonomic parameters and disease duration in PD+AF, MSA and PAF patients. A trend of association between BPRT and disease duration in PD+AF patients was apparent. This may reflect the heterogeneous progression of autonomic dysfunction in MSA patients. Nonetheless, BPRT may be a potential measure to be used for follow-up in PD patients who have autonomic dysfunction/failure. There are only a few longitudinal studies of cardiovascular autonomic function in PD patients. The results showed a significant reduction of heart rate variability and greater orthostatic blood pressure change from baseline during standing after 2.5 years (Mesec *et al.*, 1999). Another study found a significant worsening of autonomic symptoms, using the composite autonomic symptom scale (COMPASS), in MSA compared with PD after 12-months follow-up (Lipp *et al.*, 2009). Further investigation of these findings in a greater number of patients with a longitudinal approach would provide a better

understanding of whether or not these tests could be used to follow-up patients in a clinical setting.

In conclusion, despite a similar severity of OH between patient groups, a significantly prolonged BPRT and lower BRS were evident in MSA compared to PD+AF patients as well as in PAF compared to PD+AF, which may be explained by a greater impairment of autonomic function and more widespread autonomic failure in MSA and PAF. Our findings suggest that BPRT and BRS may be additional markers to help differentiate patients with MSA (who have orthostatic hypotension) from PD+AF, also between PAF and PD+AF.

4.1.2 Quantitative cardiovascular autonomic function in patients with Parkinson's disease (PD) and healthy control individuals

4.1.2.1 Introduction

Cardiovascular autonomic dysfunction, such as orthostatic hypotension (OH), is increasingly recognised as a major non-motor feature in PD (Martinez-Martin *et al.*, 2007). OH is commonly present in patients with PD and sometimes without orthostatic symptoms (Senard *et al.*, 1997). It is often evident in later stages in PD, when patients become symptomatic or have apparent autonomic dysfunction. That said, cardiovascular autonomic dysfunction can occur in early stages of PD, even before the hallmark motor symptoms occur (Goldstein, 2006), which can range from mild autonomic dysfunction to autonomic failure (Ziemssen & Reichmann, 2010). A recent study demonstrated that abnormal reuptake from cardiac MIBG can be evident even in the early stage of PD (Hoehn and Yahr stage I) (Spiegel *et al.*, 2005). Furthermore, a later study also reported an association between cardiovascular autonomic function test results; specifically, the blood pressure response in phase IV (e.g., the BP overshoot) during the Valsalva manoeuvre (VM), and the reduction of cardiac MIBG reuptake in de novo patients with PD (Oka *et al.*, 2011). As abnormal reuptake of cardiac MIBG represents the post-ganglionic autonomic lesion in PD (Amino *et al.*, 2005), cardiovascular autonomic dysfunction could be a potential non-invasive biomarker to aid diagnosis in early PD.

More recently, other cardiovascular autonomic tests, such as spectral analysis of heart rate variability, baroreflex sensitivity (BRS), have been reported to be abnormal in PD patients (Kallio *et al.*, 2000; Haapaniemi *et al.*, 2001; Szili-Torok *et al.*, 2001). These indices have been proposed to be useful for evaluating cardiovascular autonomic function in chronic autonomic failure disorders (Vogel *et al.*, 2005; Schrezenmaier *et al.*, 2007) but the efficacy of these tests remains uncertain in PD patients without cardiovascular autonomic symptoms and OH. There have been only few studies categorising patients with PD by the presence (or lack of) of OH and the assessment of quantitative CV autonomic function measures, including baroreflex sensitivity (BRS), blood pressure recovery time (BPRT), Valsalva ratio (VR) and the heart rate responses to deep breathing (HR_{DB}).

The aim of this study was to therefore investigate the usefulness of a variety of cardiovascular autonomic function indices, including BRS, BPRT, VR and HR_{DB} in PD patients without both OH and orthostatic symptoms compared with age-matched healthy

controls. We also investigated the relationship between these CV indices with disease duration, age, gender and PD motor severity.

4.1.2.2 Methods

Subjects

Patients with a confirmed diagnosis of Parkinson's disease (PD) and healthy controls were included (Chapter 3). None of them met the criteria of OH. Patients were excluded if they were unable to perform the VM or had a flat top VM response.

Clinical history

History and relevant information of all patients and participants were recorded. These include age, gender, duration of disease (time from diagnosis to testing date), dopaminergic medications and presenting symptoms. Motor severity and depression in PD were evaluated by UPDRS and Hamilton rating (HAM-D) scales, respectively.

Cardiovascular autonomic screening tests (AFT)

Cardiovascular autonomic function was assessed using Autonomic Unit protocols (Mathias *et al.*, 2013a). This included all pressor tests, Valsalva manoeuvre (VM), deep breathing, Hyperventilation, 10-minute 60 degrees HUT as described in Chapter 3. Beat-to-beat blood pressure and heart rate were recorded continuously on-line using digital photoplethysmography and electrocardiography, respectively. These variables were also recorded intermittently using upper arm sphygmomanometry. Beat-to-beat blood pressure and heart rate data were used to analyse quantitative cardiovascular autonomic function indexes including BP responses to VM, blood pressure recovery time (BPRT), Valsalva ratio (VR) and HR responses to deep breathing (HR_{DB}) off-line.

Quantitative cardiovascular autonomic function indexes

Phase 2 early (phase II_E), phase 2 late (phase II_L), phase 4 (phase IV), the Valsalva ratio (VR) and BPRT were determined from the VM as previously described (Vogel *et al.*, 2005). BRS was calculated from the slope of the linear regression between the length of the RR interval and preceding SBP during phase 2 of the VM (Goldstein *et al.*, 1982). The heart rate response during deep breathing (HR_{DB}) was measured by the mean of the heart rate change (maximum-minimum) during 6 breath cycles.

Statistical methods

Data are presented as mean (± 1 SD). Mann-Whitney U tests were used to compare between the 2 groups for non-normally distributed data and unpaired t-tests for normally distributed data. Chi-square analyses were used for analysis of categorical variables. The relationships between quantitative autonomic measures including BRS, BPRT, VR and (HR_{DB}) and disease duration were assessed by regression analysis. Quantitative autonomic measures were set as the outcome variables while other variables as covariates in univariate analysis. Multiple linear regression was performed to assess the relationship between autonomic measures and age, gender and disease severity in PD patients. Assumptions for the regression analyses were checked by the analyses of residuals. Statistical analyses were carried out using STATA 11.0 (STATA Corporation, College station, Texas, USA). All tests were 2-sided and a p value of $\leq$0.05 was considered significant.

4.1.2.3 Results

22 PD patients and 10 age-matched healthy controls were included in the analyses. Participants' demographic data are presented in Table 4.6. PD patients had a mean UPDRS part III of 14.2$\pm$6.4 and Hoehn and Yahr (HY) staging ranged from 1-3 (I: II: III, 8:13:1). The mean age at PD onset was 56$\pm$7 years. The majority of patients presented with bradykinesia (11/22; 50%), followed by tremor (8/22; 36%) and gait unsteadiness (3/22; 14%).

Table 4.6. Participant demographic data

Variable	PD (n=22)	Control (n=10)
% Male	68	40
Age at Testing, yrs	63$\pm$8	58$\pm$9
Disease duration, yrs	6$\pm$4	-
HY stage	1.7$\pm$0.6	-
Values are mean$\pm$SD HY=Hoehn and Yahr		

There was no difference in the group mean supine BP, Head-Up Tilt BP and orthostatic changes between controls and PD patients (p>0.05; see Table 4.7). None of the PD patients reported orthostatic symptoms during the 10-minute HUT.

Table 4.7. Systolic and diastolic blood pressure (SBP, DBP; mmHg) and heart rate (HR; bpm) during supine and head-up tilt and orthostatic SBP changes in PD and control groups.

Variable	PD	Control
Supine SBP	130±17	129 ±18
Supine DBP	75±12	71±10
Supine HR	66±12	64±9
Tilt SBP	123±17	126±15
Tilt DBP	75±11	77±9
Tilt HR	74±12	73±9
Orthostatic ∆SBP	-7±7	-3±11
Orthostatic ∆DBP	-1±7	-6±7
Orthostatic ∆HR	8±7	9±4
Values are mean±SD		

Baseline SBP before VM was not different between patients with PD and controls (p=0.43) but the BP change during phase 2 early of the VM was significantly greater in the PD group (p=0.029) and the BP changes during phase 2 late (p=0.037) and phase 4 (p<0.01) were significantly lower in the PD group compared to controls (see Table 4.8).

Table 4.8. Blood pressure changes (mmHg) during the various phases of the Valsalva manoeuvre in PD and control groups.

Variable	PD	Control
Baseline SBP	**121±22**	**115±19**
∆SBP Phase II_E	**36±23$^{\#}$**	**20±15**
∆SBP Phase II_L	**16±10$^{\#}$**	**31±19**
∆SBP Phase IV	**23±13$^{\$}$**	**46±19**
Values are mean±SD, SBP=Systolic BP, Phase II_E= Phase 2 early of VM, Phase II_L= Phase 2 late of VM, Phase IV=Phase 4 of VM $^{\$}$p<0.01 vs. Control , $^{\#}$p<0.05 vs. Control		

There was no difference in baroreflex sensitivity (BRS) in PD patients compared with controls (p=0.11). BPRT was significantly prolonged in PD compared with controls (p<0.01). There was no difference in VR and HR_{DB} between patients and controls (p>0.05; see Table 4.9).

Table 4.9. Cardiovascular autonomic parameters during the Valsalva manoeuvre; Baroreflex sensitivity, Blood pressure recovery time, Valsalva ratio and Heart rate response during Deep breathing; in PD and control groups.

Variable	PD	Control
BRS, ms/mmHg	**3.6±2.8**	**4.6±2.0**
BPRT, sec	**3.0±3.3#**	**0.7±0.5**
VR	**1.6±0.3**	**1.7±0.3**
HR_{DB} (bpm)	**11±5**	**15±6**

Values are mean+SD, BRS= baroreflex sensitivity, BPRT=Blood pressure recovery time, VR=Valsalva Ratio, HRDB = Heart rate response during Deep Breathing

# p<0.01 vs. Control

Autonomic function parameters, including orthostatic BP changes during HUT, BRS, VR and HR_{DB} were not correlated with disease duration in PD patients during the regression analysis. BPRT was associated with UPDRS motor score (p=0.02), age at testing (p=0.04), disease duration (p<0.01) and HY stage (p<0.01) in the univariate regression analysis. The HY stage was selected instead of UPDRS III to avoid colinearity. Multiple linear regression analysis showed that only disease duration was still significantly correlated with BPRT after adjusting for age, gender and HY scores (p=0.05; Table 4.10 and Figure 4.1).

Table 4.10. Multiple regression analysis of the correlation between BPRT and disease duration (R^2=0.44, p=0.05)

Variable	Parameter estimate	Standard error	P-value
Disease duration	0.41	0.2	0.05
Age	0.05	0.09	0.62
HY	1.26	1.34	0.36
Gender	-0.84	1.34	0.54

HY=Hoehn and Yahr

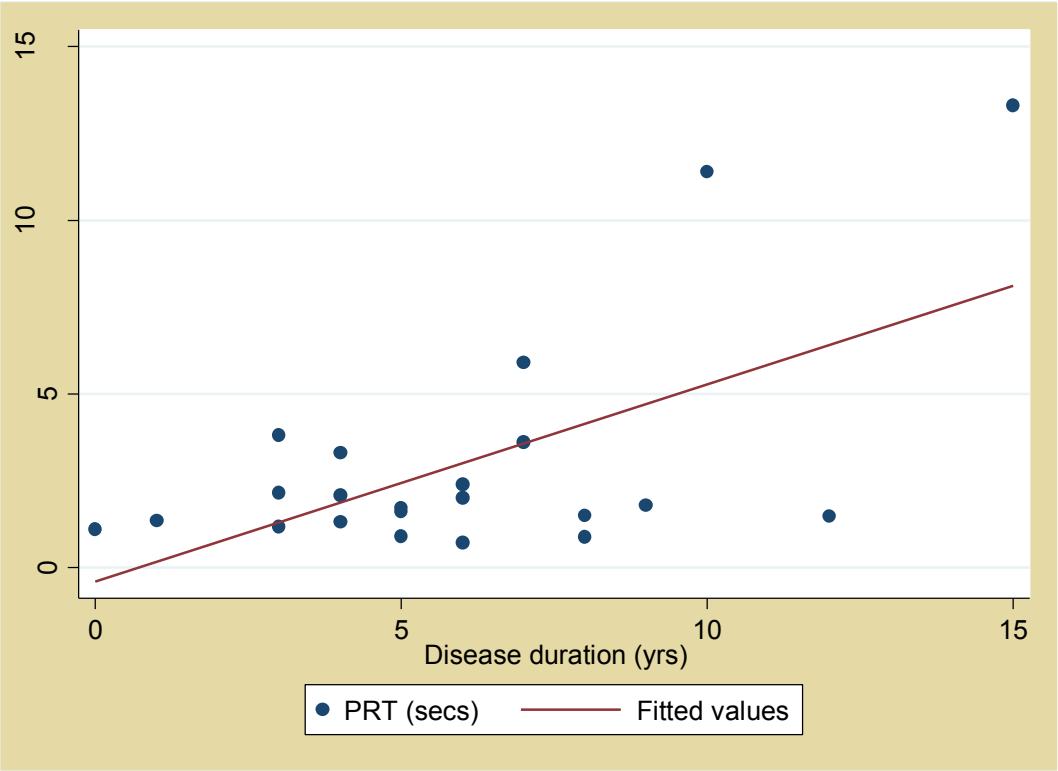


Figure 4.1. Scatterplot showing the correlation between blood pressure recovery time (BPRT; Y axis) and disease duration (X axis; R=0.60, p<0.01) in patients with PD.

4.1.2.4 Discussion

The main finding of this study was a significant prolonged blood pressure recovery time (BPRT) after the Valsalva Manoeuvre (VM) in PD patients without autonomic failure (orthostatic hypotension) compared to age-matched controls suggesting cardiovascular autonomic dysfunction may be present in PD patients even without OH. It was also demonstrated that there was a strong relationship between PD disease duration and BPRT even after taking age and motor dysfunction severity (HY stage) into account.

The mechanism of BP changes during the VM is complex but can provide useful information on the integrity of cardiovascular autonomic function. The BP and heart rate responses can broadly be divided into four phases during the VM. Phase I and III are mechanical phases, which are influenced by the performance of the manoeuvre. The BP fall during phase II early is a result of an abrupt reduction of venous return, stroke volume and reduced cardiac output (CO) as a consequence BP continues to fall despite an increase in heart rate due to a withdrawal of cardiovagal influence. A fall of BP is arrested within 4

seconds after sympathetic discharge (Delius *et al.*, 1972). Phase II early is then followed by a baroreflex mediated increase in total peripheral vascular resistance resulting from sympathetic discharge to muscle. Subsequently, BP transiently increases (1-2 seconds) during phase II late prior to phase III. During phase IV, there is a overshoot of BP at the end of the VM, as a result of the increased venous return (Wexler *et al.*, 1968) and CO (Brooker *et al.*, 1974) back to pre VM baseline while the systemic blood vessels remain vasoconstricted.

Given that BP recovery during phase II late specifically represents a sympathetic-mediated vasoconstrictor response (Vogel *et al.*, 2005) and Phase IV is influenced by the combination of sympathetic vasoconstrictor function and cardiac sympathetic function (Sandroni *et al.*, 1991), the significantly reduced BP changes (e.g., increases) in phase II late and phase IV, as well as the prolonged BPRT in the PD patients in the present study suggests that there may be subtle abnormalities in the sympathetic vasoconstrictor function in PD without classic autonomic failure and that BPRT can detect subtle sympathetic dysfunction before orthostatic hypotension becomes evident during HUT. Cardiac sympathetic dysfunction, as in abnormal cardiac MIBG uptake, is often reported in de novo PD patients (Oka *et al.*, 2011). The findings of this study implicate that there may be post-ganglionic sympathetic dysfunction in other vascular beds in addition to the heart even in the early stages of PD (Oka *et al.*, 2006).

Pathology in the central autonomic network has been proposed to be involved in cardiovascular autonomic dysfunction in PD. Indeed, the dorsal motor nucleus of the vagus nerve was found to be abnormal even in the early stages of PD and purportedly progresses to the olfactory nucleus, pontine tegmental nuclei and the locus coeruleus and ventrolateral area of the medulla (Braak *et al.*, 2003). Since the ventrolateral medulla is part of the baroreflex pathway, it could be hypothesized that BRS could be abnormal in patients with PD. However, no significant difference in BRS in PD compared to controls was evident in this study. These findings are similar to previous reports (Barbic *et al.*, 2007; Friedrich *et al.*, 2010), even though the BRS in those studies was derived from a different technique; spectral analysis. Nevertheless, there are some studies that have reporting an abnormal BRS in PD (Szili-Torok *et al.*, 2001). These inconsistent results suggest that BRS is not universally abnormal in PD. The impairment of BRS occurs commonly in patients with PD+OH (Barbic *et al.*, 2007)(see Chapter 4.1.1), whereas our results suggest that BRS is relatively normal in PD without OH compared with age-matched controls. These findings are in agreement with a proposed hypothesis that the occurrence of PD+AF depends on 3

different parts of the autonomic pathways, namely the arterial baroreflex, cardiac- and extra-cardiac sympathetic function (demonstrated by a reduction of plasma noradrenaline) (Jain & Goldstein, 2012).

Orthostatic BP changes were not different in patients with PD compared to age-matched controls. This result indicates that these hemodynamic responses during HUT cannot be used as cardiovascular autonomic function markers to distinguish PD from the general population. Despite this finding from HUT, BPRT were still found to be significantly abnormal in PD. This finding suggests that changes in BPRT are not entirely reflected in HUT BP data. The mechanism of BPRT may be different from orthostatic BP responses to HUT. Although the BRPT reflects a combination of sympathetic vasoconstrictor and cardiac sympathetic function, the BP response to orthostatic challenge using HUT involves a much greater number of neural, hemodynamic and hormonal mechanisms in the BP response, any of which could compensate for any mechanism that might be impaired, e.g., the unremarkable BP changes during HUT may be a reflection of redundancy in other BP maintaining mechanisms in PD patients. In addition, the grading of orthostatic changes could be another factor that contributes to the difference between OH and BPRT findings. OH is quite a severe feature to detect, e.g., a decrease of 20 mm Hg SBP will represent a large deficit in CV autonomic function, whereas 5-10 mm Hg suggests a subtle defect.

In the present study, there were also no differences in other cardiovascular autonomic parameters, including VR and HR_{DB}. These findings are in line with previous studies (Gross *et al.*, 1972; Mesec *et al.*, 1999; Schmidt *et al.*, 2009b). As these are measurements for cardiac parasympathetic function, these normal results may suggest either PD with no OH patients actually have an intact parasympathetic function or these parameters may not be sensitive enough to detect subtle abnormalities within this autonomic pathway. Nonetheless, there are a few studies that have reported an impairment of cardiac parasympathetic activity derived from heart rate variability in PD patients (Shibata *et al.*, 2009). A recent pathological study reported Lewy body deposition in the sinoatrial node ganglion (Okada *et al.*, 2004), which may explain why only some patients with PD have impaired baroreflex function as part of their disease progression.

The current study also described an association between BPRT and disease duration in PD patients after controlling for age and motor dysfunction severity (HY stage). Apart from BPRT, disease duration was not associated with any other cardiovascular autonomic function index. There have been only a few studies that have specifically examined the relationship between cardiovascular autonomic dysfunction and disease duration/severity in

PD. One study reported a greater degree of OH and a reduction of HR_{DB} and VR in PD patients after a 3 year-follow up (Mesec *et al.*, 1999), while other cross-sectional studies consistently found only an association between increasing severity of OH and disease duration and age (van Dijk *et al.*, 1993; Linden *et al.*, 1997; Wullner *et al.*, 2007). These findings implicate that age and disease duration are likely to play an important part in the progression of cardiovascular autonomic dysfunction, particularly OH, in PD and the relationship between cardiovascular autonomic dysfunction and disease duration is complex and may not simply progress in a homogeneous pattern in individual patients with PD, similarly to what was evident in the present study. Larger longitudinal studies are needed to confirm whether BPRT is a useful marker to monitor CV autonomic dysfunction progression in PD patients.

Comparing the BPRT and BRS results between PD (this chapter) and PD+AF patients (Chapter 4.1.1), there was no difference in BRS between these two groups ($p=0.13$) but there was a significantly prolonged BRPT in PD+AF compared to PD ($p<0.01$). Multiple linear regression analysis controlling for age and gender also demonstrated a significant association between BPRT and disease duration only with patients with PD but not in PD+AF. This finding suggests that BPRT progresses with disease duration in early PD and then plateaus when patients develop OH (e.g., PD+AF) in the later stages of disease.

In conclusion, a significantly prolonged BPRT indicates early cardiovascular autonomic dysfunction in PD patients before potentially widespread autonomic failure may develop. Our findings suggest that BPRT is a useful marker in addition to the orthostatic challenge test and should be part of autonomic investigations even in PD without CV autonomic dysfunction symptoms.

4.1.3 Pressor tests in patients with chronic autonomic failure

4.1.3.1 Introduction

Pressor tests, that include isometric handgrip exercise (IE), cold pressor (CP) and mental arithmetic (MA) stressors, are routinely performed as part of standard autonomic function screening tests (AFT) (Mathias *et al.*, 2013b). These tests are measures of sympathetic nerve function and are commonly impaired in patients with autonomic dysfunction/failure.

The IE, MA and CP manoeuvres typically increase heart rate and stroke volume and thereby cardiac output, as well as total peripheral resistance in some manoeuvres, and blood pressure as a result of sympathetic activation (Ludbrook *et al.*, 1975; Rowell & O'Leary, 1990; Nobrega *et al.*, 1997). Although these 3 pressor tests produce relatively similar haemodynamic responses, each test involves different afferent pathways. Previous studies suggested that IE causes an increase in BP and HR (Lind *et al.*, 1964) and this phenomenon was caused by the combination of central command and the peripheral activation of the exercise pressor reflex (Winchester *et al.*, 2000). An increase in cardiac output is followed by an increase in peripheral vascular resistance (Mark *et al.*, 1985). In contrast to IE, cardiovascular responses during MA are predominantly activated by central pathways. During MA, there is an increase in sympathetic outflow as a consequence of the activation of the central autonomic network, such as the right insular cortex and right anterior cingulate areas (Critchley *et al.*, 2000). Furthermore, the cardiovascular responses are mainly a result of increased cardiac output without an increase in total peripheral resistance (Hjemdahl *et al.*, 1984). The cold pressor test (CP), evoked by painful stimuli, transmits signals from temperature nociceptors and unmyelinated C-fibers and the A∂ fibers. Subsequently, these signals are integrated at the hypothalamus and vasomotor centres in the medulla causing an increase in sympathetic outflow and a rise in HR and BP (McLeod & Tuck, 1987).

Despite differences in the autonomic lesion between PD (pre- and post-ganglionic) and MSA (pre-ganglionic) patients with autonomic failure, these patients often share several autonomic abnormalities, such as sympathetic and parasympathetic dysfunction during autonomic function tests. Most studies have compared patients with PD and MSA regardless of the integrity of their cardiovascular autonomic function (e.g. PD or MSA patients with and without autonomic dysfunction/failure have been grouped together). There have been no studies specifically looking at the cardiovascular responses to pressor tests in PD with

autonomic failure (PD+AF) and MSA with autonomic failure (MSA+AF); 2 patient groups where the overlapping autonomic features are most evident and can cause the most difficulty in differentiating a correct diagnosis.

The aim of this study was to therefore specifically assess the BP and HR responses to pressor tests in PD patients with (PD+AF) and without autonomic failure (PD) and MSA patients with autonomic failure, as well as comparing these groups with healthy controls in order to see whether there is any difference in responses between disorders.

4.1.3.2 Methods

Subjects

Patients with a confirmed diagnosis of Parkinson's disease with (PD+AF) and without autonomic failure (PD) and Probable Multiple System Atrophy (MSA) with orthostatic hypotension and healthy controls were included (Chapter 3).

Test protocol

The detailed protocols of all pressor tests are described in Chapter 3. Briefly, BP and HR were taken before and at the end of each pressor test. The tests included 1) cold pressor test performed by applying an ice pack to a participant's wrist for 90 seconds. 2) Handgrip isometric exercise performed by voluntarily squeezing a partially inflated sphygmomanometer cuff and maintaining the contraction at 33% of maximum effort for 3 minutes. 3) Mental arithmetic test performed by serial subtraction for 2 minutes. After each pressor test, BP and HR were allowed to return to baseline levels before performing the next pressor test. A 10-minute 60 degree head up tilting test (HUT) was also performed after the pressor tests.

Statistical analyses

Data are presented as mean (± 1 SD) and median (interquartile range; IQR) as appropriate. Independent t-tests or ANOVA were used to compare BP and heart rate responses between groups. BP and heart rate responses before and after each pressor test as well as during supine and head-up tilting (HUT) were compared between groups using mixed models repeated measures ANOVA. If there was a significant main effect of time or group, post hoc analyses were performed using Bonferroni corrections for multiple comparisons. All data were analysed using commercial available software (STATA 11.0,

STATA Corporation, College station, Texas, USA). All tests were 2-sided and a p value of ≤0.05 was considered significant.

4.1.3.3 Results

Demographic data

101 patients (48 MSA, 22 PD+AF, 31 PD) and 10 healthy controls were included in the analyses. Patients with MSA, PD+AF and PD were significantly older than controls ($p<0.05$) but the disease duration was not different among patient groups ($p=0.11$). Dopaminergic treatment was more commonly used in patients with PD and PD+AF compared to MSA (MSA vs. PD+AF; $p<0.01$ and MSA vs. PD; $p<0.01$). There was no difference in the number of patients on dopaminergic medication among PD and PD+AF ($p=0.07$; Table 4.11).

Table 4.11. Patient demographic data

Variable	MSA	PD+AF	PD	Control
Number	48	22	31	10
% Male	50	59	68	40
Age at testing, yrs	62±8*£	69±8*	63±9*£	57±8
Disease duration Median (IQR), yrs	5 (3-6)	6 (4-10)	7 (4-10)	-
% Dopaminergic treatment	42£$	82	97	-
Values are mean±SD unless stated *p<0.05 vs. control, $p<0.05 vs. PD, £p<0.05 vs. PD+AF				

Cardiovascular responses to HUT

Supine SBP and DBP were not significantly different between MSA, PD+AF, PD patients and controls but there was a higher baseline HR in MSA patients compared with the other groups. During head-up tilting (HUT), SBP was significantly lower in patients with MSA and PD+AF compared to PD ($p<0.01$) and controls ($p<0.01$). Correspondingly, the BP changes (e.g., the decrease in BP) during HUT were significantly greater in MSA and PD+AF compared to controls (MSA vs. controls; $p<0.01$ and PD+AF vs. control; $p=0.01$).

In PD, there was a significant SBP fall with HR rise during HUT (both p<0.01) but not DBP (p=0.43) compared to corresponding supine values. In contrast, only a significant HR was significantly increased on HUT compared to supine (p<0.01), but there were no differences in SBP and DBP in healthy controls (both p>0.05). There was also no difference in the changes in BP and HR during HUT between PD and controls (both p>0.05). SBP and DBP during HUT were not different between MSA and PD+AF (p=0.35 and p=0.9, respectively) but HR was significantly higher in MSA during HUT (Table 4.12).

Table 4.12. Blood pressure and HR during supine, head-up tilting and orthostatic changes in PD, PD+AF, MSA patients and controls.

