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DISSERTATION

Autonomic examination including hand skin temperature and its response to cooling in multiple system atrophy and Parkinson's disease

to become a Doctor of Medicine (Dr. med.)

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Contents

Summary

Multiple system atrophy (MSA) clinically is characterized by prominent autonomic dysfunction with combinations of parkinsonian features (MSA-P), cerebellar ataxia (MSA-C) and possible corticospinal symptoms. Autonomic dysfunction in MSA comprises cardinal symptoms of orthostatic hypotension (OH) and urinary incontinence. Additionally, sudomotor and cardiovagal abnormalities are frequently present among the MSA patients.

Due to overlapping motor presentations it can be very challenging to distinguish MSA-P from Parkinson's disease (PD). The cardinal MSA autonomic symptoms - OH and urinary incontinence - are of limited assistance while these symptoms are common among the PD patients as well. Autonomic abnormalities for the PD patients may also include constipation, heat or cold intolerance, postprandial hypotension and relative nocturnal hypertension.

Previous studies compared hand skin temperature and its response to cooling in subjects with PD and probable MSA. Significant differences regarding hand temperature before and after cooling, as well as kinetics of natural rewarming were found indicating that disturbed neurovascular thermoregulation of distal extremities potentially belongs to MSA autonomic features and could be