Variable	MSA	PD+AF	PD	Control
Supine				
Supine SBP	134±21	136±20	129±17	129±17
Supine DBP	80±12	75±11	73±11	71±7
Supine HR	78±10*	69±10$^{\#}$	66±11$^{\#}$	61±7
Tilt SBP	88±20*$^{\$\phi}$	93±23*$^{\$\phi}$	122±18$^{\phi}$	125±15
Tilt DBP	59±13**$^{\$\phi}$	60±15**$^{\$\phi}$	74±10	77±9
Tilt HR	87±12*$^{\phi}$	78±9$^{\#\phi}$	74±11$^{\#\phi}$	71±8$^{\phi}$
Orthostatic ΔSBP	-46±20*$^{\$}$	-42±16*$^{\$}$	-7±6	-4±9
Orthostatic ΔDBP	-21±12*$^{\$}$	-15±12*$^{\$}$	-1±7	5±5
Orthostatic ΔHR	10±8	8±6	8±6	10±3

Values are mean±SD
*p<0.05 vs. control, $^{\#}$p<0.05 vs. MSA, $^{\$}$p<0.05 vs. PD
$^{\phi}$p<0.05 vs. corresponding supine value

Cardiovascular responses to pressor tests

There was a higher baseline SBP, DBP in patients with MSA compared with PD (p<0.01) and controls (p=0.03). Baseline SBP and DBP were not different between MSA and PD+AF patients (p=0.25), as well as between PD and controls (p=0.82). During IE, SBP, DBP and HR significantly rose from baseline in PD+AF, PD and controls (p<0.05) but not in

MSA who only had a significant rise in HR (SBP pre- vs. post-IE; p=0.41, DBP pre- vs. post-IE; p=0.06; HR pre- vs. post-IE; p<0.01).

There were a significant increase in SBP and DBP during MA in PD+AF, PD and controls (p<0.05) but not in MSA patients (p>0.05). HR significantly increased during MA in PD+AF, PD, MSA patients and controls (p<0.01).

During CP, BP and HR significantly rose from baseline in MSA and PD+AF (p<0.01), whereas there were an increase in SBP and DBP (both p<0.01) without a significant increase in heart rate (p=0.28) in controls. In PD, there was only an increase in SBP (p<0.01) without changes in DBP and HR (both p>0.05) during CP (Table 4.13).

Table 4.13. Blood pressure and HR responses to isometric exercise, mental arithmetic and cold pressor test in PD, PD+AF, MSA patients and controls

Variable	MSA	PD+AF	PD	Control
Isometric exercise (IE)				
Pre				
SBP	141±24*	134±17	125±17$^{\#}$	123±18
DBP	80±13*	76±11	71±11$^{\#}$	69±10
HR	73±10	62±9$^{\#}$	67±12	64±8
Post				
SBP	142±23	143±18$^{\phi}$	147±19$^{\phi}$	155±8$^{\phi}$
DBP	82±12$^{\phi}$	80±12$^{\phi}$	81±12$^{\phi}$	85±9$^{\phi}$
HR	79±10$^{\phi}$	69±10$^{\#\phi}$	76±12$^{\phi}$	70±10$^{\phi}$
Mental arithmetic (MA)				
Pre				
SBP	139±23	136±16	125±15$^{\#}$	127±17
DBP	81±12	76±11	72±12$^{\#}$	71±10
HR	73±9	66±9	67±13	62±8$^{\#}$
Post				
SBP	138±21	143±19$^{\phi}$	139±17$^{\phi}$	140±20$^{\phi}$
DBP	79±11	79±11$^{\phi}$	77±12$^{\phi}$	79±9$^{\phi}$
HR	78±9*$^{\phi}$	70±10$^{\#\phi}$	74±13$^{\phi}$	68±6$^{\phi}$
Cold pressor test (CP)				
Pre				
SBP	142±24	138±23	127±17$^{\#}$	129±21
DBP	81±13	76±11	72±11$^{\#}$	71±8
HR	74±9*	66±8$^{\#}$	67±12$^{\#}$	61±9
Post				
SBP	150±25$^{\$\phi}$	148±23$^{\phi}$	137±17$^{\phi}$	148±26$^{\phi}$
DBP	85±13$^{\phi}$	79±13$^{\phi}$	79±11	82±7$^{\phi}$
HR	77±10*$^{\phi}$	68±8$^{\#\phi}$	68±13$^{\#}$	63±9

Values are mean±SD, *p<0.05 vs. control, $^{\#}$p<0.05 vs. MSA, $^{\$}$p<0.05 vs. PD
$^{\phi}$p<0.05 vs. corresponding baseline value

With regards to BP changes from baseline during IE, MSA and PD+AF had significantly smaller BP changes when compared with PD and controls (Table 4.14). There was no difference in BP and HR changes between MSA and PD+AF (SBP; p=0.09, DBP;

p=0.32 and HR; p=0.29) and patients with PD had no difference in BP and HR changes to IE compared to healthy controls (SBP; p=0.19, DBP; p=0.13 and HR; p=0.5). HR changes were not different among groups (p>0.05).

The SBP and DBP changes in MSA were significantly less than those in PD+AF, PD and controls during MA (MSA vs. controls; p<0.01, MSA vs. PD+AF; p=0.01 and MSA vs. PD; p<0.01). PD had a greater change during MA compared to PD+AF (p<0.01) but no difference when compared to controls (p=0.70). There was no difference in HR changes among groups during MA (p>0.05). SBP changes during CP were significantly lower in MSA, PD+AF and PD compared to control but there was no difference among patient groups (p>0.05; Figures 4.2-4.4).

Table 4.14. Blood pressure and HR changes to isometric exercise, mental arithmetic and cold pressor test in PD, PD+AF, MSA patients and controls

Variable	MSA	PD+AF	PD	Control
Isometric exercise				
ΔSBP	1±10**$$	9±14**$$	23±14	32±19
ΔDBP	2±7**$$	4±7**$	10±12	16±9
ΔHR	6±5	7±4	9±6	6±11
Mental arithmetic				
ΔSBP	-1±7**$$	6±11##$$	15±10	13±11
ΔDBP	-2±6**	3±6##	5±6##	8±4
ΔHR	4±5	4±4	7±6	6±5
Cold pressor				
ΔSBP	8±9**	9±8*	11±9*	20±11
ΔDBP	4±7	4±5	6±8	10±6
ΔHR	3±5	2±3	1±8	2±5

Values are mean±SD, * p<0.05 vs. control, ** p<0.01 vs. control, # p<0.05 vs. MSA, ## p<0.01 vs. MSA, $p<0.05 vs. PD, $$p<0.01 vs. PD, £p<0.05 vs. PD+AF, ££p<0.01 vs. PD+AF

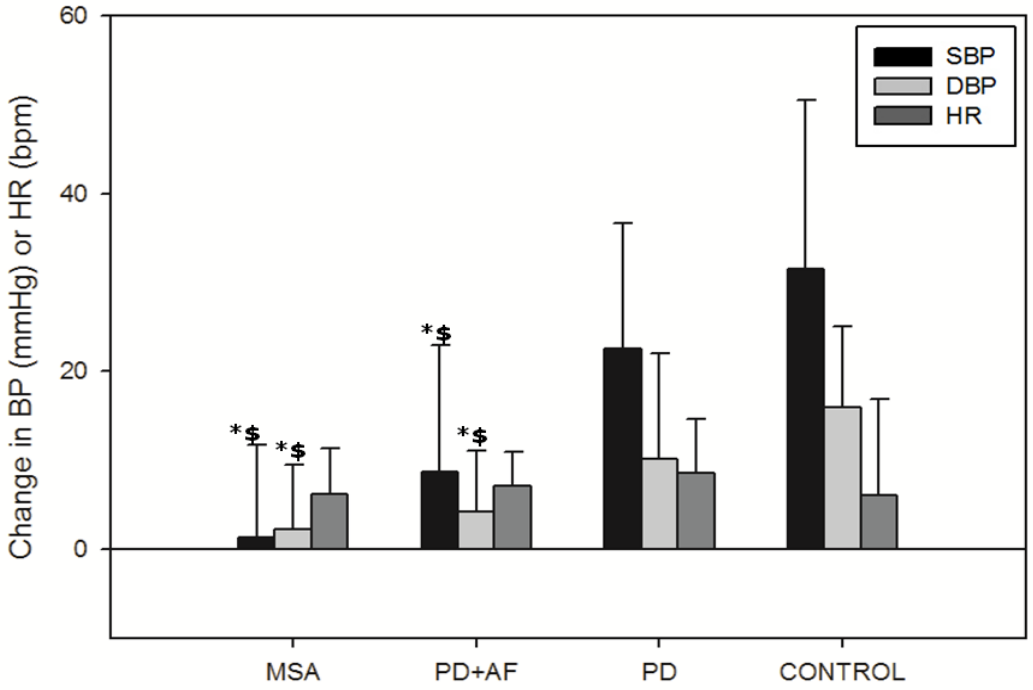


Figure 4.2. Blood pressure and HR changes to isometric exercise (IE) in MSA, PD+AF, PD patients and controls; *p<0.05 vs. control, $p<0.05 vs. PD

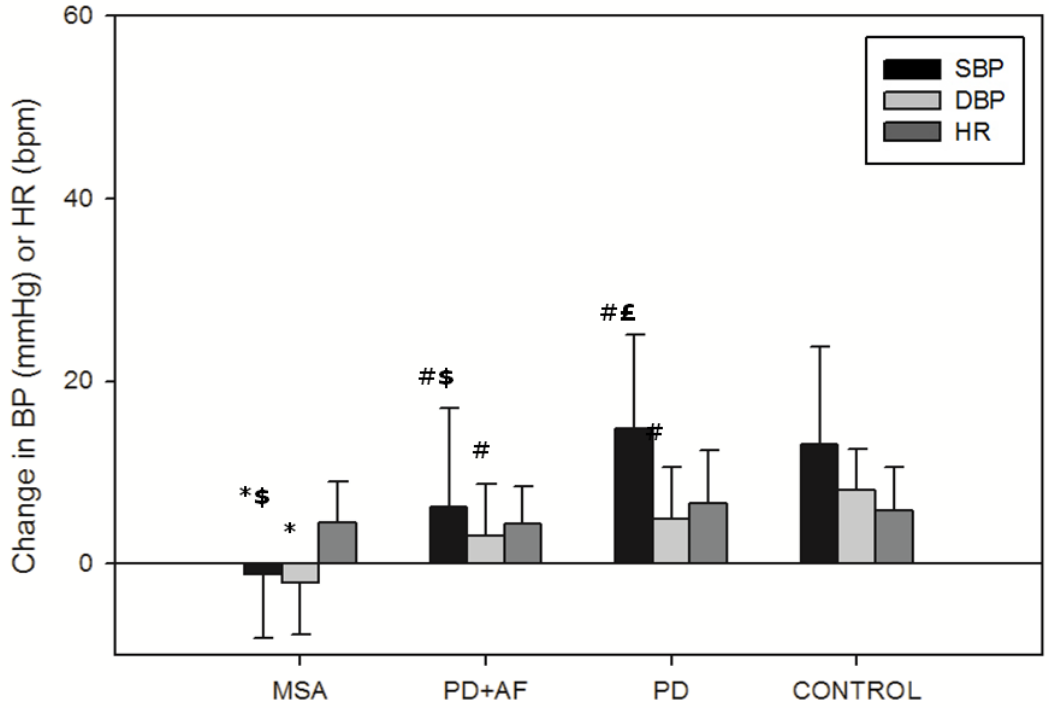


Figure 4.3. Blood pressure and HR changes to mental arithmetic (MA) test in MSA, PD+AF, PD patients and controls; *p<0.05 vs. control, $p<0.05 vs. PD, #p<0.05 vs. MSA, £p<0.05 vs. PD+AF.

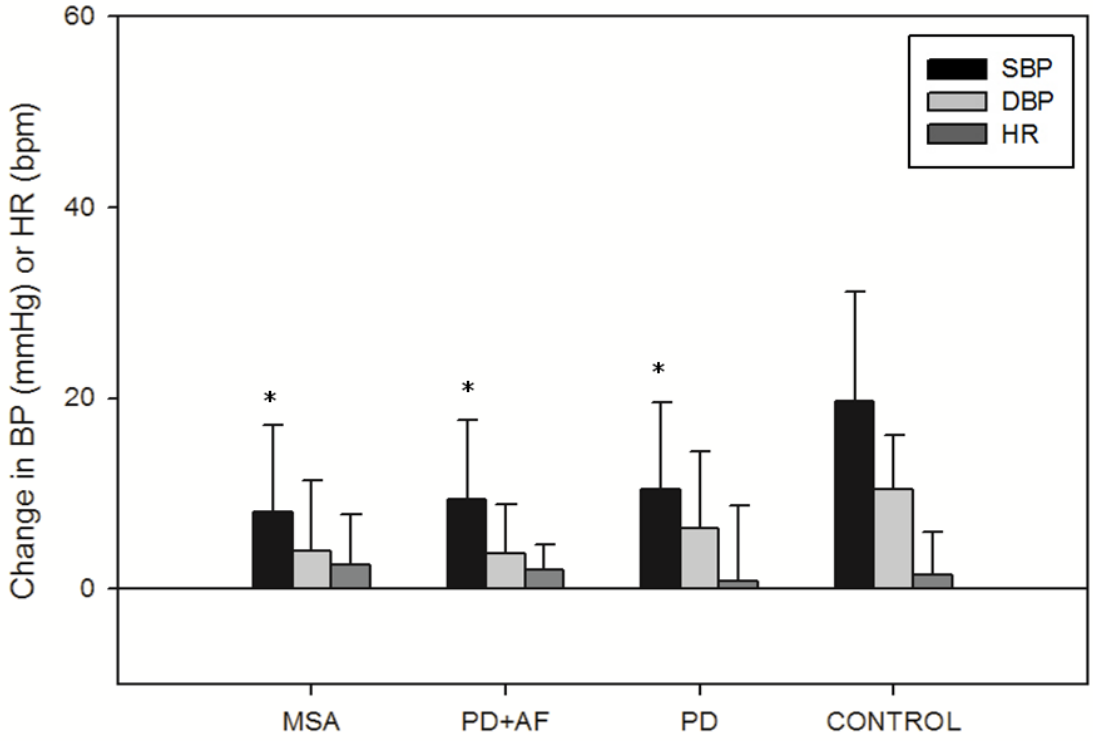


Figure 4.4. Blood pressure and HR changes to cold pressor (CP) test in MSA, PD+AF, PD patients and controls; *p<0.05 vs. control.

4.1.3.4 Discussion

The aim of this study was to compare the BP and HR responses to pressor stimuli in MSA and PD with and without autonomic failure and healthy controls using a variety of pressor tests alongside the cardiovascular responses to HUT. The main findings in this study were that despite similar degrees of orthostatic BP changes during HUT in MSA and PD+AF groups and in PD and control groups, there were differential BP and HR responses to pressor stimuli in different groups of patients. Patients with autonomic failure (MSA and PD+AF) had significantly smaller BP changes when compared with PD patients and healthy controls whilst performing isometric handgrip exercise (IE). BP responses to Cold pressor test (CP) were significantly less in all patient groups when compared with controls. BP responses were significantly attenuated in MSA and PD+AF when compared with PD patients and healthy controls during mental arithmetic test (MA) but were lower still in MSA vs. PD+AF.

There have been only few studies that have used pressor tests to investigate patients with chronic autonomic failure. Previous studies reported a reduced BP response to CP in MSA patients compared to controls (De Marinis *et al.*, 2000; Kimber *et al.*, 2000). Another study reported no difference in BP responses to MA between MSA and PAF. To our knowledge, BP and HR responses to pressor tests have not been directly compared in PD, PD+AF and MSA, who often share features of orthostatic hypotension.

Similar BP responses in MSA and PD+AF to IE were evident in the current study. This may be explained by the complex pathways involved in IE as the responses are the summation of both central and peripheral (reflex) inputs. Pathological lesions anywhere within these two pathways can result in abnormal hemodynamic responses to IE. Comparable blood pressure rises during IE between MSA and PD+AF indicate that this test may not be suitable to differentiate between these conditions particularly when orthostatic hypotension is already present.

BP responses to the cold pressor test were significantly attenuated in patients with MSA, PD+AF and PD compared with healthy controls. Given that none of our participants had problems with neuropathy and the noxious stimuli is mainly integrated in the vasomotor centers in the medulla (McLeod & Tuck, 1987), the abnormal BP and HR responses during the CP are more likely to be caused by the pathological involvement within this area which has been reported in patients with autonomic failure (Benarroch *et al.*, 2005). The decrement in the BP response to CP in MSA compared to controls is consistent with previous studies (De Marinis *et al.*, 2000; Kimber *et al.*, 2000) as well as those in PD+AF and PD. Although there was no difference in BP responses between MSA, PD+AF and PD patients, an interesting result was that patients with PD had significantly blunted BP responses compared with healthy controls during CP. This finding may indicate that a subtle sympathetic abnormality is already present in patients with PD even if they do not have orthostatic hypotension, similar to the BPRT findings in the previous study. The similar responses between the MSA, PD+AF groups compared to PD indicate that, however, the CP test cannot reveal differences between varying levels of autonomic dysfunction/failure.

Cardiovascular responses during MA are predominantly activated by central pathways. The blunted BP responses during MA in patients with MSA compared to PD+AF may reflect a greater involvement of central autonomic network dysfunction in MSA relative to PD+AF and PAF. Nevertheless, this hypothesis has not been previously confirmed and further research using MA in conjunction with functional MRI study in these patients may provide important insight in answering this question.

The results also showed that there was no difference in blood pressure and heart rate responses during orthostasis in patients with MSA compared to PD+AF. These findings are consistent with the majority of previous studies (Lipp *et al.*, 2009). Although SBP was siginiciantly fall during HUT in PD, there was no statistical difference in SBP, DBP and HR responses in patients with PD and controls. Prior studies reported significant changes in BP in patients with PD compared to controls (Gross *et al.*, 1972). This conflicting result may be explained by differences in PD disease stage and PD medications between studies. These factors were reported as contributing factors to cardiovascular autonomic dysfunction in patients with PD (van Dijk *et al.*, 1993; Mesec *et al.*, 1999). These inconsistent findings also indicate that orthostatic BP changes during HUT may not be a reliable test to differentiate PD from the age-matched healthy population.

In conclusion, this study demonstrated that patients with PD+AF and MSA, who had similar degree of orthostatic hypotension during HUT, can have different BP responses during pressor tests, namely mental arithmetic testing. These differences may help distinguish between patients with PD and MSA who present with orthostatic hypotension. Moreover, the BP response to cold pressor test was also blunted in patients with PD even though there was no orthostatic hypotension. This finding would support the idea that sympathetic dysfunction is subtly present in PD patients even in those without orthostatic hypotension.

4.1.4 24 hour-ambulatory blood pressure and heart rate monitoring (24 hr-ABPM) in patients with chronic autonomic failure

4.1.4.1 Introduction

24 hour-ambulatory blood pressure and heart rate monitoring (24 hr-ABPM) is widely used in patients with blood pressure problems, particularly in those with high blood pressure (hypertension). A number of studies have demonstrated the advantage of using this equipment to detect and follow-up patients with hypertension (Ohkubo *et al.*, 2005; Rodriguez-Roca *et al.*, 2006; Franklin *et al.*, 2012). More recently, 24 hr-ABPM has also been utilized as a screening test for patients with orthostatic hypotension in conjunction with laboratory autonomic tests. Furthermore, 24 hr-ABPM offers information not only on the average daytime blood pressure (BP) but also circadian BP revealing key information on the daytime and night-time profiles. In normal subjects, BP is normally lower during night-time which has been described as the physiological or normal pattern of nocturnal blood pressure 'dipping'. This pattern can be absent or reversed (BP night-time>BP daytime) in patients with autonomic failure. These patients tend to have lower BP during the day (due to the repeated bouts of OH) and a loss of BP dipping or even a higher BP at night, also influenced by supine hypertension, a common occurrence in autonomic failure (Goldstein *et al.*, 2003b). According to the latest guidelines from the European Society of Hypertension and the European Society of Cardiology for the management of hypertension, those who have a normal BP reduction at night (BP decrease >10%) are classified as dippers while those with a nocturnal fall of <10% BP are classified as non-dippers. Those patients with a BP increase during the night are grouped as reverse dippers, which are more likely to happen in chronic autonomic failure (O'Brien *et al.*, 2013).

24 hr-ABPM is commonly reported as abnormal in patients with PD regardless of their underlying autonomic function (Schmidt *et al.*, 2009a). The prevalence of non-dippers in PD ranges from 48 to 95% (Senard *et al.*, 1992; Ejaz *et al.*, 2006; Schmidt *et al.*, 2009a). Abnormal nocturnal circadian falls in blood pressure occurred more often in PD+AF compared to PD suggesting that there is a link of abnormal BP circadian rhythms and orthostatic hypotension (Senard *et al.*, 1992). More recently, 24 hr-ABPM was used among different forms of parkinsonian disorders, including Parkinson's disease (PD), Multiple system atrophy (MSA) and Progressive supranuclear palsy (PSP). The results showed that a number of these patients had a significantly higher proportion of absent nocturnal BP dipping compared to age-matched controls. These findings support the use of this equipment for

screening autonomic function in these patients groups, where the autonomic laboratory is not available (Schmidt *et al.*, 2009a).

Nevertheless, 24 hr-ABPM has never been specifically compared in patients with PD with and without autonomic failure and MSA with autonomic failure. Furthermore, the efficacy of ABPM with an autonomic protocol diary in detecting OH, compared with head-up tilt-table testing in these patient groups, has also never been evaluated. The aim of this study was therefore to 1) examine 24 hr-ABPM in patients with chronic autonomic failure (MSA+AF, PD+AF and PD) and 2) determine the effectiveness of 24 hr-ABPM compared to standard orthostatic challenge testing (10 minute Head-up Tilt) in diagnosing orthostatic hypotension (OH) in these patients.

4.1.4.2 Methods

Participants

Patients with a confirmed diagnosis of Parkinson's disease with (PD+AF) and without autonomic failure (PD) and Probable Multiple System Atrophy (MSA) with orthostatic hypotension were included (Chapter 3). Patients were selected only if cardiovascular autonomic screening tests (AFT) and 24 hr-ABPM were performed during the same admission. In order to verify the efficacy of 24 hr-ABPM in detecting OH using an autonomic protocol, only patients who completed a diary as part of the test were included in the sensitivity analyses.

Clinical history and evaluation of autonomic nervous system function

Demographic data, such as age at testing, dopaminergic medications and disease duration were also noted.

Cardiovascular autonomic screening tests (AFT)

All patients underwent screening autonomic function tests (AFT) using the Autonomic Unit protocols (Mathias *et al.*, 2013b) to ascertain whether or not patients had orthostatic hypotension (OH) and autonomic failure (AF).

24 hour-Ambulatory Blood Pressure and Heart Rate Monitoring (24 hr-ABPM)

All patients were fitted with the 24 hr-ABPM (model 90207, Spacelabs™ Medical, Redmond, Washington) after their AFT as part of their autonomic investigations. BP and HR

were recorded every 20 minutes during the day (0800-2300) and every 60 minutes during the night (2300-0800). The average BP and HR were calculated for daytime, night-time and the entire 24-hour period. Patients were asked to record their symptoms in the diary during the day (e.g. dizziness, light-headedness, blurred vision etc) as well as the position and activity (lying down, sitting, standing, walking, and exercising) at the same time. Patients were also asked to record additional BP readings in addition to the automated readings if they developed symptoms. Bedtime and wake-up time were also recorded in order to determine the period of sleep. Postural challenges were included in the diary for patients to complete during the 24 hr-ABPM monitoring. This included an orthostatic challenge using a 5-minute standing test. Patients were asked to press a recording button after 5-minute of lying down and again after 5-minute standing 4 times throughout the 24 hours. Symptoms (if experienced by patients) were also noted in the diary. BP and HR variabilities were derived from the standard deviations of the means of the specific period of times (24-hr, daytime and night-time).

Patients were classified into 3 groups: dipper (BP fall during night-time>10% compared to daytime), absent nocturnal BP fall or non-dipper (BP fall during night-time<10% compared to daytime) and reverse nocturnal BP (BP higher during night-time than daytime). Patients with an average daytime SBP >140 mmHg or DBP >90 were defined as daytime hypertensives and those with nighttime SBP >125 or DBP >75 as nighttime hypertensives according to international guidelines (Pickering *et al.*, 2005).

Statistical Analyses

Data are presented as mean (± 1 SD) or median (inter-quartile range), where appropriate. Analysis of covariance (ANCOVA) was used for comparisons between the 3 groups for normally distributed data while the Kruskal-Wallis test was used if data were non-normally distributed. If there was a significant difference, Mann-Whitney U tests were then used to compare between 2 groups for non-normally distributed data and unpaired t-tests for normally distributed data with Bonferroni corrections. Chi-square analyses were used for analysis of categorical variables.

SBP and DBP responses from the 24 hr-ABPM standing test with the greatest degree of BP reduction were compared with the BP and HR responses during HUT using a standard criteria of OH (SBP fall $\geq$20 mmHg or DBP fall $\geq$10 mmHg). Considering the BP responses during HUT as a gold standard test, sensitivity (Sn) of the 24 hr-ABPM to detect OH was defined as the proportion of patients who met the criteria of OH from a standing test

during 24 Hr monitoring and the proportion of patients who met the criteria of OH during laboratory HUT. Specificity (Sp) was defined as the proportion of patients who did not meet the criteria of OH in the standing test in 24 Hr monitoring and all patients who were correctly classified as no OH from HUT. ROC (Receiver operating characteristic) analysis was used to evaluate the sensitivity and specificity in detecting OH from 24 hr-ABPM and to calculate Sn and Sp using different BP cut-off points. In general, the optimal cut-off point would be both Sn and Sp that as close to 100% as possible. Nonetheless, it is less likely to happen as the Sn tend to vary inversely with Sp (Sn increases as Sp decreases, or vice versa). In this study, ROC curves were plotted Sn against 1-Sp and were used to determine the Sn and Sp of the 24 hr-ABPM in detecting a fall of 20-mmHg SBP and 10-mmHg DBP (according to a standard of OH diagnostic criteria). These were done alongside the calculation of the area under the curve (AUC).

The AUC is an overall summary of the diagnostic performance of the test. The perfect discrimination for AUC is 1, which means the diagnostic test can perfectly differentiate between two conditions with both Sn and Sp equalling 100%. The AUC of 0.9 or higher represents an outstanding discrimination. A value of AUC of 0.8-0.9 shows an excellent discrimination; a value of 0.7-0.8, an acceptable discrimination. An AUC of 0.5 or less indicates that the diagnostic accuracy is questionable and not different from random chance. The AUCs can be used to compare the diagnostic performance of the test; the higher value of the AUC shows better performance than the lower value. Statistical analyses were carried out using STATA 11.0 (STATA Corporation, College station, Texas, USA). All tests were 2-sided and a p value of $\leq$0.05 was considered significant.

4.1.4.3 Results

Demographic data

74 patients (23 MSA, 18 PD+AF, 33 PD) were included in the analyses. Patients with PD+AF were significantly older than PD and MSA (both $p<0.01$) but there was no difference in age between MSA and PD ($p=0.55$). There was no difference in disease duration among groups ($p=0.23$). Dopaminergic treatment was more commonly used in patients with PD and PD+AF compared to those with MSA (MSA vs. PD+AF; $p=0.01$ and MSA vs. PD; $p<0.01$). There was no difference in the number of patients on dopaminergic medication among PD and PD+AF ($p=0.53$, Table 4.15).

Table 4.15. Patient demographic data

Variable	MSA	PD+AF	PD
Number	23	18	33
% Male	48	44	67
Age at testing Mean (SD), yrs	62±9	72±7*$	64±10
Disease duration Median (IQR), yrs	4 (3-6)	7 (4-10)	6 (2-10)
% Dopaminergic treatment	39*#	78	85

Values are mean±SD unless stated, * p<0.05 vs. PD, # p<0.05 vs. PD+AF, $p<0.05 vs. MSA

Cardiovascular responses to HUT

Supine SBP and DBP were not significantly different between MSA, PD+AF, PD patients but there was a higher baseline HR in MSA patients. During head-up tilting (HUT), SBP was significantly lower in patients with MSA and PD+AF compared to PD (p<0.01). Correspondingly, BP changes during HUT were significantly higher in MSA and PD+AF when comparing with PD. There were no difference in SBP and DBP during HUT between MSA and PD+AF (p=0.35 and p=0.9, respectively) but HR was significantly higher in MSA during HUT (Table 4.16). During HUT, all patients with MSA and PD+AF fulfilled the criteria of OH, whereas none of the PD patients had OH during HUT.

Table 4.16. Blood pressure and HR during supine, head-up tilting and orthostatic changes in MSA, PD+AF and PD patients

Variable	MSA (n=17)	PD+AF (n=11)	PD (n=16)
HUT			
Supine SBP	137±18	140±21	130±14
Supine DBP	81±11	75±15	75±10
Supine HR	77±9	69±4$^{\$}$	69±10$^{\$}$
Tilt SBP	95±19*	101±24*	126±16
Tilt DBP	60±13*	63±17*	76±10
Tilt HR	85±13	77±6$^{\$}$	76±11$^{\$}$
Orthostatic ΔSBP	-42±17*	-39±17*	-5±8
Orthostatic ΔDBP	-20±12*	-12±10*	0±5
Orthostatic ΔHR	8±6	8±5	7±5
Values are mean±SD unless stated, *p<0.05 vs. PD, $^{\$}$p<0.05 vs. MSA			

24 hour-Ambulatory BP Monitoring data

MSA patients had significantly lower daytime SBP and DBP compared to PD patients (both p<0.01), whereas there was no difference between PD+AF and PD patients (p=0.34), as well as between MSA and PD+AF (p=0.17). Daytime average HR was not different among patient groups (p=0.28). During nighttime, there was a significantly higher SBP in PD+AF patients compared to PD (p=0.01), but no difference between MSA and PD (p=0.17) or MSA and PD+AF (p=0.11). Nighttime HR in MSA was greater than PD (p=0.03) but there was no difference between PD and PD+AF (p=0.06). The average SBP, DBP and HR in the 24-hour period were not different among groups (p>0.05). There were no differences in the number of patients with daytime and nighttime hypertension among groups (p>0.05).

The percentage nocturnal blood pressure falls in SBP and DBP were significantly lower in MSA compared to PD (both p<0.01) but there was no difference between MSA and PD+AF (SBP; p=0.71 and DBP; p=0.55). The percentage of nocturnal HR fall was not different among patient groups (p=0.06). Correspondingly, MSA and PD+AF had a higher proportion of patients with abnormal BP circadian rhythms (both absent and reversed BP circadian rhythms; non-dippers) than patients with PD (p<0.01 and p=0.04, respectively) but not between MSA and PD+AF (p=0.08). Patients with reversed BP circadian rhythms were

significantly more common in MSA and PD+AF compared to those with PD (both p<0.01) but not between MSA and PD+AF (p=0.14). There was also no difference in the number of patients with an absent BP circadian rhythm among groups (p>0.05).

Table 4.17. 24 hr-ABPM profiles in MSA, PD and PD+AF

Variable	MSA (n=23)	PD+AF (n=18)	PD (n=33)
Daytime - Mean SBP - Mean DBP - Mean HR	 116±12* 71±7* 81±9	 122±13 73±12 75±7	 127±12 77±9 79±10
Patients with daytime hypertension, % (n)	4% (1/23)	6% (1/18)	18% (6/33)
Nighttime - Mean SBP - Mean DBP - Mean HR	 119±12 70±9 72±11*	 127±16* 72±13 65±8	 115±12 67±9 66±8
Patients with nighttime hypertension, % (n)	30% (7/23)	50% (9/18)	24% (8/33)
24-hour values - Mean SBP - Mean DBP - Mean HR	 117±11 71±6 80±9	 123±13 72±11 73±7	 125±11 76±9 76±9
Patients with abnormal BP circadian rhythm, % (n)	96% (22/23)*	78%(14/18)*	48% (16/33)
Patients with absent BP circadian rhythm, % (n)	39% (9/23)	22%(4/18)	33% (11/33)
Patients with reversed BP circadian rhythm, % (n)	57% (13/23)*	56%(10/18)*	15% (5/33)
Nocturnal BP/HR Fall - Mean SBP (%) - Mean DBP (%) - Mean HR (%)	 2.8 ±9.0* -1.6 ±10.8* -11.1±9.9	 4.3 ±14.7* -0.1 ±20.7* -12.7±5.7	 -9.1 ±8.9 -12.9 ±11.0 -16.1±7.3
Values are mean±SD unless stated, * p<0.05 vs. PD, # p<0.05 vs. PD+AF, $p<0.05 vs. MSA			

24-hr SBP variability was significantly higher in MSA and PD+AF patients compared to PD (both p<0.01) but there was no difference in 24-hr DBP variability between MSA and PD+AF (p=0.32). There was a significantly lower daytime DBP variability in PD+AF compared to PD. MSA patients had significantly lower 24-hr HR variability compared to PD (p<0.01). SBP, DBP and HR variability during nighttime were not different among groups (p>0.05; Table 4.17).

Table 4.18. 24 hr-ABPM BP and HR variability in MSA, PD and PD+AF

Variable	MSA (n=23)	PD+AF (n=18)	PD (n=33)
BP variability			
- SBP daytime	15.6±5.2*	16.8±4.9*	11.6±4.4
- DBP daytime	10.0±2.9	10.8±2.8*	8.9±2.6
- HR daytime	6.7±2.7*	7.8±2.9	9.3±3.2
- SBP nighttime	11.2±6.4	12.8±5.3	9.5±4.1
- DBP nighttime	7.9±3.6	7.8±3.1	7.3±3.0
- HR nighttime	4.4±2.2	5.5±2.7	4.8±2.4
- SBP 24-hr	15.5±4.5*	17.2±4.5 *	13.1±4.6
- DBP 24-hr	10.2±2.3	11.2±2.7	9.9±3.0
- HR 24-hr	7.5±2.8*	8.4±2.6	10.2±2.9

Values are mean±SD, * $p<0.05$ vs. PD, # $p<0.05$ vs. PD+AF, $ $p<0.05$ vs. MSA

SBP=Systolic Blood Pressure, DBP=Diastolic Blood Pressure; values in mmHg, Heart Rate=HR; value in bpm.

NBPF=Nocturnal blood pressure fall

24 hr-ABPM and patient-report diary

Out of 74, 44 (59%) patients completed the diary during the 24 hr-ABPM monitoring. There was no difference in the number of patients who completed the diary among groups; 17(74%) MSA, 11(61%) PD+AF and 16 (48%) PD patients. For the purpose of the sensitivity and specificity analyses of 24 hr-ABPM in detecting OH, patients were divided into those with and those without OH according to the BP responses during HUT. MSA and PD+AF patients were combined as a single group (OH+ group) and PD patients were a control group (OH-). The average supine SBP was significantly higher in patients with OH (OH+), but the mean DBP and HR were not different compared to patients without OH (OH-). During head-up tilting (HUT), Tilt SBP was significantly lower in OH+ compared to OH- ($p<0.01$). By definition, the degree of SBP and DBP changes during HUT were significantly higher in OH+ when comparing with OH- ($p<0.01$).

Cardiovascular responses to HUT and standing test using ABPM

With 24 hr-ABPM, supine SBP, DBP and HR were not different between OH+ and OH- but the average SBP and DBP was significantly lower in OH+ during the standing test compared to OH-. The degree of orthostatic changes (both SBP and DBP) were also significantly different between OH+ and OH- ($p<0.05$) but not for HR (Table 4.18).

Table 4.19. Blood pressure and HR during supine, head-up tilting and orthostatic changes in patients without OH (PD) compared with patients with OH (MSA and PD+AF)

Variable	Patients with OH (MSA and PD+AF)	Patients without OH (PD)
Number of patients	28	16
HUT		
Supine SBP	138±16$^{£}$	127±13
Supine DBP	79±11	74±9
Supine HR	73±8	70±7
Tilt SBP	100±19$^{£}$	125±17
Tilt DBP	63±13$^{£}$	75±8
Tilt HR	81±12	78±9
∆SBP	-38±15$^{£}$	-3±9
∆DBP	-16±11$^{£}$	1±5
∆HR	8±6	8±4
Standing test		
Supine SBP	128±16	128±18
Supine DBP	75±13	74±11
Supine HR	74±11	74±10
Stand SBP	99±14$^{£}$	122±16
Stand DBP	62±11$^{£}$	78±12
Stand HR	84±12	81±11
∆SBP	-29±19$^{£}$	-6±6
∆DBP	-13±13$^{£}$	4±6
∆HR	10±7	7±5

Values are mean±SD, $^{£}$ $p<0.01$ vs. patients without OH, $^{\phi}p<0.01$ vs. corresponding Supine

ROC analysis for detecting OH comparing HUT and standing tests using ABPM with autonomic protocol diary.

Using 24 hr-ABPM, the area under the curve (AUC) that distinguishes OH+ from OH- was 0.87 (95% CI, 0.75-0.99). A fall of 20 mmHg or more in SBP showed a sensitivity and specificity of 82% and 100% (AUC 0.91, 95% CI 0.84-0.98) in differentiating OH+ from OH-, respectively. A DBP fall of 10 mmHg or more had a 57% sensitivity and a 94% specificity to discriminate OH+ from OH- with an AUC of 0.75 (0.64-0.87; Table 4.19). A 20 mmHg systolic BP fall criteria had a significantly higher efficacy in detecting OH compared to a 10-mmHg DBP fall using the 24 hr-ABPM with the autonomic protocol diary ($p<0.01$).

Table 4.20. Sensitivity analysis for 24 hr-ABPM in detecting orthostatic hypotension (OH)

	Sensitivity (%)	**Specificity (%)**	**AUC (95% CI)**
BP			
- SBP fall ≥ 20 mmHg	82	100	0.91 (0.84-0.98)
- DBP fall ≥ 10 mmHg	57	94	0.75 (0.64-0.87)

4.1.4.4 Discussion

The aim of this study was to 1) examine 24 hr-ABPM in patients with chronic autonomic failure (MSA+AF, PD+AF and PD) and 2) determine the effectiveness of 24 hr-ABPM compared to standard orthostatic challenge testing (10 minute Head-up Tilt) in diagnosing orthostatic hypotension (OH) in these patients. The main findings in this study were that an abnormal circadian BP rhythm (either a blunted nocturnal fall of BP or a reversed nocturnal fall) occurred in about half of the patients with PD. This proportion was higher in PD+AF and in patients with MSA. In comparison to a recent study (Schmidt *et al.*, 2009a), the prevalence of an abnormal circadian BP rhythm in PD in the present study was similar whereas the prevalence of an abnormal circadian BP rhythm in MSA patients in the present study was higher (68% vs. 96%, respectively). These inconsistent MSA results may be explained by the fact that this study categorized patients with regards to a patient's diagnosis *and* their autonomic function, whereas the previous study used only a diagnostic category (e.g., MSA patients without autonomic failure were included). Furthermore, a reversed nocturnal circadian BP pattern was more common in autonomic failure patients,

presenting in more than 50% of MSA and PD+AF patients compared to only 15% in PD patients. These findings suggest that even though a blunted nocturnal fall in 24-hr BP profiles can often be seen among PD patients without autonomic failure, a reversed nocturnal circadian BP pattern is much more common in patients with autonomic failure (PD+AF and MSA). Daytime SBP variability was also higher in patients with autonomic failure, which could be due to greater fluctuations in BP in MSA and PD+AF during daily activities compared to patients without autonomic failure, and supports the idea that this value could also be an additional useful measurement in 24 hr-ABPM in patients with suspected autonomic failure and/or orthostatic hypotension.

The 24-hr BP profiles in patients with MSA and PD+AF were relatively similar as indicated by no differences in the number of patients with a blunted nocturnal BP fall, a reversed nocturnal circadian BP pattern and the daytime BP variability. These results suggest that 24 hr-ABPM patterns cannot be used to discriminate between MSA and PD+AF.

Human circadian rhythms are controlled by the suprachiasmatic nucleus (SCN) in the hypothalamus (Hastings & Maywood, 2000). The SCN projects afferent input to the paraventricular nucleus (PVN), which plays an important role in controlling various autonomic functions, such as stress and metabolism (Ferguson *et al.*, 2008). Both sleep and physical activity have a large influence on BP and HR circadian changes, including the normal physiological blood pressure fall during sleep (Fabbian *et al.*, 2013). Although the cause of abnormal circadian BP rhythms in PD and MSA remains unclear, the involvement of SCN and PVN are likely to play an important contributing role (Buijs *et al.*, 1998). The abnormal circadian BP rhythms in MSA is supported by prior pathological studies showing pathological changes within both the paraventricular nucleus (Benarroch *et al.*, 2006a) and the suprachiasmatic nucleus (Ozawa *et al.*, 1998) in MSA. In contrast to MSA, the neuronal loss and pathological involvement in these structures have never been reported in PD and PD+AF. Nevertheless, sleep dysfunction is common in both patients with PD and MSA. The major cause of this problem includes REM (Rapid Eye Movement) sleep behavior disorder (RBD), obstructive sleep apnea (OSA) and excessive daytime somnolence (EDS). These features may partly explain the reason why abnormal circadian BP profiles frequently occur in both disorders. Although abnormal circadian BP rhythms are common in PD, PD+AF and MSA, it is important to note that the number of patients with a reversed circadian BP profile at night was significantly higher in PD+AF and MSA compared to PD (without AF). This finding suggests that autonomic dysfunction plays an important contributing role in the

control of circadian BP rhythms. Supine hypertension is a common feature in patients with autonomic failure (Shannon *et al.*, 1997) and is also associated with OH (e.g., the severity of autonomic failure) (Goldstein *et al.*, 2003b). It is thus very likely that supine hypertension contributed to the reversed circadian BP pattern in MSA and PD+AF patients in the present study. The cause of supine hypertension in MSA has been suggested to result from an inappropriate residual sympathetic tone (Shannon *et al.*, 2000). These findings also emphasizes that the reversed circadian BP pattern from 24 hr-ABPM is a shared phenomenon in both patients with autonomic failure from both pre- (MSA) and post-ganglionic (PD) lesions, rather than a disease-specific feature.

The present study also showed that using an autonomic protocol diary alongside 24 hr-ABPM is useful for helping diagnose patients with OH. Using the standard criteria of OH, this technique provides a reasonably high sensitivity (82%) and specificity (100%) to distinguish OH+ from OH- patients with parkinsonism. Nevertheless, a 20-mmHg fall of SBP has a significantly better efficacy in detecting OH from 24 hr-ABPM when compared with a 10-mmHg fall of DBP. To date, there has been no study that has used a diary with 24 hr-ABPM to investigate patients with OH. Given that these are non-invasive, relatively simple tests to perform without a requirement of an autonomic laboratory, this technique should be included as part of autonomic investigations in suspected OH workups. An adjunct diary to 24 Hr BP monitoring can also provide additional information with regards to the effect of patient activities on BP and HR and their relation to symptoms. Such an approach would allow the clinician/scientist to make a connection between activities and BP/HR during events if symptoms develop (Stuebner *et al.*, 2013). 24 hr-ABPM can also be used to monitor BP and HR in patients with OH/autonomic failure after starting anti-hypotensive medications.

In conclusion, this study demonstrated that patients with PD+AF and MSA, who had a similar degree of orthostatic hypotension during HUT, generally have similar circadian BP and HR patterns as revealed by 24 hr-ABPM. As abnormal circadian rhythms are similarly present in both PD+AF and MSA, this suggests that the proportion of abnormal BP circadian patterns (both absent and reversed BP circadian rhythm) in 24 hr-ABPM depends on the autonomic function rather than the diagnosis. Moreover, this study also, for the first time demonstrated that 24 hr-ABPM can offer more information regarding OH in patients with autonomic failure if a patient-completed autonomic protocol diary containing postural challenges (standing) are used. This approach has reasonably high sensitivity and specificity in detecting OH.

Chapter 5

Olfactory function, non-motor aspects and quality of life in primary chronic autonomic failure

5. Olfactory function, non-motor aspects and quality of life in primary chronic autonomic failure

5.1 Olfactory function in primary chronic autonomic failure

5.1.1 Introduction

Abnormal sense of smell is a well-known and very common feature in patients with PD. The prevalence of olfactory impairment ranges from 80-100% depending on the study population (Doty *et al.*, 1995; Hawkes *et al.*, 1997). Smell identification is the most widely used method to assess olfaction because it is more feasible and the easiest way to assess subjects both in clinical and research settings. Sniffin' Sticks is a smell identification test which was originally developed and has been widely used in Europe (Hummel *et al.*, 1997). Previous studies demonstrated that the 16-item smell identification test has been useful as a screening tool for detecting abnormal olfaction in healthy subjects (Hummel *et al.*, 1997) and is a good non-invasive instrument for distinguishing PD patients from control subjects (Ansari & Johnson, 1975; Muller *et al.*, 2002).

Post-ganglionic autonomic dysfunction is evident in PD as reductions of cardiac MIBG uptake are common, even in the early stages of PD (Spiegel *et al.*, 2005). The frequent olfactory and cardiovascular autonomic dysfunction in PD are in accordance with the Braak and Braak hypothesis, which proposed the involvement of the dorsal motor nucleus of the vagus nerve and olfactory structures at the first stage of the pathological progression of PD (Braak *et al.*, 2003). Few studies have reported the relationship between the degree of impaired olfaction and the decline in cardiac MIBG reuptake in PD patients however (Lee *et al.*, 2006). Impaired sense of smell was reported to be more severe in PD with autonomic failure (PD+AF) compared to PD patients without autonomic dysfunction (Goldstein *et al.*, 2010). These findings suggest that the impairment of olfactory dysfunction could occur in parallel to the cardiovascular autonomic dysfunction in PD but this has not been thoroughly investigated.

In contrast to PD, little is known about olfactory function in MSA and PAF, neurodegenerative disorders that have overlapping features with PD. Previous studies suggested that patients with MSA may have a mild sense of smell impairment compared to PD while olfactory dysfunction seems to be impaired among patients with PAF (Silveira-Moriyama *et al.*, 2009b). Nevertheless, these smell studies in PAF and MSA were conducted with only a limited number of patients (MSA; n=14 and PAF; n=16) (Silveira-Moriyama *et al.*, 2009b). In addition, there was only one study that specifically investigated olfactory function

in patients with autonomic failure (Garland *et al.*, 2011). The results showed that PAF had abnormal olfaction; whereas olfactory function in MSA was intact. The finding of intact olfactory function in MSA was inconsistent with some previous reports which found a mild degree of impaired olfaction in patients with MSA (Wenning *et al.*, 1995; Silveira-Moriyama *et al.*, 2009b). These different findings could be due to other differences in the MSA patients between studies, such as disease severity, disease duration or clinical presentation. This has not been documented previously and warrants further investigation of whether there are other factors that could explain these inconsistent findings in MSA patients. Furthermore, olfactory function and its associations with indices of autonomic function have also not been explored in PAF and MSA.

It is still debatable whether there is a progression of impaired olfaction with disease duration and severity in PD and it is unknown if there are alterations in olfactory function with disease severity in MSA or PAF. The few previous studies that have been performed suggested that there was no significant deterioration of smell deficit in PD patients (Doty *et al.*, 1988; Double *et al.*, 2003; Kim *et al.*, 2007) while only 1 study showed that olfactory dysfunction increased with the severity of PD (Tissingh *et al.*, 2001). In support of the former findings, there was no correlation between striatal dopaminergic terminal loss in functional brain imaging and olfactory dysfunction in PD (Siderowf *et al.*, 2005; Goldstein *et al.*, 2008).

The aim of this study was to therefore simultaneously investigate olfactory function and a range of other possible associated factors (such as age, disease duration, disease severity) in patients with MSA, PAF and PD. The sensitivity and specificity of olfactory function in differentiating between chronic autonomic disorders were also examined.

5.1.2 Methods

Participants

Patients with a confirmed diagnosis of Parkinson's disease with (PD+AF) and without autonomic failure (PD), Probable Multiple System Atrophy (MSA) with orthostatic hypotension and Pure Autonomic Failure (PAF) and healthy controls were included (Chapter 3). Patients were tested on-medication. Patients and controls had no history of severe head injury (with loss of consciousness), dementia, nasal or sinus surgery or recent history of upper respiratory tract infection. All participants also were evaluated with the mini-mental status examination (MMSE) and had scores greater than 25 out of 30. Clinical history and

relevant information of all patients and participants were recorded. For example, age, gender and duration of disease (time from first symptom to testing date).

Smell identification tests

Olfactory function was assessed with the Smell identification Test using Sniffin' Sticks (SS-16), which involves the smelling of 16 felt tip pens that are each scented with different smells. Patients were asked to sniff the tip of each pen for 30 seconds. The participant then identified 1 of 4 choices which best described the presented odour. Olfactory function was assessed as a score ranging from 0-16 (min-max).

Clinical Features

The Unified Multiple System Atrophy Rating Scale (UMSARS) was used to assess the severity of different clinical features in the MSA patients, including part 1 (activities of daily living-ADL), part 2 (Motor function) and part 4 (global disability scale). There are in total 26 questions in part 1 and part 2, with each question containing a 5-point scale from 0 (no symptom) to 4 (severe). UMSARS part 3 included measuring BP and HR before and after a 2-minute standing test. This section was not included in the UMSARS analysis in this study because of the overlap with cardiovascular autonomic function testing which was conducted (see chapters 4.1 and 4.2). UMSARS part 4 was used to measure the global disability scale ranging from 1 (completely independent) to 5 (totally dependent or bedridden).

The Unified Parkinson's Disease Rating Scale (UPDRS) was used to assess different symptoms and the ability to perform specific tasks in PD patients. The UPDRS score consists of 4 main parts: part I (Mentation, behavior and mood), part II (Activities of daily living-ADL), part III (Motor examination) and part IV (Complications of therapy). There are 44 questions in all 4 parts and each question contains a 5-point scale from 0 (no symptoms) to 4 (severe symptoms).

Hoehn and Yahr Staging (HY) was used to rate the degree of functional ability in PD patients. The score ranges from 1 (mild) to 5 (severe). The same investigator conducted all of these assessments in all patients.

Statistical Analyses

Data are presented as mean (± 1 SD) or median (inter-quartile range), where appropriate. Analysis of covariance (ANCOVA) or the Kruskal-Wallis test was used to compare the 4 groups where appropriate. If there was a significant difference, Mann-Whitney

U tests were used to compare between 2 groups for non-normally distributed data and unpaired t-tests for normally distributed data with Bonferroni corrections.

Chi-square analyses were used for analysis of categorical variables. The relationships between SS16 scores and other factors, including, age, disease duration, gender, UPDRS part III scores, were assessed by regression analysis. ROC (received operational curve) analysis was used to determine the sensitivity (Sn) and specificity (Sp) of different cut-off points for assessing the performance of a diagnostic test. In general, the optimal cut-off point would be both Sn and Sp that as close to 100% as possible. Nonetheless, it is less likely to happen as the Sn tend to vary inversely with Sp (Sn increases as Sp decreases, or vice versa). In this study, ROC curves plot Sn against 1-Sp and will evaluate the Sn and Sp of the SS-16 scores at different cut-off points in differentiating between disorders alongside the area under the curve data (AUCs). The AUC is an overall summary of the diagnostic performance of the test. The perfect discrimination for AUC is 1, which means the diagnostic test can perfectly differentiate between two conditions with both Sn and Sp equalling 100%. The AUC of 0.9 or higher represents an outstanding discrimination. A value of AUC of 0.8-0.9 shows an excellent discrimination; a value of 0.7-0.8, an acceptable discrimination. An AUC of 0.5 or less indicates that the diagnostic accuracy is questionable and not different from random chance. Statistical analyses were carried out using STATA 11.0 (STATA Corporation, College station, Texas, USA). All tests were 2-sided and a p value of $\leq$0.05 was considered significant.

5.1.3 Results

Demographics

72 patients (32 PD, 19 MSA, 21 PAF) and 20 controls were included in the analyses (see Table 5.1). Patients with PD and PAF were significantly older than controls ($p<0.01$) but there was no difference between MSA and controls ($p=0.09$). Comparing between patient groups, PAF patients were significantly older than MSA and PD (both $p<0.01$). PAF had a significantly longer disease duration compared to MSA ($p=0.022$) but no difference when compared with PD patients ($p=0.41$) who had a similar duration to MSA ($p=0.09$). Hoehn & Yahr scores were significantly higher in patients with MSA compared to PD ($p=<0.01$).

Table 5.1. Participant demographic data.

Variable	PD (n=32)	MSA(n=19)	PAF(n=21)	Control (n=20)	p-value
Age (yr)	65 ± 8* #	61 ± 7	71 ± 7*	56 ± 9	<0.01
Gender (M/F)	22/10	9/10	11/10	6/14	0.06
Disease duration (Median (IQR)), yrs	7 (1-18)	4 (2-11) $	7 (1-26)	-	0.06
% Never smoked	29	19	21	31	0.11
Hoehn & Yahr	1.7 ± 0.6	3.3 ± 0.9	-	-	<0.01
Motor scores; (UPDRS part III and UMSARS II)	14.4 ± 6.4	22.8 ± 7.0	-	-	-

Values are mean±SD, if not specified, UPDRS=Unified Parkinson's Disease Rating Scale, UMSARS=Unified Multiple System Atrophy Rating Scale

*p<0.01 vs. Control, #p=0.05 vs. PAF, $p<0.05 vs. PAF

Olfactory Function

SS-16 scores were significantly lower in all patient groups compared to controls (p<0.01; mean SS-16 scores; MSA=13.2; PD=7.6; PAF=8.4 and Controls=14.1, respectively) and this difference was still significant after adjusting for age, gender and disease duration (p<0.01; Figure 5.1). MSA patients had higher SS-16 scores compared to PAF (p<0.01) and PD (p<0.01). These differences remained significant after controlling for age, gender and disease duration (p<0.01). There was no difference in SS-16 scores between PD and PAF (p=0.34).

In a sub-group analysis of MSA sub-types, there was no difference in age, disease duration, history of smoking or UMSARS scores between MSA-C (n=11) and MSA-P (n=8). There was no difference in SS-16 scores between sub-types of MSA (p=0.90).

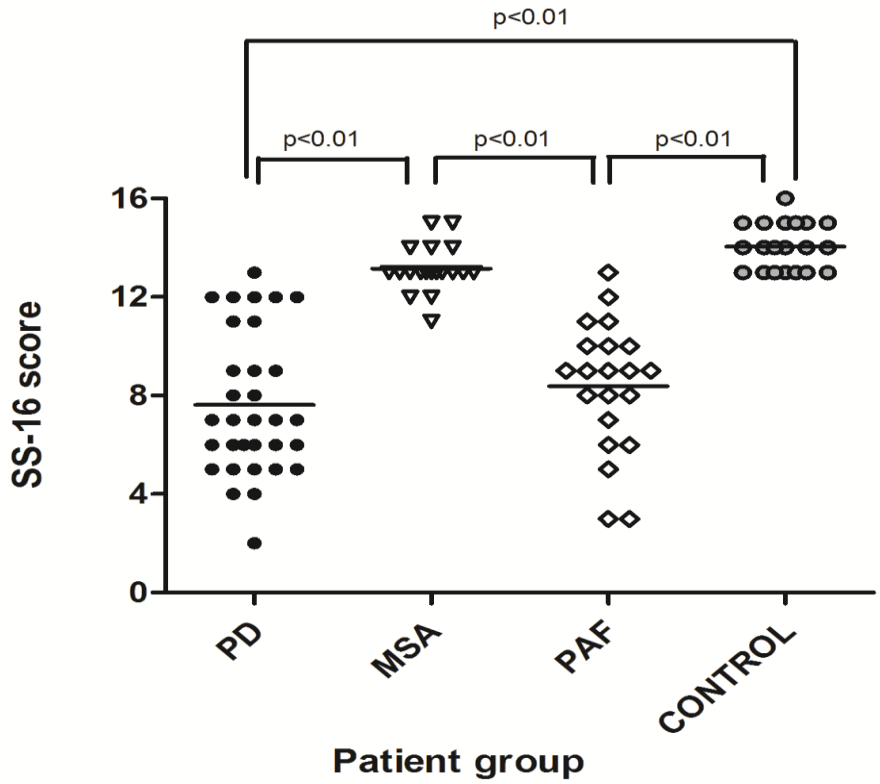


Figure 5.1. SS-16 scores in PD, MSA, PAF and controls

In patients with PD there was no association between olfactory function and age or disease duration (both p>0.05). SS-16 scores were significantly correlated with gender (p<0.01), history of smoking (p=0.03) and motor severity as assessed by the UPDRS part III (p<0.01) in the univariate analysis. There was a significant negative correlation between SS-16 scores and UPDRS part III in the multiple regression analysis after adjusting for gender and smoking history (R^2=0.44, p<0.01; Figure 5.2 and Table 5.2). Although, the number of participants was limited, there was no difference in SS-16 scores in PD patients with and without autonomic failure.

In MSA patients, SS-16 scores were only significantly associated with motor severity (UMSARS part II score) after controlling for age in the multiple regression analysis (R^2=0.36, p=0.039; Table 5.3). In contrast, SS-16 scores were not associated with age, gender, disease duration and smoking in patients with PAF (p>0.05).

Table 5.2. Multiple regression analysis of the correlation between SS-16 and UPDRS motor (part III) scores adjusted for gender and smoking in PDs

Variable	Parameter estimate	Standard error	P-value
UPDRS III score	-0.24	0.07	<0.01
Smoking	2.22	1.07	0.047
Gender	0.33	1.23	0.79

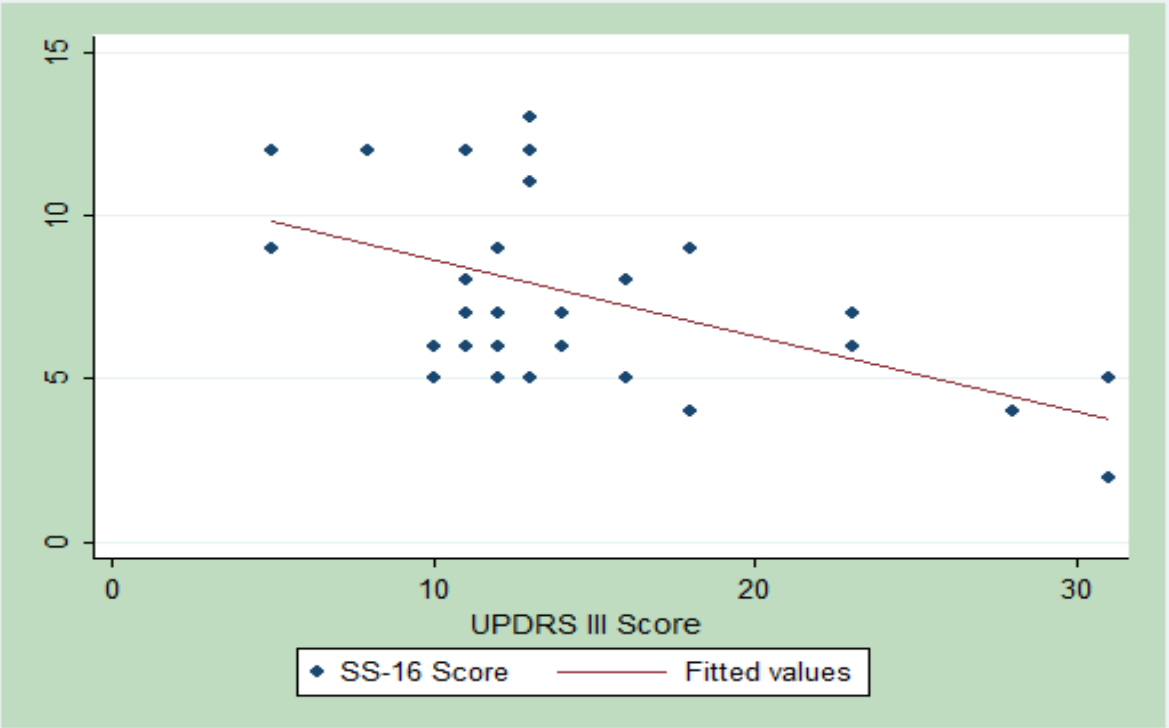


Figure 5.2. Scatterplot showing the correlation between SS-16 scores (Y axis) and UPDRS III scores (X axis; r=-0.51, p=<0.01) in patients with PD.

Table 5.3. Multiple regression analysis of the correlation between SS-16 and UMSARS motor (part II) scores adjusted for age in MSA.

Variable	Parameter estimate	Standard error	P-value
UMSARS II score	-0.06	0.03	0.039
Age	0.06	0.03	0.07

Using the SS-16 scores, the area under the curve (AUC) that distinguishes MSA from PD and PAF from PD were similar at 0.97 (95% CI, 0.93-1.00; Figure 5.3). An SS-16 score of 11 and lower showed a sensitivity and specificity of 97.4% and 81.3% (AUC 0.88, 95% CI 0.79-0.97) in differentiating PD from MSA, respectively. An SS-16 score of 11 or less had a 97.4% sensitivity and a 90.5% specificity to discriminate MSA from PAF with an AUC of 0.93 (0.84-1.00; Figure 5.3, Table 5.4).

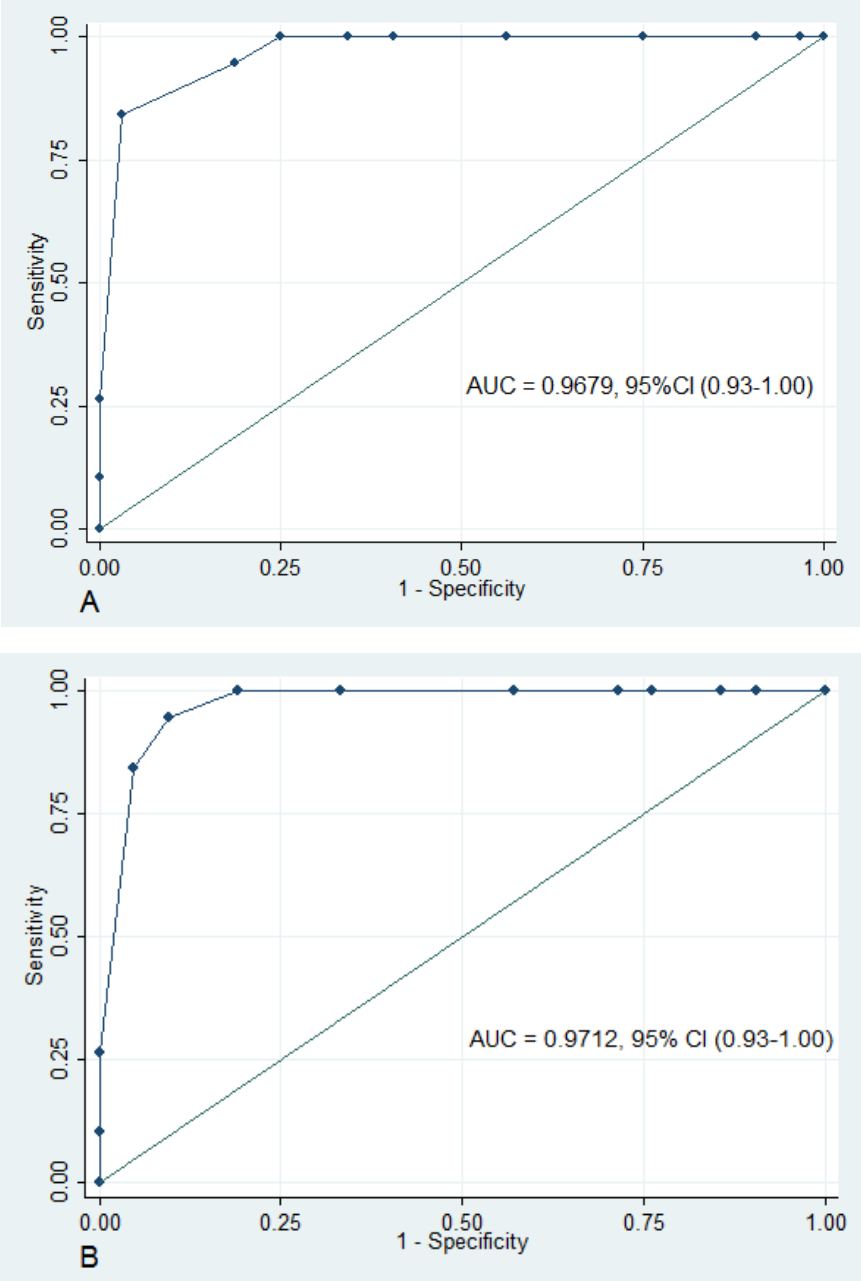


Figure 5.3. ROC curves showing the relationship between sensitivity and specificity for SS-16 scores in PD vs. MSA (A; upper) and PAF vs. MSA (B; lower).

Table 5.4. Sensitivity analysis for different cut-off points in SS-16 scores for chronic autonomic disorders.

	Sensitivity (%)	Specificity (%)	AUC (95% CI)
PD vs. MSA			
- SS-16 score ≤ 11	94.74	81.25	0.88 (0.79-0.97)
- SS-16 score ≤ 12	84.21	96.88	0.91 (0.82-1.00)
MSA vs. PAF			
- SS-16 score ≤ 11	94.74	90.48	0.93 (0.84-1.00)
- SS-16 score ≤ 12	84.21	95.24	0.90 (0.80-0.99)

5.1.4. Discussion

The aim of this study was to therefore simultaneously investigate olfactory function and a range of other possible associated factors (such as age, disease duration, disease severity) in patients with MSA, PAF and PD. The sensitivity and specificity of olfactory function in differentiating between chronic autonomic disorders were also examined. The main findings were that olfactory function was impaired in PD, PAF and MSA patients compared to controls. Sense of smell in MSA patients was better than PD and PAF but there was no difference in the degree of impaired olfaction in PD and PAF patients. Furthermore, SS-16 scores were significantly correlated with motor dysfunction scores (UPDRS III score) in PD patients after controlling for gender and history of smoking, and with motor dysfunction scores (UMSARS II score) in MSA after controlling for age.

In the present study, patients with MSA had mild olfactory impairment indicated by a lower score of SS-16 compared to healthy controls. A pathological study has reported glial cytoplasmic inclusions in the olfactory bulb in MSA (Kovacs *et al.*, 2003) that could contribute to the mild impaired sense of smell in MSA. This finding was also in line with prior studies (Wenning *et al.*, 1995; Abele *et al.*, 2003; Silveira-Moriyama *et al.*, 2009b). Nonetheless, a few studies have reported preserved olfactory function in patients with MSA

(Garland *et al.*, 2011; Glass *et al.*, 2012). These differences could be due to a small sample size in the latter studies (N=10, N=4, respectively).

The results of the present study confirm previous findings that patients with PD have marked impairment of olfactory function similar to patients with PAF (Goldstein & Sewell, 2009; Garland *et al.*, 2011). Nevertheless, another prior study reported a milder deficit of olfactory function in PAF compared to PD (Silveira-Moriyama *et al.*, 2009b). The reason for this difference in findings is unclear but may be partly explained by different etiologies between PAF patients in these studies. Many patients can initially present with symptoms similar to patients with PAF and eventually develop additional clinical features of other diagnoses, such as MSA or DLB after years of follow-up (Larner *et al.*, 2000; Iodice *et al.*, 2012). Another possible explanation is the difference in age. Our patients with PAF were older than those in the previous study (Silveira-Moriyama *et al.*, 2009b), which could potentially lower the scores of the olfactory identification test. However, age was taken into account in the final model of multiple regression analysis in the present study.

Olfactory dysfunction in PD may result from Lewy body deposition in the olfactory bulb as proposed by Braak et al (Braak *et al.*, 2003). There has been no report of olfactory bulb pathology in PAF and the cause(s) of olfactory dysfunction in PAF patients remains unclear. No previous study has ever examined the pathology of olfactory and related structures in patients with PAF. In the present study, age, gender, smoking and disease duration were not correlated with olfactory dysfunction in PAF. A recent paper reported intact olfactory function in patients with dopamine β-hydroxylase deficiency, a rare genetic disorder characterized by a lack of the Dopamine β-hydroxylase (DBH) enzyme which is required for converting dopamine into noradrenaline. An intact olfaction in these patients suggests an impaired sense of smell is not dependent on impairments in the noradrenaline pathway (Garland *et al.*, 2011). As other neurotransmitters, such as Acetylcholine, Dopamine and Serotonin, are also involved in the pathogenesis of olfactory dysfunction (Doty, 2012), it might be that olfactory dysfunction in PAF could result from abnormalities in the kinetics of any of these neurotransmitters and/or central pathology. Future research is needed to explore this hypothesis, which may provide useful information regarding the causes of impaired olfaction, particularly in comparison with patients with PD.

There is only one study reporting a more severe olfactory dysfunction in PD patients with OH, compared with PD patients without OH (Goldstein *et al.*, 2010). Unfortunately, the difference in olfactory function between PD with and without AF in the present study could not be formally examined because of the limited number of PD patients with AF (n=4).

The significant relationship between olfactory dysfunction and motor dysfunction severity in PD patients in the present study is consistent with a previous study from Tissingh et al. (Tissingh *et al.*, 2001). The results of the current study also found no correlation between disease duration and olfactory dysfunction in PD in accordance with a previous study (Hawkes *et al.*, 1997). However, some previous studies found no association between olfactory dysfunction and motor severity in PD (Doty *et al.*, 1988). A recent study suggested that olfactory dysfunction may progress over time in the early stages of PD and then possibly plateau thereafter (Berendse *et al.*, 2011). Therefore, all these findings support the hypothesis that impaired olfaction is still a possible useful marker to determine disease progression in patients with early stage PD as it could progress in parallel to nigrostriatal degeneration, particularly at the beginning of motor symptoms.

There has been no study examining the association between olfactory function and motor dysfunction function in MSA. This study is the first to reveal a correlation between UMSARS II (motor) scores and olfactory function in MSA patients. The relationship between SS-16 and UMSARS motor scores suggest that olfactory involvement is correlated with motor severity in MSA. The glial cytoplasmic inclusions in the olfactory bulb (Kovacs *et al.*, 2003) that have been reported in MSA may be a part of the disease progression and represent a widespread involvement which would have not occurred yet in early MSA patients. Given the limited number of MSA patients in the present study, a larger sample size would be needed to confirm this finding.

With regards to the efficacy of the SS-16 in distinguishing between chronic autonomic failure disorders, the SS-16 had a high sensitivity and specificity in differentiating between PD and MSA. This was consistent with a recent study (Suzuki *et al.*, 2011). This study is the first to report the efficacy of SS-16 in differentiating between MSA and PAF using ROC analysis. Cut-off values of 11 and 12 were used which provided reasonably high sensitivity and specificity. Moreover, the results of the area under the curve (AUC) analyses using the same cut-off points also suggested that this test has almost similar values in helping differentiating the diagnosis between PD and MSA as well as between MSA and PAF. Given the fact that MSA can initially present with isolated autonomic failure resembling PAF, impaired olfaction from SS-16 would support the diagnosis of PAF rather than MSA.

In conclusion, this study confirmed the similar degree of olfactory dysfunction in patients with PD and PAF and a mild impaired sense of smell in MSA. Motor severity scores (UPDRS part III) were strongly associated with olfactory dysfunction in PD. In MSA, there was evidence to suggest an association between UMSARS (motor) part II and olfactory

dysfunction. The findings from the ROC analysis also indicated that the SS-16 could also be a useful investigation for differentiating between these chronic autonomic failure disorders.

5.2 Non-motor aspects and quality of life in primary chronic autonomic failure

5.2.1 Introduction

Non-motor symptoms in PD patients have recently received much more attention and are now recognized as important features of parkinsonian syndromes. These non-motor symptoms can vary widely between patients, for example olfactory dysfunction, sleep disturbances, neuropsychiatric features and autonomic dysfunction can occur to various extremes and with various prevalences between patients (Chaudhuri *et al.*, 2006a). A variety of autonomic symptoms, such as orthostatic hypotension (OH), constipation, bladder and sexual dysfunction, were consistently reported as important non-motor symptoms in PD (Chaudhuri *et al.*, 2006a). Depression was also reported as a common symptom in PD (Karlsen *et al.*, 1999) and it is also considered to be part of disease progression in patients with PD and can predate motor symptoms in PD (Nilsson *et al.*, 2001; O'Sullivan *et al.*, 2008). Importantly, non-motor features can have a significant impact on quality of life in patients with PD (Schrag *et al.*, 2000b). A number of non-motor symptom questionnaires have recently been developed to be used as tools to interrogate these symptoms (Visser *et al.*, 2004; Chaudhuri *et al.*, 2007), but only a few specifically examine autonomic symptoms. The Scale for Outcomes in Parkinson's disease for Autonomic Symptoms (SCOPA-AUT) has been developed since 2004 in order to detect a range of autonomic symptoms in PD (Visser *et al.*, 2004). It has been validated and is one of the questionnaires recommended by The Movement Disorders Society as a tool for screening autonomic symptoms in PD (Pavy-Le Traon *et al.*, 2011).

Data from population-based epidemiological studies confirmed that non-motor symptoms are common in other chronic autonomic failure syndromes, such as MSA and PAF, in addition to PD, but they are generally milder compared in those with atypical parkinsonian disorders, such as MSA or PSP, relative to PD (Colosimo *et al.*, 2010). Nevertheless, there have been only a few studies directly examining non-motor symptoms, including autonomic symptoms, and quality of life in patients with MSA (Meissner *et al.*, 2012) and also no previous report of this aspect in PAF patients. Other non-motor aspects of autonomic disorders, such as depression, have also been investigated in isolation in PD, MSA and PAF patients but have not been compared between these disorders in the same study or related to other indices of autonomic function.

The purpose of this study was to therefore further examine the relationship between quality of life and non-motor aspects in primary chronic autonomic failure disorders by

correlating quality of life scores in MSA, PD and PAF patients with a variety of factors, including, age, gender, disease duration, motor symptoms (in MSA and PD) and depression. The relationship between autonomic symptoms in PD using the SCOPA-AUT instrument and these factors were also investigated.

5.2.2 Methods

Participants

Patients with a confirmed diagnosis of Parkinson's disease with (PD+AF) and without autonomic failure (PD), Probable Multiple System Atrophy (MSA) with orthostatic hypotension and Pure Autonomic Failure (PAF) and healthy controls were included (Chapter 3). Patients were tested on-medication. History and relevant information of all patients and participants were recorded. These included, age, gender and duration of disease (time from motor dysfunction/first symptom onset to testing date).

Protocol

A range of assessments of motor and non-motor symptoms were conducted as follows:

Motor Function and Clinical Features

The Unified Multiple System Atrophy Rating Scale (UMSARS) was used to assess the severity of different clinical features of MSA patients, including part 1 (activity daily living-ADL), part 2 (Motor function) and part 4 (global disability scale). There are a total of 26 questions in part 1 and part 2, with each question containing a 5-point scale from 0 (no symptoms) to 4 (severe symptoms). UMSARS part 3 was for measuring BP and HR before and after a 2-minute standing test. UMSARS part 4 was used to measure the global disability scale ranging from 1 (completely independent) to 5 (totally dependent or bedridden). This section was not included in the UMSARS analysis in this study because of the overlap with cardiovascular autonomic function testing which was conducted (see chapters 4.1 and 4.2).

The Unified Parkinson's Disease Rating Scale (UPDRS) was used to assess different symptoms and the ability to perform specific tasks in PD patients. The UPDRS consists of 4 main parts: part I (Mentation, behavior and mood), part II (Activities of daily living-ADL), part III (Motor examination) and part IV (Complications of therapy). There are a total of 44

questions in all 4 parts, with each question containing a 5-point scale from 0 (no symptoms) to 4 (severe symptoms).

Hoehn and Yahr Staging (HY) was used to rate the degree of functional ability in MSA and PD patients. The score ranges from 1 (mild) to 5 (severe). The same investigator conducted all these assessments in all patients.

Non-motor Questionnaires

The Hamilton Depression Rating Scale (HAM-D) was used to assess symptoms of depression and its severity in all groups. The scale consists of 17-item questions. A score between 0-7 is generally accepted as a normal range but a score of 20 or higher suggests depression.

The Schwab and England Activities of Daily Living Scale (SE) was used by all patient groups to rate how much their condition affects their functional capability. The SE score ranges from 100 (Completely independent) to 0 (bedridden).

The SCOPA-AUT was used by all patient groups to rate their symptoms on different domains of autonomic function, including, cardiovascular, gastrointestinal (GI), urinary (GU), thermoregulatory, pupillomotor and sexual symptoms. The SCOPA-AUT ranges from 0 (no symptoms) to 100 (severe symptoms) in each domain.

The Mini-Mental State Examination (MMSE) rating scale was used to rate all participants' cognitive function. The MMSE score ranges from 0-30, with a lower score indicating greater impairment of cognitive function. Given that cognitive impairment is a confounder for the validity of quality of life questionnaires, all patients with an MMSE score of less than 24 were not included in the study.

The same investigator conducted all these assessments in all patients.

Quality of life

Patient population specific scales were used to assess Quality of Life.

The 39 item Parkinson's Disease Questionnaire (PDQ-39) was used in PD patients, the MSA Health-Related Quality of Life (MSA-QoL) Scale in MSA patients and the 36 item Short Form Health Survey (SF-36) in PAF patients (see Chapter 3 for further details).

Healthy control participants completed the Short Form Health Survey (SF-36), the Mini-Mental State Examination (MMSE) and the Hamilton Depression Rating Scale (HAM-D).

The same investigator conducted all these assessments in all patients.

Statistical Analyses

Data are presented as mean (± 1 SD) or median (IQR), where appropriate. Analysis of covariance (ANCOVA) was used for comparing 3 or 4 groups for normally distributed data, while the Kruskal-Wallis test was used if data were non-normally distributed. If there was a significant difference, Mann-Whitney U tests were then used to compare between 2 groups for non-normally distributed data and unpaired t-tests for normally distributed data with Bonferroni corrections. Chi-square analyses were used for analysis of categorical variables. The relationships between PDQ-39 scores and other variables, including age, disease duration, gender, UPDRS part III scores, were assessed by Pearson's correlation coefficients or Spearman's Rank Correlations, where appropriate. Similar methods were also used to determine the relationship between MSA-QoL and other potential associated factors and the PAF SF-36 data and other potential associated factors. Statistical analyses were carried out using STATA 11.0 (STATA Corporation, College station, Texas, USA). All tests were 2-sided and a p value of ≤ 0.05 was considered significant.

5.2.3 Results

Demographics

75 patients (32 PD, 23 MSA and 20 PAF) and 20 controls were included in the analyses. Patients with MSA were younger than PAF and PD patients ($p<0.05$) but there was no difference in age between MSA and controls ($p=0.31$). Patients with MSA had a shorter disease duration compared to PAF ($p=0.01$) and PD ($p=0.05$) but there was no difference between PD and PAF ($p=0.36$). Hoehn & Yahr (HY) scores were significantly

lower in PD when compared with MSA patients (p<0.01). Age, disease duration and gender were not different between MSA sub-groups (Table 5.5).

Table 5.5. Participant demographic data

Variable	PD (n=32)	MSA(n=23)	PAF(n=21)	Control (n=20)	p-value
Age, yrs	65 ± 8	60 ± 7[#$]	70 ± 7	56 ± 9	<0.01
Gender (M/F)	23/9	12/11	14/6	6/14	0.03
Disease duration Median (IQR), yrs	7 (4-10)	4 (3-7)[#$]	8 (6-15)	-	0.04
Hoehn & Yahr	1.7±0.6	3.3 ± 0.9[#]	-	-	-
Motor scores; UPDRS part III or UMSARS II	14.4 ± 6.3	22.8 ± 7.4	-	-	-
HAM-D	6.4 ± 2.9*	11.4 ± 5.5*[#$]	7.4 ± 3.2*	0.5 ± 0.7	<0.01
SE	86 ± 12	57 ± 27[#$]	85 ± 6	-	<0.01

*p<0.05 vs. Controls, [#]p<0.05 vs. PD, [$]p<0.05 vs. PAF
Values are mean±SD unless stated, UPDRS=Unified Parkinson's Disease Rating Scale, UMSARS=Unified Multiple System Atrophy Rating Scale, SE=Schwab and England Activities of Daily Living Scale, HAM-D=Hamilton Depression Rating Scale

Depression and Activities of Daily Living Scale

Patients with chronic autonomic failure had significant higher depression scores compared to healthy controls (p<0.01). MSA patients had a significantly higher HAM-D score compared to those with PAF and PD (both p<0.01). The depression score in PAF was not different from PD (p=0.28). Schwab and England Activities of Daily Living Scale scores (SE) were significantly lower in patients MSA compared to PD and PAF (both p<0.01) but there was no difference between PD and PAF (p=0.15, Table 5.5).

Parkinson's Disease Questionnaire (PDQ-39) and the Scale for Outcomes in Parkinson's disease for Autonomic Symptoms (SCOPA-AUT)

On the PDQ-39 the highest scores were reported in bodily discomfort domain, followed by the cognitive and mobility domains. On the other hand, social support and stigma were the two domains with the lowest scores. With regards to the SCOPA-AUT, sexual dysfunction and bladder symptoms were scored highest. Other data of the PDQ-39 and SCOPA-AUT, including sub-scores, are presented in Table 5.6.

Table 5.6. PDQ-39 and SCOPA-AUT, including subscores, responses in patients with PD

Item Scores	Mean ± SD
PDQ-39	
- PDQ-Summary Index (PDQ-SI)	28.2 ± 17.4
- Mobility	30.1 ± 26.2
- Activities of daily living (ADL)	28.0 ± 23.7
- Emotional wellbeing	26.6 ± 20.7
- Stigma	17.0 ± 17.0
- Social support	16.5 ± 23.3
- Cognitive	36.9 ± 27.2
- Communication	29.6 ± 21.0
- Bodily discomfort	38.5 ± 25.7
SCOPA-AUT	
- Total	20.9 ± 12.9
- Gastrointestinal	21.1 ± 12.4
- Genitourinary	27.4 ± 17.5
- Cardiovascular	8.7 ± 10.0
- Thermoregulatory	17.8 ± 19.2
- Pupil	17.4 ± 26.3
- Sexual	35.1 ± 36.4
Values are mean±SD	

Correlation Analyses of Quality of life and non-motor symptom scales in PD

There was a strong significant correlation between the PDQ Summary Index score (PDQ-SI) and UPDRS part I and part II scores (r=0.61 and r=0.62, respectively, both p<0.01). HAM-D and SE scores were also significantly correlated with PDQ-SI (r=0.58 and r=-0.49, respectively, p<0.01). There was a trend of correlation between PDQ-SI and disease duration (r=0.34, p=0.08) and between PDQ-SI and Total SCOPA-AUT (r=0.36, p=0.08). In contrast, Total SCOPA-AUT was significantly correlated with disease duration (r=0.66, p<0.01), UPDRS part II (r=0.52, p<0.05), HAM-D scores (r=0.51, p<0.05) and gender (r=0.46, p<0.05; Table 5.7).

Table 5.7. Spearman's Rank correlations between PDQ-39 Summary Index (PDQ-SI), SCOPA-AUT and age, gender, disease duration, UPDRS and HY scores, HAM-D and SE in patients with PD.

	PDQ-SI	SCOPA-AUT
Age	0.17	0.26
Gender	-0.04	0.46*
Disease duration	0.34	0.66**
Hoehn and Yahr (HY)	0.28	-0.04
UPDRS		
- part I (Mood and behavior)	0.61**	0.21
- part II (ADL)	0.62**	0.52*
- part III (Motor)	0.33*	0.26
Other non-motor features		
HAM-D	0.58**	0.51*
SE	-0.49**	-0.31
SCOPA-AUT	0.37	-

SE=Schwab and England Activities of Daily Living Scale, HAM-D=Hamilton Depression Rating Scale, UPDRS=Unified Parkinson's Disease Rating Scale

**p<0.01, *p<0.05

Quality of life and UMSARS scores and Correlation Analyses in MSA

Quality of life (QOL) and UMSARS scores in MSA are presented in Table 5.8. There was a strong correlation between MSA-QoL total score and Activities of daily living scale (SE) scores (r=-0.71, p<0.01), UMSARS part I (ADL) (r=0.66, p<0.01) and part IV (Disability) (r=0.63, p=0.017) scores. No correlation was evident between MSA-QoL and age, disease duration and UMSARS part II (motor) scores (Table 5.9).

Table 5.8. MSA-QoL and UMSARS scores, including subscores, in patients with MSA

Item Scores	Mean ± SD
UMSARS	
- part I (ADL)	23.8 ± 8.3
- part II (Motor)	22.8 ± 7.4
- part IV (Disability)	2.7 ± 1.2
MSA-QoL	
- Motor	46.0 ± 23.1
- Nonmotor	35.0 ± 19.0
- Emotional/social	45.5 ± 20.2
- Total	42.2 ± 17.8
Values are mean±SD	

Table 5.9. Spearman's Rank Correlations between MSA-QoL scores and age, gender, disease duration, UMSARS scores, HAM-D and SE in patients with MSA

	Spearman's Rho	p-value
Age	-0.049	0.87
Gender	0.47	0.09
Disease duration	0.45	0.10
Hoehn and Yahr (HY)	0.19	0.51
UMSARS		
- part I (ADL)	0.66	<0.01
- part II (Motor)	0.28	0.35
- part IV (Disability)	0.63	0.02
Other features		
HAM-D	0.42	0.16
SE	-0.71	<0.01
SE=Schwab and England Activities of Daily Living Scale, HAM-D=Hamilton Depression Rating Scale, UMSARS=Unified Multiple System Atrophy Rating Scale		

Quality of life and non-autonomic symptom scales in PAF

There were significantly lower SF-36 subscores in PAF patients relative to healthy controls, including physical function, role limitation physical, bodily pain, general health

perceptions, vitality, social functioning, role limitation emotional as well the physical health summary scores ($p<0.01$; Table 5.10). Mental health subscores and the mental health summary scores were not different between groups ($p=0.13$ and $p=0.1$, respectively).

Table 5.10. SF-36 domain sub-scores and summary measure scores in patients with PAF and controls

	PAF (n=20)	Control (n=20)	p-value
SF-36 sub-score dimensions			
- Physical Functioning (PF)	38±27	91±20	<0.01
- Role Limitation Physical (RP)	16±37	92±29	<0.01
- Bodily Pain (BP)	55±31	85±22	0.01
- General Health Perceptions (GH)	40±23	82±12	<0.01
- Energy/Vitality (VT)	34±22	69±16	<0.01
- Social Functioning (SF)	55±29	94±13	<0.01
- Role Limitation Emotional (RE)	62±44	100±0	<0.01
- Mental Health (MH)	66±23	81±7	0.13
SF-36 Summary Measures			
- Physical Health	30±9	53±10	<0.01
- Mental Health	47±12	55±4	0.10
Values are mean±SD			

Correlation Analyses of Quality of life and non-autonomic symptoms in PAF

SF-36 Physical Health summary measure scores (PH-SM) were moderately correlated with SE scores ($r=0.48$, $p=0.032$) and gender ($r=-0.45$, $p=0.04$) but not with age or disease duration (Table 5.11).

Table 5.11. Spearman's Rank Correlations between SF-36 Physical Health summary measures and age, gender and disease duration, HAM-D and SE in patients with PAF

	Spearman's rho	p-value
Age	0.11	0.64
Gender	-0.45	0.04
Disease duration	-0.05	0.82
Other features		
HAM-D	-0.26	0.27
SE	0.48	0.03

SE=Schwab and England Activities of Daily Living Scale, HAM-D=Hamilton Depression Rating Scale

5.2.4 Discussion

The purpose of this study was to further examine the relationship between quality of life and non-motor aspects in primary chronic autonomic failure disorders by correlating quality of life scores in MSA, PD and PAF patients with a variety of factors, including, age, gender, disease duration, motor symptoms (in MSA and PD) and depression. The relationship between autonomic symptoms in PD using the SCOPA-AUT instrument and these factors were also investigated. The main findings of this study were that the difference in the severity of depression in a group of patients with chronic autonomic failure; MSA patients had higher HAM-D scores than both PAF and PD patients but these scores were comparable between PAF and PD. The measurement of Activity daily living (ADL) using the SE scale also indicated that ADL were significantly limited in MSA compared to other disorders, whereas no difference was found between PD and PAF.

Other main findings were the differences in correlations between quality of life and non-motor symptoms in different chronic autonomic failure patients. In PD, PDQ-SI were correlated with depression, UPDRS part I (mood and behavior) and part II (ADL) (all $p<0.01$) but less strong correlations were evident with disease duration, UPDRS part III (motor) and SCOPA-AUT (both $p=0.08$). These findings suggest that non-motor features have more influence on quality of life in patients with PD, compared to motor components. These findings are supported by a number of previous studies showing consistent evidence of the impact of various non-motor symptoms/features on quality of life in patients with PD (Schrag *et al.*, 2000b; Martinez-Martin *et al.*, 2009; Havlikova *et al.*, 2011; Martinez-Martin *et al.*, 2011; Santos-Garcia & de la Fuente-Fernandez, 2013; Song *et al.*, 2013).

A correlation analysis between demographic factors and autonomic symptom scores using SCOPA-AUT in PD was also performed. SCOPA-AUT is a patient-completed questionnaire that measures different domain of autonomic symptoms including cardiovascular autonomic, gastrointestinal (GI) function, genitourinary (GU) function, sudomotor function and sexual function. In the present study, there was a strong relationship between the SCOPA-AUT total score and disease duration in PD. There were also some evidence showing that a higher SCOPA-AUT total score (e.g., greater autonomic symptoms) correlated with female gender and higher motor and depression scores. These correlations including, disease duration, greater motor and depressive symptoms are in accordance with a prior study (Verbaan *et al.*, 2007), except female gender that has never been previously reported. This may be partly explained by a low number of female PD (N=9) patients in the present study's cohort.

Depression was common among patients with PD (Karlsen *et al.*, 1999)and MSA. Previous studies showed that the prevalence of depression in MSA is in the range 40-60% in large cohorts of patients (Benrud-Larson *et al.*, 2005; Tison *et al.*, 2006). The findings of the present study suggested that MSA had a higher severity of depression than patients with PD, which was consistent with a prior study (Tison *et al.*, 2006). Greater depression in MSA may be explained by a higher degree of symptom severity, such as autonomic dysfunction and motor severity, in MSA compared to PD. Even though MSA had the highest score of depression among patient groups, there was no correlation between the MSAQoL and HAM-D scores suggesting that the relationship between quality of life and depression in MSA may be non-linear, e.g., it may increase exponentially early or later in the course of the disease. In fact, MSAQoL scores were positively correlated with UMSARS part I (ADL), UMSARS part IV (Global disability scale) and negatively correlated with SE scores. As the UMSARS part I mainly consists of different activities in daily living, it is more likely to have an impact on the global disability scale (UMSARS part IV) as well as SE, e.g., some co-linearity may be evident in these indices. In addition, this study also firstly demonstrates a significantly higher HAM-D score in patients with PAF compared to healthy controls, which is comparable to PD. Unlike PD, there has been no report of pathological brain lesions that could explain the symptoms of depression in patients with PAF. This higher score of HAM-D in PAF compared to controls could reflect the disease-related factors which are caused by the autonomic dysfunction.

Schwab and England Activities of Daily Living Scale scores (SE) were significantly lower in patients MSA compared to PD and PAF. Given that SE reflects how much the

disease limits patients' daily functional capability, the lower score in MSA in this study clearly demonstrated a greater impact in this domain, compared to PD and PAF. The findings of the present study also imply that the severity of motor dysfunction in MSA may not exclusively contribute toward an MSA patient's quality of life as there was no association between UMSARS part II (motor) and MSAQoL. Supportive treatments, particularly those which are relevant to MSA patients' ADL (e.g. management of swallowing and speech, walking aids, treatment for orthostatic symptoms etc.), may provide a better quality of life in MSA patients even in those with a greater motor severity.

The present study reported for the first time that a quality of life measure was lower in patients with PAF, who have only autonomic problems without motor symptoms, compared to age-matched healthy controls. PAF patients had lower SF-36 sub-scores in almost every domain except for the mental health domain. The latter finding is slightly in contrast to the higher depression scores in PAF compared to controls. Possible explanations for this discrepancy could relate to the clear differences in the design of the 2 instruments and that the mental health domain of the SF-36 may assess various aspects of mental health as opposed to depression specifically on the HAM-D. Furthermore, there are some questions in the HAM-D which contain generic symptoms, such as fatigue, tiredness as well as anxiety-related symptoms (sweating, bladder symptoms etc), which can be caused by autonomic dysfunction and are prominent in PAF patients. These findings also perhaps highlight a need for a disease-specific QOL questionnaire for PAF.

There were no association between SF-36 scores and age, disease duration and HAM-D scores in PAF but Schwab and England Activities of Daily Living Scale (SE) scores and female gender were found to be correlated with the SF-36 Physical Health summary measures (PH-SM; an overall index of the physical health related sub-score domains) in PAF. The association between SE and SF-36 scores in PAF could be due to the fact that orthostatic hypotension has a huge impact on patients' activities and mobility, which in turn affects their quality of life by limiting their physical capabilities. The association between lower PH-SM scores and female gender may need further investigation because of the small sample size of female PAF patients (N=6). Interestingly, although statistical comparisons were not possible, the PAF patients in the present study had lower scores compared to PD patients from a previous study in every SF-36 domain except Role limitation Emotional (RE) (Riazi *et al.*, 2003). These observations suggest that autonomic dysfunction can significantly affect quality of life in patients with PAF, and perhaps more so than in PD.

In conclusion, this study indicates that there are different severities of depression and ADL scores among patients with chronic autonomic failure. MSA patients had more severe depression and lower ADL scores than those in PD and PAF. In contrast, PD and PAF seem to have a similar degree of depression, as well as ADL dysfunction, but both are more evident than in healthy controls. Depression, UPDRS part I and II correlated with quality of life in PD rather than UPDRS part III (motor severity), whereas autonomic symptoms, indicated by the SCOPA-AUT scores, clearly correlated with disease duration in PD, further supporting the contribution of non-motor symptoms to quality of life in PD. Despite higher depression scores in MSA, quality of life was strongly correlated with ADL scores, as reflected by the correlations with UMSARS part I, part IV and SE scores. Finally, quality of life in patients with PAF was clearly impaired compared with healthy controls.

Chapter 6

Initial symptoms and clinical characteristics in Pure Autonomic Failure

6. Initial symptoms and clinical characteristics in Pure Autonomic Failure

6.1 Introduction

Pure autonomic failure (PAF) is an idiopathic, sporadic, relatively rare neurodegenerative disorder characterized by post-ganglionic autonomic failure without other neurological symptoms and signs (Kaufmann, 1996). PAF has a substantially better prognosis than other primary chronic autonomic failure disorders, such as Multiple System Atrophy (Mabuchi *et al.*, 2005). The clinical features of PAF, however, have not been well defined and often overlap with other autonomic failure disorders such as MSA and PD, thus sometimes making a judicious diagnosis difficult. Supine and nocturnal hypertension is commonly observed in PAF alongside with orthostatic hypotension (Shannon *et al.*, 1997). There is only one report that details symptoms exclusively associated with orthostatic hypotension in PAF (Mathias *et al.*, 1999). There is minimal research that has examined the presenting symptoms and features of patients that have later obtained a confirmed diagnosis of PAF. Assessing the presenting symptoms of confirmed PAF patients is important as it will provide an objective analysis of features at a time when the diagnosis is often still unclear, and other disorders, such as MSA, can be suspected, and thus may generate key information that may help improve the accuracy and speed of diagnosis in PAF and other chronic autonomic failure disorders

The prognosis of PAF is generally much longer than other chronic autonomic failure disorders, e.g., MSA. The chronic effects of the features of PAF, especially the cardiovascular autonomic dysfunction, have not been thoroughly investigated however. Patients with PAF who have supine hypertension, like patients with essential hypertension, have been reported to develop ventricular hypertrophy (Vagaonescu *et al.*, 2000). The long-term vascular damage in different vascular territories (e.g., renal, retinal, peripheral and cerebral vasculature) must also be considered, but have as yet been fully investigated. For example, the frequent episodes of orthostatic hypotension may cause ischemic damage in several organs, particularly cerebral ischemia, but this has not been adequately researched.

The aim of this study is to therefore further examine the clinical characteristics of PAF by assessing the frequency of initial and presenting autonomic symptoms in confirmed PAF patients, as well as examine the intracranial imaging results and evaluate what factors (if any) are associated with ischemic small vessel lesions in cerebral white matter (WML) in this group of patients.

6.2 Methods

Patients

Medical records of 70 confirmed PAF patients who had been diagnosed between 1998 and 2009 were retrospectively reviewed. PAF patients were diagnosed using existing international consensus criteria (Kaufmann, 1996). Patients who had been diagnosed with or had suspected autoimmune related autonomic failure, such as autoimmune autonomic ganglinopathy (AAG) or paraneoplastic syndromes were excluded. All patients had been tested and had confirmed cardiovascular autonomic failure and had been followed up for at least 3 years without other emerging neurological features, such as cerebellar dysfunction or parkinsonism.

Initial symptoms, clinical history, presenting symptoms and signs

History and relevant information of all patients were retrospectively evaluated from medical records. Factors investigated included, age, gender, initial symptoms, clinical history, presenting symptoms and signs, duration of disease (time from first symptom to the autonomic testing date), current pressor medications (Fludrocortisone, Midodrine, Ephedrine) and number of medications were recorded,

Symptoms were divided into initial symptoms (the first symptom reported by patients) and presenting symptoms (all reported symptoms described by patients in their first visit to hospital/consultation). The initial symptoms were classified as cardiovascular autonomic symptoms (e.g. dizziness, lightheadness, visual disturbances, syncope, fatigue, chest pain and coat-hanger ache), erectile dysfunction, bladder symptoms, gastrointestinal (GI) symptoms (constipation and diarrhoea) and abnormal sweating in line with a previous study (Mathias *et al.*, 1999). The same classification was used for the presenting symptoms but bladder symptoms were subdivided into urinary frequency, urgency and frequent nocturia.

Evaluation of autonomic nervous system function

Results of cardiovascular autonomic function (supine and head up tilting and 24 hr ambulatory monitoring), plasma noradrenaline concentrations assessments and brain MRI reports were reviewed. Patients were asked to stop anti-hypotensive medications 12 hours prior to their cardiovascular autonomic function tests.

Cardiovascular autonomic screening tests (AFT)

AFT was performed using Autonomic Unit protocols (Mathias *et al.*, 2013a). These included pressor tests, Valsalva manoeuvre (VM), deep breathing, Hyperventilation, 10-minute 60 degrees HUT and standing tests as described in Chapter 3. Blood pressure was recorded continuously online using photoplethysmography and intermittently offline using upper arm automated sphygmomanometry. Continuous beat-to-beat cardiac activity was recorded continuously online with ECG monitoring.

24 hour-Ambulatory Blood Pressure and Heart Rate Monitoring (24 hr-ABPM)

All patients were fitted with a 24 hr-ABPM monitor (model 90207, Spacelabs™ Medical, Redmond, Washington) after their AFT as part of their autonomic investigations. BP and HR were recorded every 20 minutes during the day (0800-2300) and every 60 minutes during the night (2300-0800). The average BP and HR were calculated for daytime, night-time and the entire 24-hour period. Patients were classified into 3 groups: dipper (BP fall during night-time>10% compared to daytime), absent nocturnal BP fall or non-dipper (BP fall during night-time<10% compared to daytime) and reverse nocturnal BP profile (BP during night-time higher than daytime). Patients with an average daytime SBP >140 mmHg or DBP >90 were defined as daytime hypertensives and those with nighttime SBP >125 or DBP >75 as nighttime hypertensives according to international guidelines (Pickering *et al.*, 2005).

Plasma noradrenaline (NA) concentrations

Blood samples were collected during supine and head-up tilt tests to assess catecholamine (noradrenaline) concentrations as a biochemical marker of sympathetic neural activity. The plasma level of noradrenaline (NA) was measured using high-performance liquid chromatography with an electrochemical detector.

Brain MRI

Brain MRI was conducted using a 1.5 or 3-tesla MRI scans following a standard MRI protocol which includes an axial T1-weighted, T2-weighted and Fluid-attenuated inversion recovery (FLAIR) images as well as midsagittal T1 in all patients.

Statistical analyses

Normally distributed data were presented as mean (± 1 SD), whereas median (inter-quartile range) were used for non-normally distributed data. Frequency of initial and

presenting symptoms were reported in percentages. Statistical analyses were performed using non-parametric tests; Chi-square analyses and Fisher exact tests were used for analysis of categorical variables and Mann-Whitney U tests for quantitative data.

In order to estimate the risk of cerebral white matter lesions (WML), patients were classified into 2 groups according to their brain MRI results; PAF with WML and without WML. Variables were compared between these two sub-groups with univariate analysis. Potential confounders, including age, disease duration, supine hypertension and nocturnal BP dipping and other factors with significant differences from the univariate analysis were then included in the multiple variable logistic regression analysis in order to determine what factors are associated with an increased risk of cerebral WML.

Relationships between quantitative cardiovascular autonomic function, including plasma supine NA and orthostatic NA changes, and clinical features, as well as relationships between supine hypertension (SHT) and other factors were assessed using multiple linear regression analysis. Statistical analyses were carried out using STATA 11.0 (STATA Corporation, College station, Texas, USA). All tests were 2-sided and a p value of ≤ 0.05 was considered significant.

6.3 Results

A total of 70 records of PAF patients were identified. PAF was more common in males (44 males vs. 26 females) and the age of diagnosis was between 54 and 83 years old. Disease duration ranged from 3-28 years. Other details including anti-hypotensive medications are presented in Table 6.1.

Table 6.1. Demographic data of Pure Autonomic Failure patients

	Total	**Male**	**Female**
Number (N)	70	44	26
Age (yrs)	69 ± 7	69 ± 7	70 ± 8
Disease duration median (IQR), yrs	12 (7-16)	14 (7-17)	12 (7-16)
Pressor drugs (%)			
Fludrocortisone	55	34	21
Midodrine	30	17	13
Ephedrine	17	9	8
Number of pressor drugs (%)			
- 0	11	13	8
- 1	33	39	23
- 2	46	41	54
- 3	10	7	15

Initial symptoms

Most patients had multiple initial symptoms. Out of the 70 patients, the incidence of pre-syncopal symptoms as the initial symptom, including faintness (lightheadedness, dizziness) and visual disturbances (blurring, greying-out, enhanced brightness, darkening, tunnel vision), was the highest (61.4%). Syncope (loss of consciousness, blackouts or fainting) was present in 11/70 (16.9%) of patients. Erectile dysfunction was a common first symptom in males (44%). Decreased sweating was described in 7 (10%) patients while urinary symptoms were reported as an initial symptom in 5 (7.1%) patients. In addition, fatigue induced by standing or walking was an initial symptom in approximately 4% (see Figure 6.1). With regards to gender, there were no difference between males and females in the majority of initial symptoms, including syncope, anhidrosis, bladder and GI symptoms,

coat-hanger ache and chest pain (p>0.05). Interestingly, presyncopal symptoms were reported more in females compared to males (p=0.01), and patients who reported fatigue symptoms are all female.

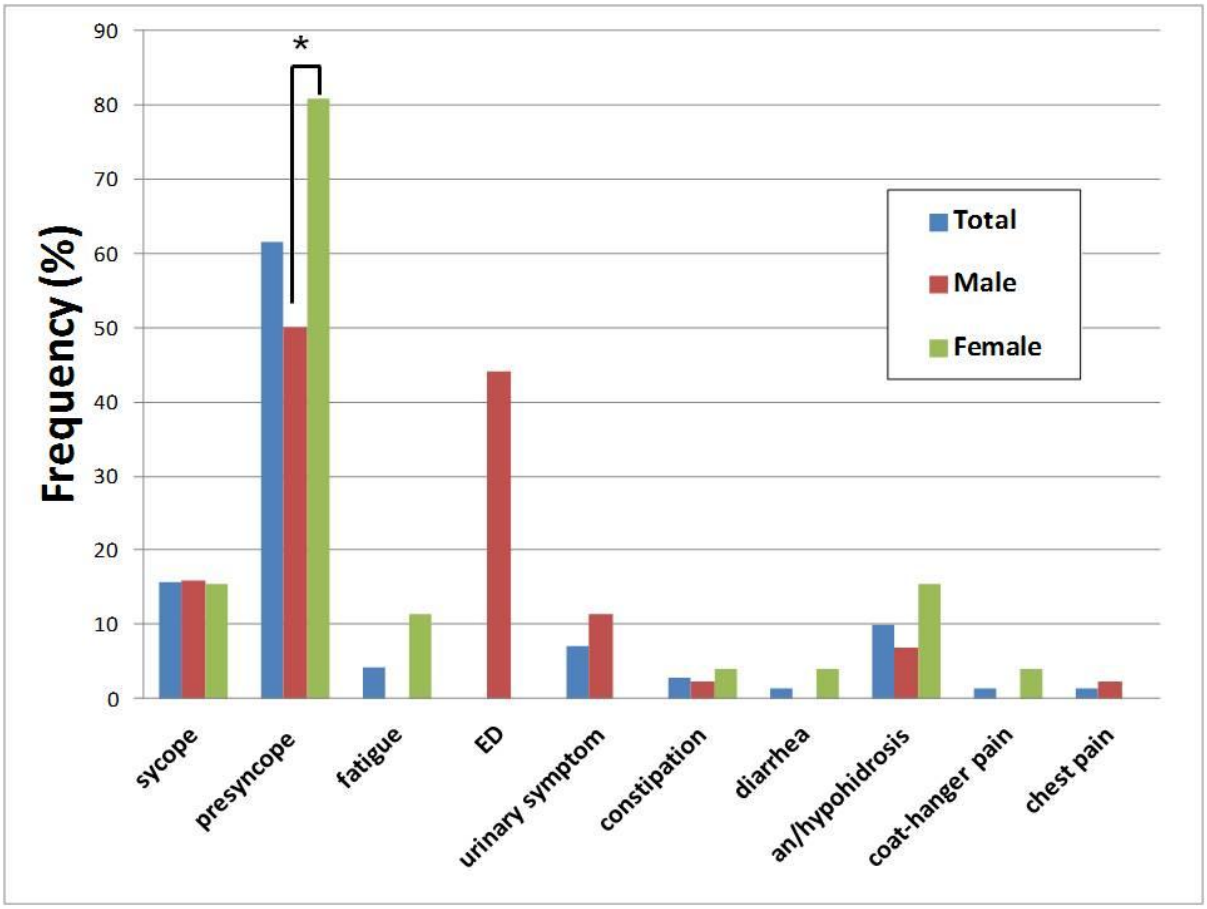


Figure 6.1. Initial symptoms in 70 PAF patients. *p<0.05 male vs. female.

Presenting symptoms at clinic

The most common presenting symptoms at clinic were pre-syncope and syncope (100% and 77.1%, respectively). Erectile dysfunction was prevalent, presenting in 31/44 (70%) of male patients. Hypohidrosis and hyperhidrosis were present in 43/70 (61.4%) and 5/70 (7.1%) patients, respectively. Pain in the suboccipital/paracervical and shoulder region ("coat-hanger" ache), which was typically relieved after lying flat, was reported in 54% of patients. Constipation and urinary frequency were less common, presenting in less than half of the patients. Non-specific symptoms like fatigue occurred in 20/70 (28.6%) of patients while chest pain was reported in 14/70 (20%) patients (see Figure 6.2). With regards to gender, there was no difference in almost all presenting symptoms between male and

female PAF patients. The exception was coat-hanger ache, which was more common in females compared to males ($p<0.05$).

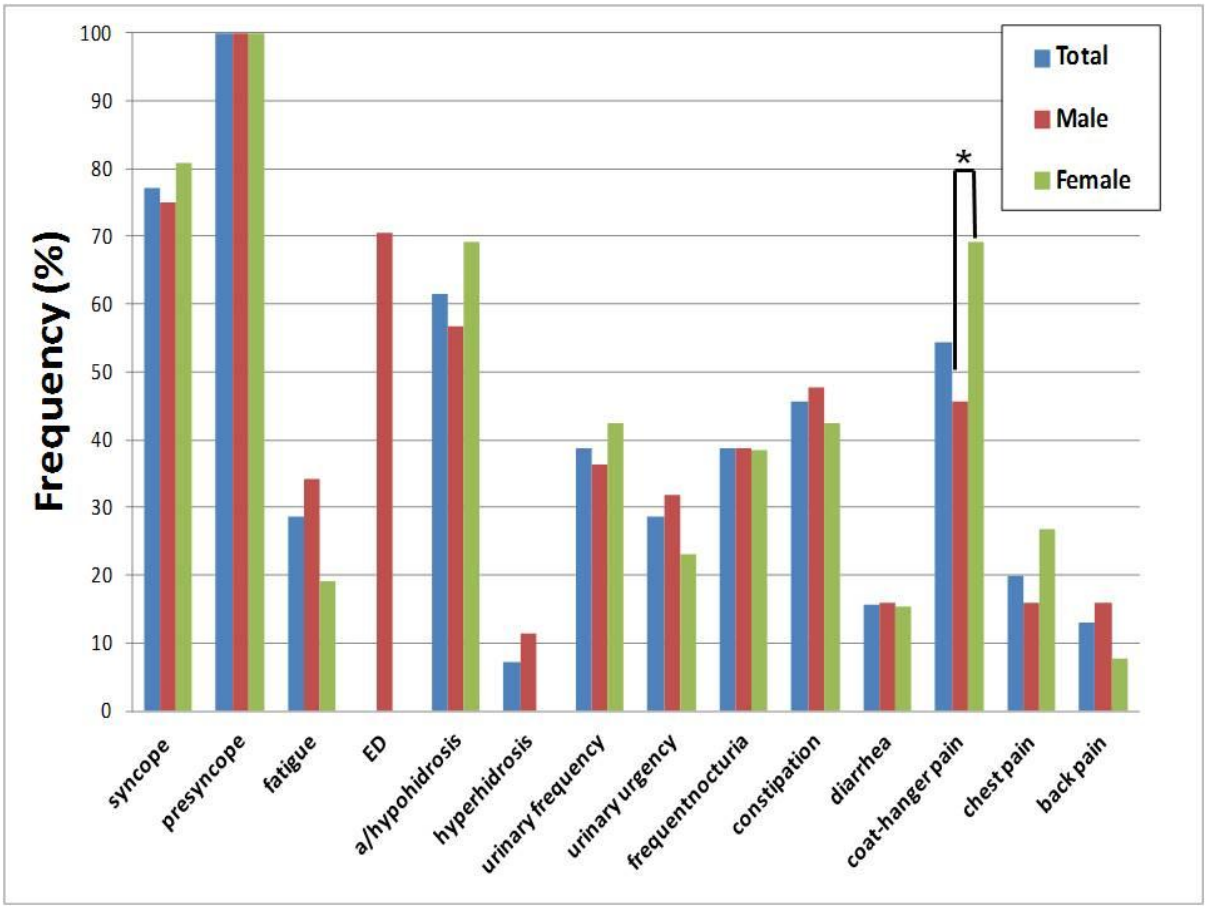


Figure 6.2. Presenting symptoms at clinic reported by PAF patients. *p<0.05 male vs. female.

Laboratory Examinations

Orthostatic hypotension was found in all PAF patients on head-up tilting. Supine hypertension (SBP>150 and/or DBP>90 mmHg) was evident in 51 out of 70 patients (73%). There were no difference in SBP, DBP and HR during supine and HUT, as well as the number of patients with supine hypertension (SHT), between male and female patients (all $p>0.05$).

24 hour-ambulatory blood pressure monitoring (24 hr-ABPM) was performed in 62 patients. A reversed circadian blood pressure rhythm was present in 40 patients (65%), whereas 14 patients (22%) had an absent nocturnal BP fall. Only 8 patients (13%) had a normal BP fall during nighttime. In addition, there were 13 patients (21%) with daytime hypertension and 41 patients (66%) with nighttime hypertension. There were no difference in

daytime or nighttime SBP, DBP and HR, as well as the number of patients with abnormal BP circadian rhythms, between male and female patients (all p>0.05).

Plasma noradrenaline (NA) concentrations were measured in 52 patients. A low concentration (<200 pg/ml) was found in 48 patients (92%) with a minimal rise in plasma NA on head-up tilting (increase of 11.4 ± 20.4 pg/ml, see Table 6.2). There were no differences in plasma NA during supine, HUT, as well as the HUT change between male and female patients (all p>0.05).

Table 6.2. Cardiovascular autonomic function and biochemical results from 70 Pure Autonomic Failure patients.

Cardiovascular autonomic function			
Tilt Table Test (N=70)	**SBP (mmHg)**	**DBP (mmHg)**	**HR (bpm)**
-Supine	166 ± 30	90 ± 14	69 ± 11
-Head-Up Tilting	84 ± 27*	54 ± 15*	75 ± 11*
24 hr-ABPM (N=62)			
-Daytime	134 ± 22	80 ± 13	74 ± 9
- Nighttime	141 ± 23	84 ± 14	70 ± 10
Biochemical analyses			
Plasma Noradrenaline (N=52)	**(NA) (pg/ml)**		
-Supine NA	129.8 ± 58.0		
-HUT NA	141.2 ± 68.0		
-Δ NA change	11.4 ± 20.4		

SBP; Systolic Blood Pressure, DBP; Diastolic Blood pressure, HR; Heart Rate, NA; Noradrenaline

*p<0.05 vs. corresponding supine value

MRI findings

Brain MRI studies were performed in 52 patients. There were radiographic ischemic changes, particularly deep white matter hyperintensity areas on T2 and FLAIR (see Figure 7.3), in 45 patients (86.5%). There was no difference in the number of patients with abnormal white matter lesions between male and female (p>0.05).

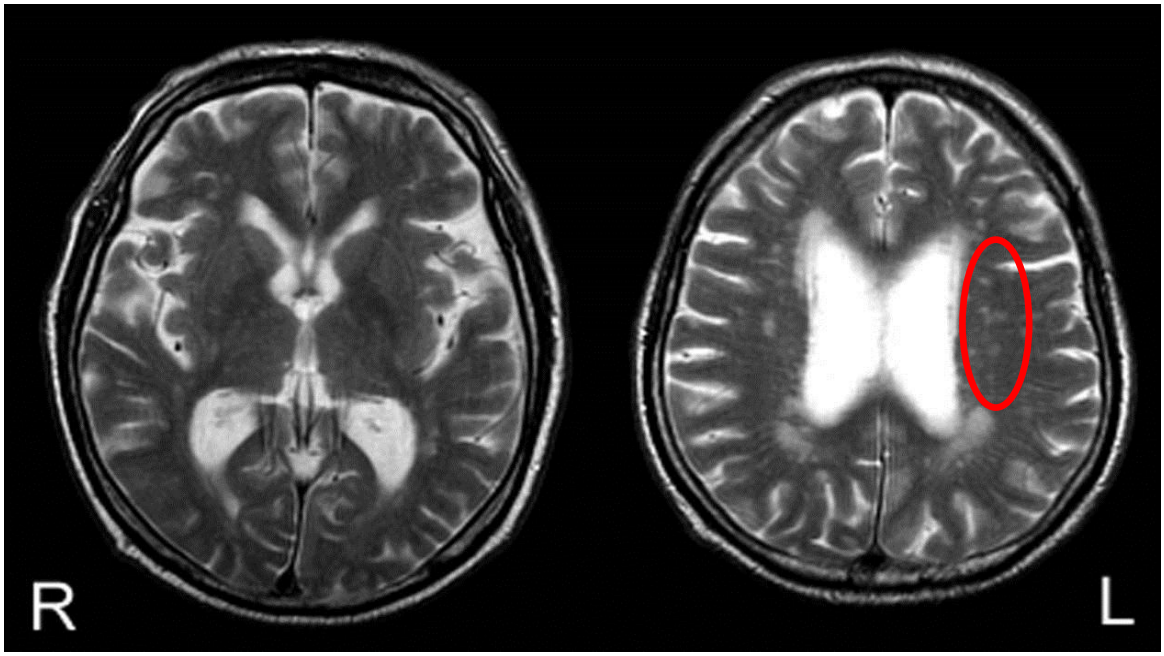


Figure 6.3. An example of a Brain MRI in a patient with PAF (74 years old, male) demonstrating ischemic small vessel lesions in cerebral white matter in the area circled.

Comparison of PAF patients with and without cerebral WML lesions

Patients with cerebral WML were significantly older than those without WML ($p<0.01$). There were no differences in gender and disease duration between sub-groups (see Table 6.3). With regards to autonomic function, there was no difference in supine and HUT BP and HR responses between PAF patients with and without WML (all $p>0.05$). Mean daytime and nighttime BP and HR were not different between sub-groups ($p>0.05$). Supine hypertension was also not different between sub-groups ($p>0.05$). Supine and tilt plasma NA, as well as the orthostatic changes in plasma NA, were not different between the PAF with and without WML sub-groups. With regards to medication, there was no difference in the different types and the number of anti-hypotensive medications between the sub-groups (see Table 6.4).

Table 6.3. Comparison of PAF patients with and without ischemic small vessel lesions in cerebral white matter (WML).

	PAF with WML (n=45)	PAF without WML (n=7)	P-value
Gender, n (%)			
- **Female**	17 (38)	1 (14)	0.4
- **Male**	28 (62)	6 (86)	
Age, yrs, mean ± SD	70 ± 8	62 ± 4	<0.01
Disease duration median (IQR), yrs	12 (7-16)	12 (8-15)	0.66
BP responses, mmHg			
- Supine SBP	168 ± 30	168 ± 35	0.96
- Supine DBP	90 ± 14	87 ± 11	0.59
- Supine HR	68 ± 12	70 ± 11	0.86
- HUT SBP	84 ± 28	80 ± 22	0.85
- HUT DBP	55 ± 15	50 ± 10	0.34
- HUT HR	76 ± 12	72 ± 12	0.29
Supine Hypertension, n (%)	33 (73%)	5 (71%)	0.92
24 hr-ABPM, mmHg			
- Daytime SBP	133 ± 23	132 ± 20	0.98
- Daytime DBP	80 ± 13	82 ± 14	0.79
- Daytime HR	73 ± 9	71 ± 8	0.63
- Nighttime SBP	142 ± 21	146 ± 32	0.94
- Nighttime DBP	83 ± 13	90 ± 17	0.35
- Nighttime HR	70 ± 10	71 ± 11	0.63
24 hr-ABPM profile			
Non-dipper, n (%)	29 (73%)	5 (57%)	0.41
Plasma Noradrenaline (NA), pg/dl			
Supine	137.4 ± 59.8	105.1 ± 35.8	0.12
HUT	151.5 ± 71.3	115.1 ± 36.1	0.18
Δ NA change	12.9 ± 20.3	10.0 ± 9.8	0.82

Table 6.4. Drug comparison of PAF patients with and without ischemic small vessel lesions in cerebral white matter (WML).

	PAF with WML (n=45)	PAF without WML(n=7)	P-value
Anti-hypotensive Medications*			
-Fludrocortisone	36 (80%)	5 (71%)	0.61
-Midodrine	20 (44%)	5 (71%)	0.18
-Ephedrine	10 (22%)	2 (29%)	0.71
Number of anti-hypotensive medications			
-no drug	5 (11%)	1 (14%)	0.81
-1 drugs	19 (42%)	1 (14%)	0.33
-2 drugs	16 (36%)	4 (58%)	0.18
-3 drugs	5 (11%)	1 (14%)	0.81
*Some patients on medications more than one			

Predictive factors of PAF patients with cerebral WML lesion

Age was the only factor that was associated with brain WML in the univariate analyses. Disease duration, a reversed nocturnal BP circadian rhythm and supine HT were included in the multivariate analysis. The results showed that increasing age was associated with a 28% increased risk of having cerebral WML in patients with PAF (OR 1.28; CI 1.04-1.58). There was no association with disease duration, reversed nocturnal BP rhythm and supine hypertension and the occurrence of WML in this group of patients (Table 7.5).

Table 6.5. Predictive factors of ischemic small vessel lesions in cerebral white matter in the PAF WML group.

	Odds ratio (OR)	95% Confidence Interval	P-value
Age	1.28	1.04-1.58	0.02
Supine Hypertension	2.85	0.25-31.92	0.39
Reversed nocturnal circadian rhythm	1.40	0.17-11.80	0.76
Disease duration	1.00	0.88-1.14	0.95

Relationships between autonomic function parameters and disease duration

All autonomic function parameters, including supine, HUT BP and BP changes during HUT, were not correlated with disease duration (all $p>0.05$), except the HUT change in plasma NA (Δ NA change; $p<0.01$). After controlling for age, gender, the presence of supine

HT and Δ SBP change on HUT, there was still a significant correlation between Δ NA HUT change and disease duration (p=0.01; see Table 6.6).

Table 6.6. Multiple regression analysis of the correlation between the changes in plasma noradrenaline (NA) during HUT (pg/ml) and disease duration in PAF patients.

Variable	Parameter estimate	Standard error	P-value
Plasma NA change	-1.28	0.50	0.01
Age	0.02	0.41	0.96
Gender	-8.90	5.87	0.14
Supine Hypertension	5.06	7.64	0.51
Δ SBP change on HUT	-0.01	0.11	0.95

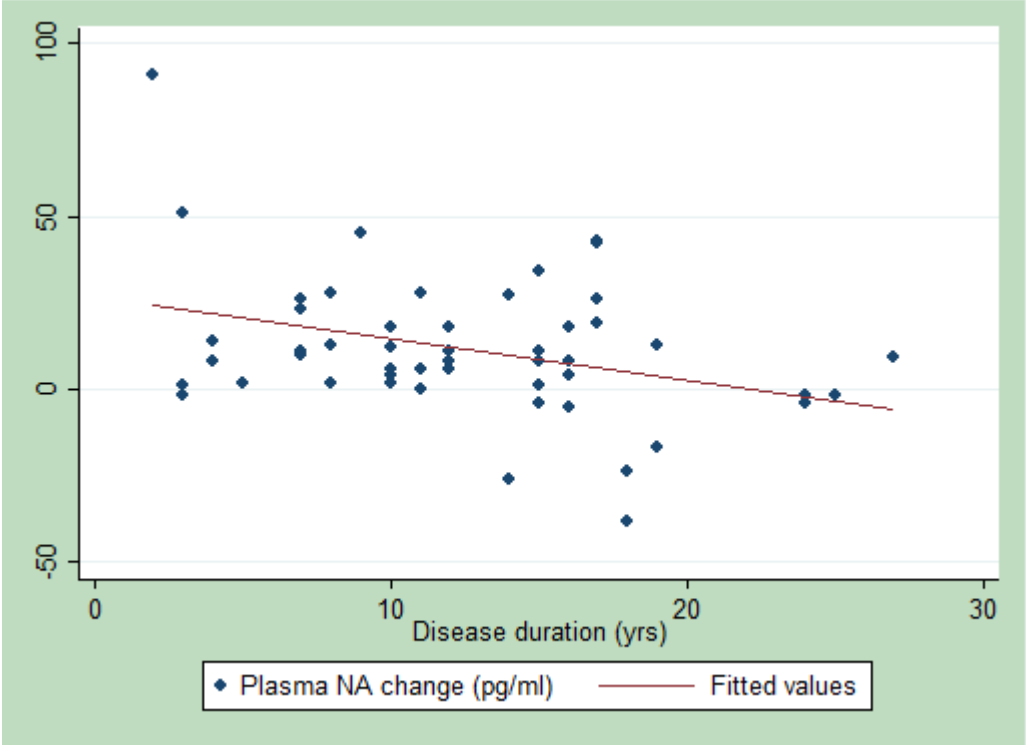


Figure 6.4. Scatterplot showing the correlation between HUT Δ Plasma NA change (Y axis) and disease duration (X axis; R=-0.34, p=0.01) in patients with PAF.

<u>Supine Hypertension (SHT) and associated factors</u>

There was no correlation between SHT and age, gender, disease duration, plasma NA and the number of anti-hypotensive medications (all p>0.05). Nevertheless, the change

in SBP (Δ SBP) during HUT was strongly associated with the presence of SHT in the univariate regression model ($p<0.01$). A change of SBP equal to or greater than 80 mmHg during HUT meant an 11.64 odds ratio of having SHT compared to a SBP fall less than 80 mmHg after taking into account age, gender, disease duration (OR=11.64; CI 2.82-48.02, $p<0.01$; see Table 6.7).

Table 6.7. Predictive factors of supine hypertension in patients with PAF.

	Odds ratio (OR)	95% Confidence Interval	P-value
Δ SBP change on HUT			
≥ 80 mmHg	11.64	2.82-48.02	<0.01
< 80 mmHg	1		
Age			
≥ 68 years	0.99	0.28-3.48	0.99
< 68 years	1		
Gender			
Female	2.91	0.73-11.7	0.13
Male	1		
Disease duration			
≥ 12 years	1.03	0.95-1.11	0.49
< 12 years	1		

6.4 Discussion

The aim of this study was to further examine the clinical characteristics of PAF by assessing the frequency of initial and presenting autonomic symptoms in confirmed PAF patients, as well as examine the intracranial imaging results and evaluate what factors (if any) are associated with ischemic small vessel lesions in cerebral white matter (WML) in this group of patients. The main findings were that orthostatic symptoms (e.g. dizziness and lightheadedness) are universal initial symptoms in PAF and presyncopal symptoms were more commonly reported in females. In addition, up to 60% of patients reported sudomotor symptoms in their first consultation. The results also indicated that erectile dysfunction (ED) was prevalent in males with almost half reporting it as an initial symptom and up to 70% at the first hospital visit. Non-specific symptoms, such as fatigue and chest pain were experienced in approximately 20% of the present study's cohort.

The study also showed that supine hypertension was commonly observed and strongly associated with orthostatic hypotension (OH). Plasma noradrenaline (NA) was usually less than 200 pg/ml whilst supine and the plasma NA change during HUT was also less than 100 pg/ml. Additionally, more than half of PAF patients also revealed a pathological reversed BP circadian rhythm and brain MRI studies revealed white matter lesions (WML) were typical presenting in more than 80% of patients.

The results of the current study are consistent with a previous study that reported presyncopal symptoms and syncope as the most common symptoms in PAF patients at the out-patient clinic or as in-patients (Mathias *et al.*, 1999). “Coat-hanger” ache was also frequently reported in the present study with a similar figure to that previous study (Mathias *et al.*, 1999). However, the presenting symptoms of chest pain and fatigue were less common in the current study, which could be due to a lower accuracy of records as often seen in retrospective data analyses. Other possible explanations are that non-specific symptoms (e.g. chest pain and fatigue) were less likely to be voluntarily reported from the patients without questioning from the clinicians. Similarly, anhidrosis and hypohidrosis were uncommon as an initial symptom in contrast to the reported symptoms at the first consultation. Nevertheless, abnormal sweating may have an insidious onset and may be less likely to be detected and/or reported by patients without a direct question. Urinary symptoms were less commonly reported as an initial symptom but these become more prominent during the disease progression, e.g., at the first consultation. This finding is in concordance with previous studies showing that urinary dysfunction seems to appear after orthostatic symptoms in PAF, which is contrary to patients with Multiple System Atrophy (Mabuchi *et al.*, 2005). This difference in bladder progression may aid in differentiating between these two conditions. Interestingly, coat-hanger ache was more frequently reported in females compared to males in this study’s cohort. The cause of coat-hanger ache is thought to be due to a transient reduction in perfusion of muscles in the neck and shoulder areas during standing in patients with OH. Therefore, the higher prevalence of this symptom could be due to a relative small muscle mass in females compared to males. This may increase the chance of developing this symptom. Nonetheless, no study has investigated this hypothesis and this warrants further investigation.

Not surprisingly, plasma NA was found to be lower with a smaller rise during HUT in the majority of the patients which is a typical finding in PAF (Goldstein *et al.*, 2003a). These findings reflect the involvement of a post-ganglionic sympathetic lesion in PAF. This is in contrast to MSA patients, generally accepted as a pre-ganglionic autonomic disorder, who

tend to have a normal supine plasma NA level (Mathias *et al.*, 2013a). On the other hand, Esler et al reported a nearly normal plasma NA levels in 4 patients with PAF but there was no information regarding the disease duration (Esler *et al.*, 1980). The reason for this difference in plasma NA is unclear. Given that the patients in the present study had isolated autonomic failure without any additional neurological features for at least 3 years, they may have had a longer disease duration compared to those in the previous study.

There was a significant association between the plasma NA HUT change (between supine and upright) and disease duration in PAF patients in the current study. There has been no study that has investigated this relationship and this is the first report of such a relationship in PAF. This finding suggests that the plasma NA HUT change may be lower as the disease progresses possibly reflecting a greater impairment of post-ganglionic sympathetic nerve activity. Furthermore, this finding also implies that the measurement of the plasma NA HUT change should be cautiously interpreted if such measurements are taken from PAF patients with short disease duration as the results could be relatively normal.

Although the exact prevalence of SHT in PAF is unknown it was reported in more than 50% of patients with primary chronic autonomic failure (Shannon *et al.*, 1997). The prevalence was higher in this cohort (70%), which could be due to a variety of factors, including, differences in patient cohorts, amount and frequency of medications and assessment protocols. Supine hypertension was also strongly associated with the degree of OH in patients with PAF in the current study, consistent with previous work (Goldstein *et al.*, 2003b) highlighting a link between the degree of autonomic failure and the severity of supine hypertension. The mechanism of supine hypertension in PAF remains unclear. Supine hypertension was not associated with disease duration, age or gender in the current study. In MSA, supine hypertension results from an inappropriate residual sympathetic tone but this finding is inconsistent in patients with PAF (Shannon *et al.*, 2000). Other suggested mechanisms of supine hypertension include the movement of intra- and extra-vascular fluid from the peripheral to the central vascular compartment, impaired baroreflexes and supersensitivity to circulating catecholamines or to pressor agents (Iodice *et al.*, 2011). Regardless of the pathophysiology, supine hypertension has a significant impact on OH treatment by limiting the use of pressor medications.

Previous studies suggested that PAF patients are at higher risk of end-organ damage due to their cardiovascular autonomic dysfunction. Garland et al found a declined renal function in patients with PAF (Garland *et al.*, 2009). Serum creatinine was significantly higher with a lower estimated glomerular filtration rate in PAF patients with supine hypertension

(SHT) compared with those without SHT. These findings are similar to a report of hypertensive cardiac ventricular hypertrophy in PAF (Vagaonescu *et al.*, 2000). As BP fluctuations are universal in patients with PAF, the frequent cerebral WML among patients with PAF in this study may reflect an increase in the risk of stroke in this patient group. The risk factors that could be associated with WML in PAF patients in this cohort were investigated. Age was found to be the only predictive factor that was independently associated with WML in this study. Supine and HUT blood pressure, mean BP daytime and nighttime, a reversed circadian BP profile and supine hypertension were not associated with WML. Interestingly, there was also no difference in the risk of WML and the types and the number of pressor medications and cerebral WML in this study. Although there is no record of how many of the PAF patients developed symptomatic stroke, this finding supports the idea that the WML lesions in patients with PAF were less likely to be related to medications. Nonetheless, it is routine practice to monitor and re-evaluate autonomic function tests before adjusting/increasing medications.

The WML findings in the current study are consistent with a previous study from Struhal et al. which showed an association between WML and age in patients with PAF. This study also found that the WML incidence was not increased in PAF in comparison with age-matched control data from large prospective studies (de Leeuw *et al.*, 2001; Launer, 2004). It is not clear whether individuals with PAF and WML have a higher risk of cerebrovascular diseases due to limited follow-up data. A recent study reported a small number of PAF patients who developed stroke (Struhal *et al.*, 2013). Nevertheless, this finding needs further investigation by using a longitudinal design to follow-up how many patients actually develop symptomatic stroke to confirm this hypothesis.

The limitations of this study are the retrospective design and the lack of detailed follow-up and also other metabolic profiles that could potentially increase a risk of cardiovascular disorders in patients with PAF. A long-term follow-up in these patients would provide more information regarding cardiovascular events in this patient group.

In conclusion, this large cohort of PAF patients demonstrated that the most common initial symptoms in both male and females were of orthostatic intolerance, while erectile dysfunction was prevalent in males. Urinary symptoms, sweating abnormalities and gastrointestinal symptoms were more likely to gradually develop in the course of the disease. Cerebral WML were a very frequent finding in brain MRI imaging and age was an independent factor of these WM changes. Plasma NA was lower during supine with only a

minimal rise on HUT and longer disease duration of PAF was negatively correlated with the plasma NA HUT change.

Chapter 7

Electrogastrography (EGG) in primary chronic autonomic failure

7. Electrogastrography (EGG) in primary chronic autonomic failure

7.1 Introduction

Gastrointestinal (GI) symptoms can sometimes be prominent in autonomic disorders, such as PAF, MSA and PD. Abdominal pain, bloating and nausea/vomiting are commonly reported in MSA, as a result of delayed gastric emptying times (Thomaides *et al.*, 2005). The gastrointestinal tract is controlled by extrinsic (sympathetic and parasympathetic) and intrinsic (enteric nervous system-ENS) pathways. These systems are vital for maintaining effective co-ordination of digestion. The extrinsic pathways, projecting from the dorsal motor nucleus of the vagus nerve has a crucial role in the initiation of gastric motility (Travagli *et al.*, 2006). This is followed by peristaltic waves, which propel food contents towards the pylorus. These peristaltic waves are coordinated by gastric electrical activity, which is generated by the interstitial cells of Cajal (ICCs) on the greater curvature of the stomach (Sanders *et al.*, 2006). The ICCs are considered gastric pacemaker cells because they generate rhythmic depolarization with the same frequency as slow waves of gastric myoelectrical activity (Camborova *et al.*, 2003) and also relay signals between the ENS and the smooth muscle cells (Huizinga *et al.*, 2004; Ward & Sanders, 2006). The frequency of gastric myoelectrical activity is approximately 3 cycles per minute in healthy subjects (Chang, 2005) and can be easily detected by cutaneous electrogastrography (EGG).

A number of studies have used EGG in various gastric disorders, for instance diabetic gastropathy (Koch, 2001), functional dyspepsia (van der Voort *et al.*, 2003) and recently in Parkinson's disease and Multiple System Atrophy (Sakakibara *et al.*, 2009). Gastric electrical dysrhythmia was detected in patients with PD (Lu *et al.*, 2004) but not in MSA (Suzuki *et al.*, 2005). Although no pathological studies of the involvement of ICC in autonomic failure disorders have previously been reported, there is evidence of alpha-synuclein deposition in Auerbach's and Meissner's plexuses, which are closely linked to the ICC, in patients with PD (Braak *et al.*, 2006b). Abnormal gastric slow waves in PD might reflect gastrointestinal involvement which is considered a potential early marker of the pre-motor stage of PD and is consistent with a neuropathological study of Braak *et al* (Braak *et al.*, 2003).

PAF is characterized by orthostatic hypotension (OH) without other neurological features (Kaufmann, 1996). Symptoms of dysfunction of other autonomic domains, e.g., sexual and gastrointestinal function, are also common in PAF. Although there has been no study on the prevalence and pathophysiology of gastrointestinal (GI) symptoms in PAF,

these features (e.g. abdominal fullness, nausea/vomiting and constipation) are frequently reported among patients with PAF (see Chapter 6). GI dysfunction can even be a presenting feature in PAF patients (Yamanaka *et al.*, 2006) (see Chapter 6). PAF and PD share a few common features, such as hyposmia, reduced cardiac MIBG reuptake and a presence of Lewy bodies, both in skin and the central nervous system (Hirayama *et al.*, 1995; Ikemura *et al.*, 2008; Silveira-Moriyama *et al.*, 2009b; Shishido *et al.*, 2010). These features raise a question of whether or not PAF is a limited form of PD without motor features (Kaufmann & Goldstein, 2010). Given these common features of PD and PAF and abnormal EGG findings previously reported in PD (Sakakibara *et al.*, 2009), PAF may also have a similar pattern of EGG dysfunction as to patients with PD. Furthermore, MSA represents a pre-ganglionic autonomic disorder whilst PAF has mostly post-ganglionic autonomic pathology (Kaufmann *et al.*, 2001). Comparing between these disorders would thus be an ideal model to evaluate autonomic disorders with different pathological lesions that present with similar clinical characteristics. To date, there have not been any studies investigating EGG activity in patients with PAF. The aim of this study was therefore to evaluate gastric myoelectrical activity (GMA) before and after standard liquid meal ingestion in PAF patients. It was hypothesized that PAF patients would have gastric dysrhythmia in line with the nature of post-ganglionic autonomic pathology in this disorder, similar to PD. MSA patients were also examined in order to compare an autonomic disorder with a different lesion to PAF.

7.2 Methods

Participants

Patients with PAF and MSA (with confirmed autonomic failure) were recruited. All patients fulfilled international consensus diagnostic criteria (as described in Chapter 3). Each participant was asked to refrain from eating for at least three hours before testing. All patients continued their current medications as usual. None of patients had history of dyspepsia, functional dyspepsia or gastrointestinal dysmotility disorders. Patients with a previous history of gastrointestinal surgery were excluded.

Clinical history

History and relevant information of all patients were recorded. These included, age, gender, duration of disease (time from diagnosis to testing date), medications and presenting symptoms. Patients were asked whether or not they had any following autonomic symptoms within the last 3 months including; upper gastrointestinal (UGI) symptoms

(nausea/vomiting, abdominal fullness), constipation, orthostatic intolerance symptoms (lightheadedness or dizziness when standing), bladder symptoms.

Measurements and protocols

Orthostatic BP and HR responses were assessed using a liquid meal challenge test with a 10-minute head-up tilt test (HUT) at 60° before and after the liquid meal. All participants rested for 15 minutes before undergoing a HUT challenge for up to 10 min. After returning to the supine position, participants rested for 15 minutes before a standard liquid meal was ingested through a straw whilst supine. The liquid meal consisted of 20g glucose and 60 g Complan® made up to 300 ml with full fat milk. All participants then rested for 45 minutes before the orthostatic challenge was repeated. BP and HR were measured using upper arm sphygmomanometry (GE Medical Systems, Tampa, FL, USA).

A four-channel cutaneous EGG device (Nipro EG; Nipro, Japan) was used to record gastric myoelectrical activity at a sampling rate of 1 Hz. Five surface electrodes were placed on 5 different sites on the abdomen as previously described (Sakakibara *et al.*, 2009). EGG was recorded in the supine position during the period after the first and before the second HUT, which included 15-minute before (pre-prandial EGG recording) and 45-minute after meal ingestion (post-prandial EGG recording, Figure 7.1).

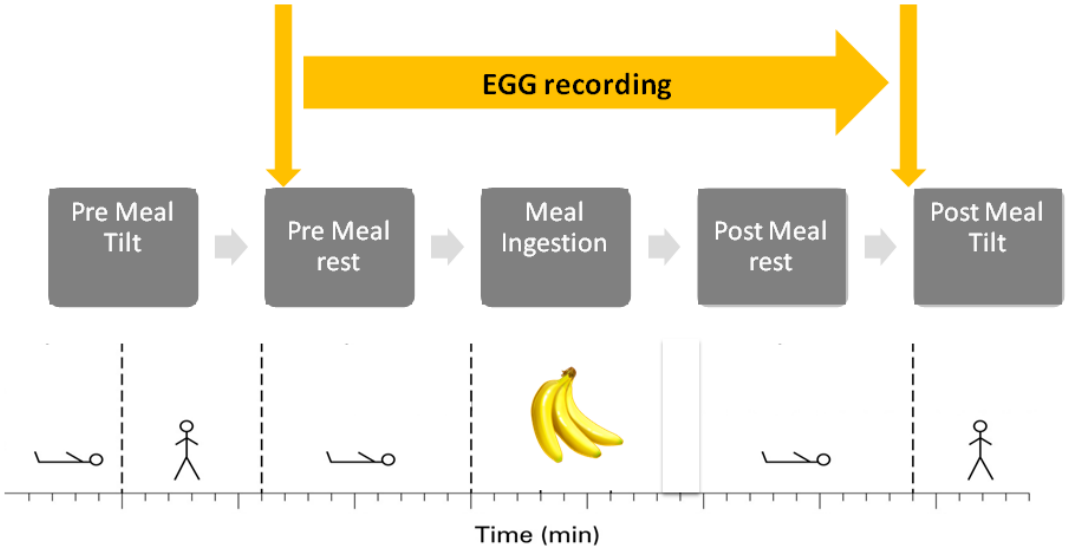


Figure 7.1. Schematic showing the standard liquid meal protocol and EGG recording periods.

EGG analysis

EGG data were analysed offline (EGS2 Ver 3.1 software, EG, Gram Co., Japan). Running spectral analysis was performed using Fast Fourier Transformation (FFT in the range 1.6 to 9.0 cycles per minute (cpm)). FFT was applied to consecutive 512-second data periods with a 392-second overlap. The dominant frequency (DF) was defined as the frequency at which the overall power spectrum showed peak power between 2.0 and 4.0 cpm. The frequency ranges were also divided into 3 different types, including low frequency range (LFR; 1.6-2.0 cpm), normal frequency range (NFR; 2.0-4.0 cpm), and high frequency range (HFR; 4.0-9.0 cpm). LFR%, NFR% and HFR% were calculated from the ratios of LFR, NFR and HFR components of total power, respectively. The instability coefficient of dominant frequency (ICDF) is an indicator of the variability of the DF and was calculated as the ratio of the standard deviation and the mean of the EGG dominant frequencies. ICDF was calculated in 15-minute segments; pre- and post (30 min)-prandial periods.

Statistical analyses

Normally distributed data are presented as mean (± 1 SD), whereas median (inter-quartile range) were used for non-normally distributed data. Independent t-tests or repeated measures ANOVA were used to compare BP and heart rate responses and the DF and ICDF responses between PAF and MSA patients before and after the liquid meal challenge. If there was a main effect of patient group or time, post hoc analyses were performed using Bonferroni corrections for multiple comparisons. All data were analysed using commercial available software (STATA 11.0; STATA Corporation, College station, Texas, USA). All tests were 2-sided and a p value of $\leq$0.05 was considered significant.

7.3 Results

23 patients (15 PAF and 8 MSA) were recruited. Patients with PAF were significantly older and had a longer disease duration compared with MSA (both $p<0.01$, see Table 6.1). Twenty seven percent (4/15) of the PAF patients had upper gastrointestinal (UGI) symptoms, including nausea and abdominal fullness, while these were reported in 38% (3/8) of patients with MSA. Constipation was common in both PAF and MSA (53% and 63%, respectively). All patients with PAF and MSA both had orthostatic hypotension and bladder symptoms (urinary frequency/urgency). Other demographic features are presented in Table 7.1.

Table 7.1. Patient demographic data

Variable	PAF	MSA
Number	15	8
% Female	67	63
Age at testing, Mean (SD), yrs	69 (8)*	59 (6)
Disease duration, Median (Interquartile range), yrs	9 (6-11)*	4 (3-7)
UGI symptoms		
Nausea	2 (13%)	2 (25%)
Abdominal fullness	2 (13%)	1 (12.5%)
Autonomic dysfunction		
Orthostatic intolerance symptoms	15 (100%)	8 (100%)
Constipation	8 (53%)	5 (63%)
Bladder symptoms	15 (100%)	8 (100%)
*p<0.05 vs. MSA		

Cardiovascular responses to HUT

Supine blood pressure (BP) and heart rate (HR) were not significantly different between PAF and MSA patients. There were also no differences in HR and BP during head-up tilting (HUT) between groups. All MSA and PAF patients had lower blood pressure during HUT ($p<0.05$) and fulfilled the criteria for OH (Kaufmann, 1996) but the degree of OH was not different between the two disorders ($p>0.05$).

After liquid meal ingestion, supine BP was significantly reduced compared with supine pre-prandial BP in both MSA and PAF patients (both $p<0.05$). There was no change in post-prandial HR however ($p>0.05$). BP was significantly lower during post-prandial HUT compared to pre-prandial HUT but there was no difference between MSA and PAF patients (both $p<0.05$). The post-prandial orthostatic BP change was not different in MSA compared to PAF ($p>0.05$; Table 7.2).

Table 7.2. Blood pressure and HR during supine, head-up tilting and orthostatic changes before and after liquid meal ingestion in MSA and PAF patients.

Variable	PAF (n=15)	MSA (n=8)
Pre-prandial		
Supine SBP	153±29	145±21
Supine DBP	87±18	84±13
Supine HR	63±10	65±11
Tilt SBP	80±22$^{\$}$	94±25$^{\$}$
Tilt DBP	52±11$^{\$}$	60±14$^{\$}$
Tilt HR	71±16$^{\$}$	73±16$^{\$}$
Orthostatic ΔSBP	-73±31	-51±28
Orthostatic ΔDBP	-36±18	-24±20
Orthostatic ΔHR	9±19	9±8
Post-prandial		
Supine SBP	120±35$^{\#}$	128±21$^{\#}$
Supine DBP	69±15$^{\#}$	68±15$^{\#}$
Supine HR	70±11	72±10
Tilt SBP	66±17$^{\$\#}$	75±23$^{\$\#}$
Tilt DBP	43±9$^{\$\#*}$	53±10$^{\$\#}$
Tilt HR	77±14$^{\$}$	79±13$^{\$}$
Orthostatic ΔSBP	-54±31	-53±17
Orthostatic ΔDBP	-26±15	-15±12
Orthostatic ΔHR	7±13	7±6

Values are mean±SD
*p<0.05 vs. MSA, $^{\$}$p<0.05 vs. corresponding Supine value, $^{\#}$p<0.05 vs. corresponding pre-prandial value

EGG responses

Baseline (Pre-prandial)

There was no significant difference in pre-prandial DF between groups (p>0.05). ICDF was significantly higher in PAF compared to MSA during baseline (p<0.05).

Post-prandial

Post-prandial DF was significantly higher after meal ingestion in both PAF and MSA groups ($p<0.01$). There was no difference in the change in DF between groups ($p>0.05$). ICDF was significantly higher post-prandial ($p<0.05$) and was still significantly higher in PAF compared to MSA ($p<0.05$). The change in ICDF post-prandial was not different between groups (>0.05). The LFR%, NFR% and HFR% were not different between pre- and post-prandial conditions as well as between the two disorders (Table 7.3).

Table 7.3. Mean (SD) Pre- and post-prandial and changes in Dominant frequency (DF), instability coefficient of dominant frequency (ICDF), low frequency range (LFR%), normal frequency range (NFR%), high frequency range (HFR%) in PAF and MSA patients.

Variable	PAF (N=15)	MSA (N=9)
DF (cpm)		
- Pre-prandial	2.58±0.24	2.61±0.32
- Post-prandial	2.75±0.23**	2.77±0.31**
- Δ Pre-post prandial	0.17±0.21	0.16±0.34
ICDF (%)		
- Pre-prandial	12.84±4.23$^{\$}$	6.09±2.39
- Post-prandial	14.98±3.90*$^{\$}$	7.82±1.57*
- Δ Pre-post prandial	2.14±3.66	1.73±2.89
LFR%		
- Pre-prandial	6.41±2.75	6.07±5.09
- Post-prandial	5.84±3.49	5.00±3.56
- Δ Pre-post prandial	-0.56±3.73	-1.07±5.56
NFR%		
- Pre-prandial	88.09±5.88	89.06±4.30
- Post-prandial	90.57±4.75	91.65±4.14
- Δ Pre-post prandial	2.48±6.80	2.58±5.43
HFR%		
- Pre-prandial	5.46±4.10	4.96±1.86
- Post-prandial	3.59±1.52	3.44±1.12
- Δ Pre-post prandial	-1.88±4.43	-1.53±2.30
**p<0.01 vs. pre-prandial, *p<0.05 vs. pre-prandial, $^{\$}$p<0.05 vs. MSA		

7.4 Discussion

The aim of this study was to evaluate gastric myoelectrical activity (GMA) before and after standard liquid meal ingestion in PAF patients. It was hypothesized that PAF patients would have gastric dysrhythmia in line with the nature of post-ganglionic autonomic pathology in this disorder, similar to PD. MSA patients were also examined in order to

compare an autonomic disorder with a different lesion to PAF. The main findings were that PAF patients had gastric dysrhythmia as demonstrated by a significantly higher ICDF compared to MSA patients.

Abnormal EGG findings were previously reported in patients with MSA and Parkinson's disease (PD) but investigations have not been performed in PAF, an autonomic failure disorder with overlapping features with MSA and PD. Prior studies reported a low ICDF and normal post-prandial DF in MSA (Sakakibara *et al.*, 2009). On the other hand, patients with PD had a high ICDF and a blunted increase in DF after meal ingestion compared with controls (Soykan *et al.*, 1999). A subsequent study also demonstrated a reduction of the percentage of 'normal' gastric slow waves, as well as a lack of an increase in DF post-prandial, in PD compared with controls (Lu *et al.*, 2004).

Even though there has been no previous pathological study reporting the involvement of the ENS in patients with PAF, various clinical and pathological features in PAF, which are also common to PD patients, are suggestive of ENS dysfunction. Apart from parkinsonism, PAF and PD have several clinical features in common. These include hyposmia and various domains of autonomic dysfunction (e.g. OH, bladder dysfunction, sweating dysfunction, constipation) (Goldstein & Sewell, 2009; Mathias & Bannister, 2013). In addition, both PD and PAF also share abnormal pathological findings in the central nervous system (Lewy body deposition in the substantia nigra or locus ceruleus (Hague *et al.*, 1997)) and in the periphery (Lewy bodies and neuritis in the dermis of a patient with PAF (Shishido *et al.*, 2010)). These findings lead to the term "Lewy body synucleinopathies", which include both PD, PAF and dementia with Lewy bodies (DLB) (Kaufmann & Goldstein, 2010). Some experts proposed that these disorders may actually be the same entity but the site of Lewy body pathology will determine the clinical manifestation (Kaufmann & Goldstein, 2010).

Given these common features between PD and PAF, gastric dysrhythmia, as evidenced by a high ICDF, during baseline and after the liquid meal ingestion in PAF patients in the present study may have resulted from an impaired gastric pacemaker in line with the finding of high ICDF in a patient with PD supporting the post-ganglionic autonomic pathology in these disorders (Arai *et al.*, 2000). Nevertheless, there is no previous study specifically looking at the pathology of gastric pacemaker cells in PAF. Given similar gastrointestinal symptoms and ICDF findings in both disorders, gastric dysrhythmia in patients with PAF might reflect the involvement of these cells similar to that in PD patients.

Previous studies demonstrated a transient increase of DF after meal ingestion which returns to pre-meal levels in healthy controls while ICDF remains relatively unchanged (Sakakibara *et al.*, 2009). Although an age-matched healthy control group as a comparison group was not used in the present study, the findings in MSA patients were consistent with previous studies in that DF was normal and ICDF was significantly lower in MSA (Suzuki *et al.*, 2005; Sakakibara *et al.*, 2009). ICDF is thought to reflect parasympathetic function (Muth *et al.*, 1998). The decrease of ICDF in MSA may be caused by a reduced parasympathetic vagal outflow. Comparing with the same control data from these previous studies, the DF values were similar and the ICDF values were higher in the PAF patients in the present study (Sakakibara *et al.*, 2009). Gastric dysrhythmia was previously reported in patients with functional dyspepsia (Lin *et al.*, 1999). There was no difference in the number of gastrointestinal (GI) symptoms between MSA and PAF patients in the present study. In addition, the majority of participants did not report upper GI symptoms. This would indicate that the abnormal ICDF in PAF cannot entirely be explained by their underlying GI symptoms and that there might be a slight disproportion between the pathophysiology and symptomology of GI dysfunction in PAF. The present study also demonstrated that the orthostatic BP and HR changes were not different between MSA and PAF patients indicating that the abnormal ICDF findings in PAF were not due to differences in cardiovascular autonomic function.

MSA patients can present with a variety of autonomic features, such as orthostatic hypotension, urinary frequency/urgency and gastrointestinal dysfunction, which are also evident in PAF. Overlapping autonomic features in MSA and PAF can sometimes make it difficult to differentiate PAF and MSA, particularly in early stages (Iodice *et al.*, 2012). The difference between ICDF in MSA and PAF in our study warrants further evaluation of whether or not evaluating gastric slow wave activity in conjunction with a liquid meal challenge may be a potential non-invasive investigation in patients who present with autonomic failure. There is no consensus on the amount and/or variety of autonomic investigations in order to distinguish MSA and PAF, apart from assessing hemodynamic responses after clonidine infusion (Young *et al.*, 2006). Vasodepressor effects of clonidine were reported to be more prominent in MSA patients in contrast to those in PAF which may reflect the difference in remaining function between these disorders. Some experts use a criterion of isolated autonomic failure without additional neurological features after years of follow-up to ensure the diagnosis of PAF (Young *et al.*, 2006). Nevertheless, there are a few

reports of some patients with PAF who developed MSA features 5 years after the onset of autonomic failure (Iodice *et al.*, 2012).

In conclusion, a significantly higher ICDF in PAF patients indicates abnormal gastric myoelectric activity and gastric pacemaker function in PAF and supports the hypothesis of post-ganglionic impairment of PAF, similar to EGG findings and pathology in PD patients. In contrast, MSA patients appear to have normal or even lower ICDF. These findings also suggest that EGG may potentially be a non-invasive adjunct investigation to help differentiate patients with MSA from PAF.

Chapter 8

Discussion and Future Directions

Chapter 8

8. Discussion and Future Directions

8.1 Discussion

The overall aim of this thesis was to compare and contrast various aspects of autonomic function, non-motor features and quality of life between the most common forms of primary chronic autonomic disorders, Multiple System Atrophy (MSA), Parkinson's disease with and without autonomic failure (PD+AF and PD) and Pure Autonomic Failure (PAF). A variety of non-invasive autonomic and allied, as well as clinical, investigations were selected and used to compare disorders and examine how these aspects of autonomic disease contribute to quality of life in these different disorders and achieve the specific aims of the thesis.

Patients with chronic autonomic disorders, namely MSA, PAF and PD+AF, share a fundamental feature, orthostatic hypotension (OH) as part of cardiovascular autonomic failure/dysfunction. Given that reason, the diagnosis in these conditions can sometimes be difficult if patients present with isolated autonomic failure without other neurological features. Both PD and MSA patients often present with autonomic failure and parkinsonian features, while patients with PAF commonly present with autonomic failure but can develop parkinsonian features in the later stage of disease after years of follow-up. These heterogeneous progressions can lead to difficulties in diagnosis of these disorders. It was not clear whether there were any measures that could be useful in aiding such a diagnosis. In fact, there were only a few studies comparing cardiovascular autonomic function between these disorders.

In a series of studies in Chapters 4, cardiovascular autonomic function was examined using a variety of methods, including, traditional laboratory autonomic laboratory tests (e.g., head-up tilting and pressor tests), novel assessment of indices of cardiovascular autonomic function and 24 hr-ambulatory blood pressure monitoring, in MSA, PAF and PD patients (with and without autonomic failure). The first study (Chapter 4.1) systematically examined cardiovascular autonomic function in PD+AF, MSA and PAF patients who all met the criteria of OH. There was no difference in the degree of OH between MSA and PD+AF but it was more severe in PAF. This study confirmed previous studies reporting similar impairments in almost all cardiovascular autonomic function measures in these disorders, except the blood pressure recovery time (BRPT) and baroreflex sensitivity (BRS), derived from the Valsalva Manoeuvre (VM), which were the only 2 cardiovascular autonomic function indices that were

more severely impaired in MSA compared to PD+AF. This result suggests that BPRT and BRS could be additional autonomic markers to help distinguish MSA from PD (particularly in those with orthostatic hypotension). This study was also showed that cardiovascular autonomic function was also similar among MSA sub-types (e.g., the parkinsonian vs. the cerebellar sub-types), who have OH. It was also apparent that cardiovascular autonomic symptoms (such as dizziness or lightheadedness) were poorly correlated with the presence of OH, suggesting an absence of orthostatic symptoms in more than 20% of MSA patients with OH. This finding would support a referral for autonomic function tests in patients with suspected MSA, even without orthostatic symptoms.

Apart from a trend of association between BPRT and disease duration in PD+AF patients, there was no clear relationship between disease duration and other cardiovascular autonomic function measures in MSA, PD+AF and PAF patients. These results indicated a diverse pattern of cardiovascular autonomic function progression in patients with chronic autonomic failure. Nevertheless, a previous study suggested a correlation between disease duration and cardiovascular autonomic dysfunction, as well as older age and medications in patients with PD (van Dijk *et al.*, 1993) but these were not present in a group of patients with chronic autonomic failure in this thesis.

When PD patients without autonomic failure were examined (Chapter 4.2), a significantly prolonged BPRT was also found in these PD patients without orthostatic hypotension, in comparison to age-matched controls. This finding suggests that subtle cardiovascular sympathetic dysfunction may occur in PD, even in asymptomatic patients without OH. This finding was supported by an abnormal BP response to the cold pressor test in PD patients without autonomic failure (Chapter 4.3). In agreement with the previous study of van Dijk et al., (1993) a significant relationship between PD disease duration and BPRT was also evident even after taking age and motor severity into account. This finding could be further investigated in prospective longitudinal studies of whether or not BPRT could be used as a cardiovascular autonomic function marker in patients with PD.

Pressor tests, more conventional cardiovascular autonomic function tests that include isometric exercise, cold pressor and mental arithmetic manoeuvres, provide information on cardiovascular autonomic function, in particular sympathetic neural pathways, in patients with suspected or confirmed autonomic failure/dysfunction. Studies in this thesis (Chapter 4.3) showed that the BP and HR responses to pressor stimuli are different among chronic autonomic disorders. With regards to isometric exercise, BP responses were significantly attenuated in PD+AF and MSA but not in PD, suggesting that the hemodynamic responses

to this manoeuvre are mainly dependent on the severity of autonomic dysfunction. These findings are in contrast to the BP and HR responses to cold pressor and mental arithmetic stimuli. During cold pressor stimuli, BP increased in all patient groups but less so than in controls. While the attenuated BP responses to CP were diminished in MSA and PD+AF as expected, a blunted increase in PD patients who do not have apparent autonomic dysfunction (as shown by no orthostatic hypotension) suggest that this test may not be sensitive enough to distinguish between varying severities of autonomic dysfunction. In response to mental arithmetic the BP responses were, as expected, diminished in MSA and PD+AF relative to healthy controls. Given that the BP changes during head-up tilting were similar between MSA and PD+AF, one would speculate that the hemodynamic responses to mental arithmetic would be similar in these patient groups. However, the BP response was significantly lower in MSA relative to PD+AF and thus might be a useful index for helping to distinguish between these conditions.

24 hr-ABPM has been widely used in patients with hypertension but it is less well used in patients with chronic autonomic failure. It is known that BP is generally lower at night-time compared to daytime in healthy individuals. This normal circadian BP rhythm is also referred to as a “nocturnal BP rhythm” or “BP dipping”. Autonomic failure can affect this physiological response via a disruption of this pattern, either by an absent nocturnal BP fall (non-dipping) or even an increase in BP at night in compared to daytime (“reversed nocturnal BP”). In Chapter 4.4, circadian BP rhythms in MSA, PD with and without autonomic failure using 24 hr-ABPM were examined. The results confirmed that abnormal circadian BP rhythms (both non-dipping and reversed nocturnal BP) were common in all patient groups and most common in MSA patients with confirmed OH and PD+AF and then PD. These findings indicate that abnormal circadian BP rhythms are not specific features for a MSA vs. PD+AF diagnosis, but instead, are influenced by a patient’s autonomic function. The use of 24 hr-ABPM may provide an alternative assessment of OH in contrast to the traditional laboratory head-up tilting protocol. The efficacy of 24 hr-ABPM in detecting OH when used in conjunction with a diary in comparison to laboratory head-up tilting was examined and 24 hr-ABPM with a diary clearly showed a high sensitivity and specificity in detecting OH in patients with parkinsonism suggesting that 24 hr-ABPM might provide an alternative clinical assessment of OH in suspected autonomic failure patients.

Impaired olfaction in PD has been increasingly studied in the last decade. It is partly due to growing evidence on the impact of non-motor symptoms on quality of life in patients with PD. Olfactory dysfunction is a well-known non-motor feature in PD. In contrast, the

evidence of olfactory dysfunction in MSA and PAF is inconsistent due to the rarity of these disorders. In chapter 5.1., this thesis confirmed mild olfactory dysfunction in MSA and markedly impaired olfaction in PD and PAF. The similar degree of impaired olfaction between PD and PAF lend support to the hypothesis that PAF may be a restricted form of a Lewy body disease. Furthermore, this study confirmed that the SS-16 olfactory function test is a useful tool to differentiate between PD and MSA, as well as between MSA and PAF with high sensitivity and specificity. The degree of impaired olfaction in PD was also correlated with motor severity; UPDRS part III, consistent with some previous studies. It was also demonstrated, for the first time, that olfactory dysfunction and motor severity (UMSARS part II) were also significantly correlated in patients with MSA. These results could potentially explain the discrepant findings between studies of olfactory function in patients with MSA.

Quality of life is a crucial factor for patients with chronic autonomic disorders whose daily activities and physical and psychological health can be severely limited. In Chapter 5.2 indices of quality of life (specific to each disorder), activities of daily life and depression were evaluated in chronic autonomic disorders (MSA, PD, PD+AF and PAF). All patients had significantly higher scores of depression relative to healthy controls and the level of depression was most severe in patients with MSA. In PD, there was a strong association between quality of life and several non-motor domains, including, depression, mood and behaviour, activities of daily life, reinforcing the link between quality of life and a number of non-motor features in PD. A limitation of this study must be noted. Only selected domains of non-motor symptoms, including HAM-D (depression), SE (ADL) and SCOPA-AUT (Autonomic), were assessed. There are a number of other non-motor symptoms of chronic autonomic disorders, for instance, cognitive dysfunction, fatigue, sleep disorders and pain that were not investigated. Alternative instruments, such as the Non-motor Symptoms Scale (NMSS) or additional specific questionnaires (e.g. fatigue severity scale) would have allowed a more extensive investigation of non-motor features of these disorders.

In contrast to the findings in PD, there was no correlation between depression and quality of life in the MSA patients. Instead, and consistent with other findings in PD patients, quality of life was strongly associated with MSA patients' physical function, global disability and activities of daily living but not with motor function. These results indicate a greater impact of activities of daily living on an MSA patient's quality of life rather than the severity of their motor dysfunction. As there is no curative treatment in MSA, supportive care or interventions which aid a patient's activities of daily living may be better strategies to improve quality of life in MSA.

With regards to PAF, for the first time, a mild degree of depression and lower quality of life compared to healthy controls was described. There was also an association between quality of life and activities of daily living in PAF. Comparing with SF-36 data from a previous study (Riazi *et al.*, 2003), the quality of life scores in the PAF patients were, at least, impaired as much or even more than in patients with PD. These data suggest that although PAF patients do not have motor dysfunction, the restriction of activities of daily living and the level of depression were comparable to those with PD. As PAF patients have no other obvious neurological impairments, apart from orthostatic hypotension as well as disturbed autonomic function in other domains, their quality of life is therefore mainly contributed by a restriction to their daily living activities via episodes of autonomic dysfunction, e.g., OH.

Despite the first patient with PAF being described almost a hundred years ago by Bradbury and Eggleston, there has been limited information regarding the presenting symptoms, clinical features and laboratory investigations in this disorder because of its low prevalence. In Chapter 6, a range of important initial and presenting symptoms alongside autonomic laboratory and brain imaging investigations in a large cohort of PAF patients were assessed. Pre-syncopal and syncopal symptoms were the most common initial symptoms in the majority of PAF patients, whereas erectile dysfunction was also prevalent in almost a half of male patients. With regards to symptoms at the first consultation (e.g., after the period of initial symptoms), a greater range and frequency of symptoms were evident compared with the initial symptoms presentation. Pre-syncope, syncope and erectile dysfunction were again most prevalent. This was followed by hypohidrosis and “coat-hanger” pain in about 60% of patients. Bladder and bowel symptoms were reported in almost half of patients followed by non-specific symptoms such as fatigue or chest pain. These results indicate that when taking a clinical history from PAF patients it is vital to take history related to sudomotor, genitourinary, gastrointestinal and other non-specific symptoms, in addition to orthostatic symptoms such as dizziness or lightheadedness. Furthermore, bladder symptoms appear to develop as disease progresses in PAF. This is contrast to bladder symptoms in MSA, which are often present before orthostatic symptoms (Mabuchi *et al.*, 2005). With regards to the laboratory investigations, 75% of patients had supine hypertension with a reversed circadian BP rhythm in more than 60%. A low level of supine plasma NA concentration (less than 200 pg/ml) with a small rise during HUT was found in more than 90% of patients. Furthermore, longer disease duration was significantly associated with lower plasma NA HUT changes possibly reflecting greater impairments in sympathetic neural function as the disease progresses.

Paraventricular white matter lesions (WML) in brain MRI were a typical feature in PAF patients, with a prevalence of more than 80%, which might be similar to aged matched healthy individuals. Increasing age was identified as an independent risk factor that can predict cerebral white matter lesions in PAF, the number and type of anti-hypotensive medications were not associated with an increased risk of WML. The causes of WML in PAF remain unclear but wide fluctuations of BP in PAF may be a potential explanation. Further studies are needed to determine whether WML actually increase the future risk of stroke in PAF.

Gastrointestinal (GI) symptoms are a common non-motor feature in PD and are also prevalent in MSA and PAF. GI dysfunction in PAF has been relatively under-researched however. The majority of GI investigations is relatively invasive and involves sophisticated laboratory procedures. Cutaneous electrogastrography (EGG) is a non-invasive method of recording gastric myoelectrical activity (GMA). To date there has been no study that has evaluated GMA in patients with PAF. In Chapter 7, EGG was used to record GMA before and after a standard liquid meal challenge test in PAF and MSA patients. Abnormal EGG patterns, indicating gastric dysrhythmia, were evident in PAF, but not in MSA. As both MSA and PAF had similar degrees of OH, these EGG differences appear to be independent from cardiovascular autonomic dysfunction. The difference in EGG findings between these two disorders may also help define the GI pathophysiology based on their differing lesion sites (pre- vs. post-ganglionic, respectively). The exact mechanism of gastric dysrhythmia in PAF is uncertain. It might reflect a balance of gastric pacemaker cells (Interstitial cells of Cajal; ICC) and vagal output from the brainstem. As patients with PAF have no pathological abnormalities in the brainstem, the presence of gastric dysrhythmia may be caused by the involvement of the ICC as part of post-ganglionic autonomic denervation, similar to that which occurs in patients with PD who also have impaired EGG profiles (Sakakibara *et al.*, 2009). Furthermore, abnormal EGG findings in both PAF and PD are in line with the proposed hypothesis that PAF is part of a restricted “Lewy body disorders spectrum”(Kaufmann & Goldstein, 2010). Future studies focusing on gastrointestinal pathological involvement in PAF may grant further insight of why the majority of PAF patients do not develop symptoms of Parkinson’s disease. This study also supports the use of EGG in conjunction with a liquid meal challenge as a non-invasive test to differentiate between PAF and MSA. Nevertheless, larger studies are needed to confirm whether or not this can be used in a clinical setting.

8.2 Future Directions

Although there has been a significant progress in the understanding of autonomic function and non-motor symptoms in patients with primary chronic autonomic failure, the number of studies specifically investigating these disorders is still relatively few as they are hampered by several limitations and there are several questions that need to be further addressed in future studies.

Firstly, the progression of cardiovascular autonomic function in chronic autonomic failure patients has not been adequately studied in the past. Most studies, including those in this thesis, used a cross-sectional design study to assess cardiovascular function in association with disease duration. Only a few prior studies conducted in a longitudinal approach. Further investigations using novel autonomic function assessment techniques, such as BRPT or BRS, in these patients with a prospective study design would close a knowledge gap in this area. As autonomic dysfunction seems more prevalent with age, medications and disease duration, follow-up studies will be able to overcome these problems and may be able to identify the associated factors that increase the risk of developing autonomic dysfunction in patients with PD. This approach also offers a better opportunity to determine why cardiovascular autonomic function can variably progress and also determine the prognostic factors among patients with MSA. As shown from the findings that autonomic failure affects quality of life in patients with PAF, future studies in these patients as well as in those with other forms of autonomic failure should not only measure their autonomic function (e.g. BP or HR) but also include their quality of life at the same time. This will provide a real benefit toward the patients particularly when conducting an interventional trial, such as drug treatments.

Most cardiovascular autonomic function tests rely on individual co-operation. Some tests require participants engaging in complicated tasks, such as the Valsalva manoeuvre. Other tests, for example isometric handgrip exercise, involve an individual's ability to adequately perform a task to a reasonable level in order to interpret the results with confidence. Patients with a certain degree of cognitive dysfunction or those with movement difficulties may not be able to complete these tests adequately. The accuracy of these tests cannot therefore thoroughly be evaluated in these patient groups as a result. A poor correlation between orthostatic symptoms and autonomic dysfunction is another limitation, which is often encountered during testing. This example was clearly shown in the present studies that a reasonable number of patients had OH without any symptoms during HUT. This highlights an important autonomic laboratory investigation as part of the diagnostic

pathway. Development of new techniques in autonomic function testing, which require less co-operation from patients is also needed. The use of non-invasive tests, such as EGG or novel cardiovascular autonomic measures, may help to improve the diagnostic accuracy and facilitate a diagnosis in patients with autonomic dysfunction at earlier stages before the development of autonomic failure.

With regards to patients with PAF, a prospective design would provide more accurate outcomes in determining the risk of end-organ damage. Considering the findings of WML in PAF on brain MRI, it is tempting to explore whether a significant fluctuation of BP in PAF patients would actually increase the risk of stroke or damage to other organs, such as renal failure, myocardial infarction. In addition, the similarities in a variety of clinical features and investigation findings between PAF and PD offers a great opportunity to examine why patients with PAF have a much lower prevalence of cognitive dysfunction and parkinsonism. As PAF is a rare autonomic disorder with a favourable life expectancy, a multi-centre longitudinal study would give better answers to these questions.

In conclusion, all current works in this thesis provide further insight regarding cardiovascular autonomic function and other non-motor symptoms, as well as quality of life, in patients with primary chronic autonomic disorders. Additionally, some pathophysiological differences between disorders described in the studies, could help facilitate an earlier diagnosis, improve diagnostic accuracy and help prognostication of these disorders. These findings may also supply important information for developing future interventional studies, for instance drug or other therapeutic trials, on patients with primary chronic autonomic disorders.

Chapter 9

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9. References

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Appendix

Parkinson's Disease Questionnaire (PDQ-39)

Please tick <u>one</u> box for each question

Due to having Parkinson's disease, how often <u>during the last month</u> have you....

	Never	Occasionally	Sometimes	Often	Always
1. Had difficulty doing the leisure activities which you would like to do?	☐	☐	☐	☐	☐
2. Had difficulty looking after your home, e.g. DIY, housework, cooking?	☐	☐	☐	☐	☐
3. Had difficulty carrying bags of shopping?	☐	☐	☐	☐	☐
4. Had problems walking half a mile?	☐	☐	☐	☐	☐
5. Had problems walking 100 yards?	☐	☐	☐	☐	☐
6. Had problems getting around the house as easily as you would like?	☐	☐	☐	☐	☐
7. Had difficulty getting around in public?	☐	☐	☐	☐	☐
8. Needed someone else to accompany you when you went out?	☐	☐	☐	☐	☐
9. Felt frightened or worried about falling over in public?	☐	☐	☐	☐	☐
10. Been confined to the house more than you would like?	☐	☐	☐	☐	☐
11 Had difficulty washing yourself?	☐	☐	☐	☐	☐
12 Had difficulty dressing yourself?	☐	☐	☐	☐	☐
13 Had problems doing up your shoe laces?	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐
*Please check that you have ticked **one box for each question** before going on to the next page*					

Please tick <u>one</u> box for each question

Due to having Parkinson's disease, how often <u>during the last month</u> have you....

	Never	Occasionally	Sometimes	Often	Always
14. Had problems writing clearly?	☐	☐	☐	☐	☐
15. Had difficulty cutting up your food?	☐	☐	☐	☐	☐
16. Had difficulty holding a drink without spilling it?	☐	☐	☐	☐	☐
17. Felt depressed?	☐	☐	☐	☐	☐
18. Felt isolated and lonely?	☐	☐	☐	☐	☐
19. Felt weepy or tearful?	☐	☐	☐	☐	☐
20. Felt angry or bitter?	☐	☐	☐	☐	☐
21. Felt anxious?	☐	☐	☐	☐	☐
22. Felt worried about your future?	☐	☐	☐	☐	☐
23. Felt you had to conceal your Parkinson's from people?	☐	☐	☐	☐	☐
24. Avoided situations which involve eating or drinking in public?	☐	☐	☐	☐	☐
25.Felt embarrassed in public due to having Parkinson's Disease?	☐	☐	☐	☐	☐
26. Felt worried by other people's reaction to you?	☐	☐	☐	☐	☐
27. Had problems with your close personal relationships?	☐	☐	☐	☐	☐
28. Lacked support in the ways you need from your spouse or partner? **If you do not have a spouse or partner tick here ☐**	☐	☐	☐	☐	☐
29. Lacked support in the ways you need from your family or close friends?	☐	☐	☐	☐	☐

*Please check that you have ticked **one box for each question** before going on to the next page*

Please tick <u>one</u> box for each question

Due to having Parkinson's disease, how often <u>during the last month</u> have you....

	Never	Occasionally	Sometimes	Often	Always
30 Unexpectedly fallen asleep during the day?	☐	☐	☐	☐	☐
31 Had problems with your concentration, e.g. when reading or watching TV?	☐	☐	☐	☐	☐
32 Felt your memory was bad?	☐	☐	☐	☐	☐
33 Had distressing dreams or hallucinations?	☐	☐	☐	☐	☐
34 Had difficulty with your speech?	☐	☐	☐	☐	☐
35 Felt unable to communicate with people properly?	☐	☐	☐	☐	☐
36 Felt ignored by people?	☐	☐	☐	☐	☐
37 Had painful muscle cramps or spasms?	☐	☐	☐	☐	☐
38 Had aches and pains in your joints or body?	☐	☐	☐	☐	☐
39 Felt unpleasantly hot or cold?	☐	☐	☐	☐	☐
*Please check that you have ticked **one box for each question***					

Thank you for completing the PDQ-39 questionnaire

MSA-QoL Questionnaire

Having a health problem can affect a person's quality of life in many different ways. In order to understand how your illness affects your life, we are interested which, if any, of the following problems you have experienced. We would also like to know how problematic each has been for you.

<u>Please note that this list includes many problems that you may never experience.</u>

In the last 4 weeks have you	No Problem	Slight Problem	Moderate Problem	Marked Problem	Extreme Problem	Not appl.
1. Had difficulty moving?	☐	☐	☐	☐	☐	
2. Had difficulty walking?	☐	☐	☐	☐	☐	
3. Had problems with your balance?	☐	☐	☐	☐	☐	
4. Had difficulty standing up without support?	☐	☐	☐	☐	☐	
5. Had difficulty speaking?	☐	☐	☐	☐	☐	
6. Had difficulty swallowing food?	☐	☐	☐	☐	☐	
7. Had too much saliva or drooling?	☐	☐	☐	☐	☐	
8. Had difficulty with handwriting?	☐	☐	☐	☐	☐	
9. Had difficulty feeding yourself?	☐	☐	☐	☐	☐	
10. Had difficulty drinking fluids?	☐	☐	☐	☐	☐	
11. Had difficulty dressing yourself?	☐	☐	☐	☐	☐	
12. Needed help to go to the toilet?	☐	☐	☐	☐	☐	
13. Had to stop doing things that you liked to do, e.g. your hobbies?	☐	☐	☐	☐	☐	
14. Had difficulty doing things around the house, e.g. housework?	☐	☐	☐	☐	☐	
15. Experienced bladder problems?	☐	☐	☐	☐	☐	

16. Experienced problems with constipation?	☐	☐	☐	☐	☐	
In the last 4 weeks have you	**No Problem**	**Slight Problem**	**Moderate Problem**	**Marked Problem**	**Extreme Problem**	**Not appl.**
17. Experienced dizziness when standing up?	☐	☐	☐	☐	☐	
18. Suffered from cold hands or feet?	☐	☐	☐	☐	☐	
19. Experienced pain in your neck or shoulders?	☐	☐	☐	☐	☐	
20. Experienced pain elsewhere, e.g. in your legs or your back?	☐	☐	☐	☐	☐	
21. Had difficulty getting comfortable during the night?	☐	☐	☐	☐	☐	
22. Had difficulty breathing during the night?	☐	☐	☐	☐	☐	
23. Been feeling tired very quickly (without exerting yourself)?	☐	☐	☐	☐	☐	
24. Experienced lack of energy?	☐	☐	☐	☐	☐	
25. Experienced slowness of thinking?	☐	☐	☐	☐	☐	
26. Had difficulty with your concentration, e.g. reading or watching TV?	☐	☐	☐	☐	☐	
27. Felt frustrated?	☐	☐	☐	☐	☐	
28. Felt depressed?	☐	☐	☐	☐	☐	
29. Experienced a loss of motivation?	☐	☐	☐	☐	☐	
30. Been feeling incapable?	☐	☐	☐	☐	☐	
31. Worried about the future?	☐	☐	☐	☐	☐	
32. Worried about your family?	☐	☐	☐	☐	☐	

33. Felt on your own or isolated?	☐	☐	☐	☐	☐	
34. Experienced loss of confidence when interacting with others?	☐	☐	☐	☐	☐	
35. Felt that your role in your family or among friends has changed?	☐	☐	☐	☐	☐	
36. Experienced difficulty seeing your friends?	☐	☐	☐	☐	☐	
In the last 4 weeks have you	**No Problem**	**Slight Problem**	**Moderate Problem**	**Marked Problem**	**Extreme Problem**	**Not appl.**
37. Had to give up social activities, e.g. going out for a meal, participating in events?	☐	☐	☐	☐	☐	
38. Had difficulty talking to friends about your illness?	☐	☐	☐	☐	☐	
39. Been embarrassed to talk to people?	☐	☐	☐	☐	☐	
40. Felt that life has become boring?	☐	☐	☐	☐	☐	

Short Form Health Survey (SF-36)

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.

Please answer these questions by "check-marking" your choice. Please select only one choice for each item.

1- In general, would you say your health is:

1. Excellent 2. Very good 3. Good 4. Fair 5. Poor

2- Compared to ONE YEAR AGO, how would you rate your health in general NOW?

1. MUCH BETTER than one year ago.
2. Somewhat BETTER now than one year ago.
3. About the SAME as one year ago.
4. Somewhat WORSE now than one year ago.
5. MUCH WORSE now than one year ago.

3- The following items are about activities you might do during a typical day. **Does your health now limit you** in these activities? If so, how much?

Activities	**1. Yes, Limited A Lot**	**2. Yes, Limited A Little**	**3. No, Not Limited At All**
a) **Vigorous activities,** such as running, lifting heavy objects, participating in strenuous sports?	1. Yes, limited a lot	2. Yes, limited a little	3. No, not limited at all
b) **Moderate activities,** such as moving a table, pushing a vacuum cleaner, bowling, or playing golf?	1. Yes, limited a lot	2. Yes, limited a little	3. No, not limited at all
c) Lifting or carrying groceries?	1. Yes, limited a lot	2. Yes, limited a little	3. No, not limited at all

d) Climbing **several flights** of stairs?	1. Yes, limited a lot	2. Yes, limited a little	3. No, not limited at all
e) Climbing **one** flight of stairs?	1. Yes, limited a lot	2. Yes, limited a little	3. No, not limited at all
f) Bending, kneeing or stooping?	1. Yes, limited a lot	2. Yes, limited a little	3. No, not limited at all
g) Walking **more than a mile**?	1. Yes, limited a lot	2. Yes, limited a little	3. No, not limited at all
h) Walking **several** blocks?	1. Yes, limited a lot	2. Yes, limited a little	3. No, not limited at all
i) Walking **one** block?	1. Yes, limited a lot	2. Yes, limited a little	3. No, not limited at all
j) Bathing or dressing yourself?	1. Yes, limited a lot	2. Yes, limited a little	3. No, not limited at all

4- During the **past 4 weeks**, have you had any of the following problems with your work or other regular activities *as a result of your physical health*?

	Yes	No
a) Cut down on the **amount of time** you spent on work or other activities?	1. yes	2. No
b) **Accomplished less** than you would like?	1. yes	2. No
c) Were limited in the **kind** of work or other activities?	1. yes	2. No
d) Had **difficulty** performing the work or other activities (for example it took extra effort)?	1. yes	2. No

5. During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

	Yes	No
a) Cut down on the **amount of time** you spent on work or other activities?	1. yes	2. No
b) **Accomplished less** than you would like?	1. yes	2. No
c) Didn't do work or other activities as **carefully** as usual?	1. yes	2. No

6. During the **past 4 weeks**, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

1. Not at all 2. Slightly 3. Moderately 4. Severe 5. Very severe

7. How much **bodily pain** have you had during the **past 4 weeks**?

1. None 2. Very mild 3. Mild 4. Moderate 5. Severe 6. Very severe

8. During the **past 4 weeks**, how much did **pain** interfere with your normal work (including both work outside the home and housework)?

1. Not at all 2. A little bit 3. Moderately 4. Quite a bit 5. Extremely

9. These questions are about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the **past 4 week** …

	1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. None of the time
a) Did you feel full of pep?	1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. None of the time
b) Have you been a very nervous person?	1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. None of the time
c) Have you felt so down in the dumps that nothing could cheer you up?	1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. None of the time
d) Have you felt calm and peaceful?	1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. None of the time
e) Did you have a lot of energy?	1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. None of the time
f) Have you felt downhearted and blue?	1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. None of the time
g) Do you feel worn out?	1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. None of the time
h) Have you been a happy person?	1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. None of the time
i) Did you feel tired?	1. All of the time	2. Most of the time	3. A good bit of the time	4. Some of the time	5. A little of the time	6. None of the time

10. During the **past 4 weeks**, how much of the time has your **physical health or emotional problems** interfered with your social activities (like visiting with friends, relatives, etc.)?

1. All of the time
2. Most of the time.
3. Some of the time
4. A little of the time.
5. None of the time.

11. How TRUE or FALSE is **each** of the following statements for you?

	1. Definitely true	2. Mostly true	3. Don't know	4. Mostly false	5. Definitely false
a) I seem to get sick a little easier than other people?	1. Definitely true	2. Mostly true	3. Don't know	4. Mostly false	5. Definitely false
b) I am as healthy as anybody I know?	1. Definitely true	2. Mostly true	3. Don't know	4. Mostly false	5. Definitely false
c) I expect my health to get worse?	1. Definitely true	2. Mostly true	3. Don't know	4. Mostly false	5. Definitely false
d) My health is excellent?	1. Definitely true	2. Mostly true	3. Don't know	4. Mostly false	5. Definitely false

Hamilton Rating Scale for Depression (HDRS or HAM-D)

PLEASE COMPLETE THE SCALE BASED ON A STRUCTURED INTERVIEW
Instructions: for each item select *the one "cue" which best characterizes the patient. Be sure to record the answers in the appropriate spaces (positions 0 through 4).*

1 DEPRESSED MOOD *(sadness, hopeless, helpless, worthless)*
0 |__| Absent.
1 |__| These feeling states indicated only on questioning.
2 |__| These feeling states spontaneously reported verbally.
3 |__| Communicates feeling states non-verbally, i.e. through facial expression, posture, voice and tendency to weep.
4 |__| Patient reports virtually only these feeling states in his/her spontaneous verbal and non-verbal communication.

2 FEELINGS OF GUILT
0 |__| Absent.
1 |__| Self reproach, feels he/she has let people down.
2 |__| Ideas of guilt or rumination over past errors or sinful deeds.
3 |__| Present illness is a punishment. Delusions of guilt.
4 |__| Hears accusatory or denunciatory voices and/or experiences threatening visual hallucinations.

3 SUICIDE
0 |__| Absent.
1 |__| Feels life is not worth living.
2 |__| Wishes he/she were dead or any thoughts of possible death to self.
3 |__| Ideas or gestures of suicide.
4 |__| Attempts at suicide (any serious attempt rate 4).

4 INSOMNIA: EARLY IN THE NIGHT
0 |__| No difficulty falling asleep.
1 |__| Complains of occasional difficulty falling asleep, i.e. more than half an hour.
2 |__| Complains of nightly difficulty falling asleep.

5 INSOMNIA: MIDDLE OF THE NIGHT
0 |__| No difficulty.
1 |__| Patient complains of being restless and disturbed during the night.
2 |__| Waking during the night – any getting out of bed rates 2 (except for purposes of voiding).

6 INSOMNIA: EARLY HOURS OF THE MORNING
0 |__| No difficulty.
1 |__| Waking in early hours of the morning but goes back to sleep.
2 |__| Unable to fall asleep again if he/she gets out of bed.

7 WORK AND ACTIVITIES
0 |__| No difficulty.
1 |__| Thoughts and feelings of incapacity, fatigue or weakness related to activities, work or hobbies.
2 |__| Loss of interest in activity, hobbies or work – either directly reported by the patient or indirect in listlessness,indecision and vacillation (feels he/she has to push self to work or activities).
3 |__| Decrease in actual time spent in activities or decrease in productivity. Rate 3 if the patient does not spend at least three hours a day in activities (job or hobbies) excluding routine chores.
4 |__| Stopped working because of present illness. Rate 4 if patient engages in no activities except routine chores, or if patient fails to perform routine chores unassisted.

8 RETARDATION (slowness of thought and speech, impaired ability to concentrate, decreased motor activity)
0 |__| Normal speech and thought.
1 |__| Slight retardation during the interview.
2 |__| Obvious retardation during the interview.
3 |__| Interview difficult.
4 |__| Complete stupor.

9 AGITATION
0 |__| None.
1 |__| Fidgetiness.
2 |__| Playing with hands, hair, etc.
3 |__| Moving about, can't sit still.
4 |__| Hand wringing, nail biting, hair-pulling, biting of lips.

10 ANXIETY PSYCHIC
0 |__| No difficulty.
1 |__| Subjective tension and irritability.
2 |__| Worrying about minor matters.
3 |__| Apprehensive attitude apparent in face or speech.
4 |__| Fears expressed without questioning.

11 ANXIETY SOMATIC (physiological concomitants of anxiety) such as:
gastro-intestinal – dry mouth, wind, indigestion, diarrhea, cramps, belching
cardio-vascular – palpitations, headaches
respiratory – hyperventilation, sighing
urinary frequency
sweating
0 |__| Absent.
1 |__| Mild.
2 |__| Moderate.
3 |__| Severe.
4 |__| Incapacitating.

12 SOMATIC SYMPTOMS GASTRO-INTESTINAL
0 |__| None.
1 |__| Loss of appetite but eating without staff encouragement. Heavy feelings in abdomen.
2 |__| Difficulty eating without staff urging. Requests or requires laxatives or medication for bowels or medication for gastro-intestinal symptoms.

13 GENERAL SOMATIC SYMPTOMS
0 |__| None.
1 |__| Heaviness in limbs, back or head. Backaches, headaches, muscle aches. Loss of energy and fatigability.
2 |__| Any clear-cut symptom rates 2.

14 GENITAL SYMPTOMS (symptoms such as loss of libido, menstrual disturbances)
0 |__| Absent.
1 |__| Mild.
2 |__| Severe.

15 HYPOCHONDRIASIS
0 |__| Not present.
1 |__| Self-absorption (bodily).
2 |__| Preoccupation with health.
3 |__| Frequent complaints, requests for help, etc.
4 |__| Hypochondriacal delusions.

16 LOSS OF WEIGHT *(RATE EITHER a OR b)*
a) According to the patient:
0 |__| No weight loss.
1 |__| Probable weight loss associated with present illness.
2 |__| Definite (according to patient) weight loss.
3 |__| Not assessed.
b) According to weekly measurements:
0 |__| Less than 1 lb weight loss in week.
1 |__| Greater than 1 lb weight loss in week.
2 |__| Greater than 2 lb weight loss in week.
3 |__| Not assessed.

17 INSIGHT
0 |__| Acknowledges being depressed and ill.
1 |__| Acknowledges illness but attributes cause to bad food, climate, overwork, virus, need for rest, etc.
2 |__| Denies being ill at all.

Total score: |__|__|

SCOPA-AUT

By means of this questionnaire, we would like to find out to what extent in the past month you have had problems with various bodily functions, such as difficulty passing urine, or excessive sweating. Answer the questions by placing a cross in the box which best reflects your situation. If you wish to change an answer, fill in the 'wrong' box and place a cross in the correct one. If you have used medication in the past month in relation to one or more of the problems mentioned, then the question refers to how you were while taking this medication. You can note the use of medication on the last page.

1. In the past month, have you had difficulty swallowing or have you choked?

☐	☐	☐	☐
never	sometimes	regularly	often

2. In the past month, has saliva dribbled out of your mouth?

☐	☐	☐	☐
never	sometimes	regularly	often

3. In the past month, has food ever become stuck in your throath?

☐	☐	☐	☐
never	sometimes	regularly	often

4. In the past month, did you ever have the feeling during a meal that you were full very quickly?

☐	☐	☐	☐
never	sometimes	regularly	often

5. *Constipation is a blockage of the bowel, a condition in which someone has a bowel movement twice a week or less.*
In the past month, have you had problems with constipation?

☐	☐	☐	☐
never	sometimes	regularly	often

6. In the past month, did you have to strain hard to pass stools?

☐	☐	☐	☐
never	sometimes	regularly	often

7. In the past month, have you had involuntary loss of stools?

☐ never ☐ sometimes ☐ regularly ☐ often

Questions 8 to 13 deal with problems with passing urine. If you use a catheter you can indicate this by placing a cross in the box "*use cathether*".

---

8. In the past month, have you had difficulty retaining urine?

☐ never ☐ sometimes ☐ regularly ☐ often ☐ *use catheter*

9. In the past month, have you had involuntary loss of urine?

☐ never ☐ sometimes ☐ regularly ☐ often ☐ *use catheter*

10. In the past month, have you had the feeling that after passing urine your bladder was not completely empty?

☐ never ☐ sometimes ☐ regularly ☐ often ☐ *use catheter*

11. In the past month, has the stream of urine been weak?

☐ never ☐ sometimes ☐ regularly ☐ often ☐ *use catheter*

12. In the past month, have you had to pass urine again within 2 hours of the previous time?

☐ never ☐ sometimes ☐ regularly ☐ often ☐ *use catheter*

13. In the past month, have you had to pass urine <u>at night</u>?

☐ never ☐ sometimes ☐ regularly ☐ often ☐ *use catheter*

14. In the past month, when standing up have you had the feeling of either becoming lightheaded, or no longer being able to see properly, or no longer being able to think clearly?

☐ never ☐ sometimes ☐ regularly ☐ often

15. In the past month, did you become light-headed after standing for some time?

☐ never ☐ sometimes ☐ regularly ☐ often

16. Have you fainted in the past 6 months?

☐ never ☐ sometimes ☐ regularly ☐ often

17. In the past month, have you ever perspired excessively during the day?

☐ never ☐ sometimes ☐ regularly ☐ often

18. In the past month, have you ever perspired excessively during the night?

☐ never ☐ sometimes ☐ regularly ☐ often

19. In the past month, have your eyes ever been over-sensitive to bright light?

☐ never ☐ sometimes ☐ regularly ☐ often

20. In the past month, how often have you had trouble tolerating cold?

☐ never ☐ sometimes ☐ regularly ☐ often

21. In the past month, how often have you had trouble tolerating heat?

☐ Never ☐ sometimes ☐ regularly ☐ often

The following questions are about sexuality. Although we are aware that sexuality is a highly intimate subject, we would still like you to answer these questions. For the questions on sexual activity, consider every form of sexual contact with a partner or masturbation (self-gratification). An extra response option has been added to these questions. Here you can indicate that the situation described has not been applicable to you in the past month, for example because you have not been sexually active. Questions 22 and 23 are intended specifically for **men**, 24 and 25 for **women**.

The following 3 questions are only for men

22. In the past month, have you been impotent (unable to have or maintain an erection)?

☐ never ☐ sometimes ☐ regularly ☐ often ☐ *not applicable*

23. In the past month, how often have you been unable to ejaculate?

☐ never ☐ sometimes ☐ regularly ☐ often ☐ *not applicable*

23a. In the past month, have you taken medication for an erection disorder? (If so, which medication?)

☐ no ☐ yes: _____

Proceed with question 26

The following 2 questions are only for women

24. In the past month, was your vagina too dry during sexual activity?

☐ never ☐ sometimes ☐ regularly ☐ often ☐ *not applicable*

25. In the past month, have you had difficulty reaching an orgasm?

☐ never ☐ sometimes ☐ regularly ☐ often ☐ *not applicable*

The following questions are for everyone

The questions below are about the use of medication for which you may have or have not needed a doctor's prescription. If you use medication, also give the name of the substance.

26. In the past month, have you used medication for:

a. constipation?	☐ no	☐ yes: _____
d. urinary problems?	☐ no	☐ yes: _____
e. blood pressure?	☐ no	☐ yes: _____
f. other symptoms *(not symptoms related to Parkinson's disease)*	☐ no	☐ yes: _____

_____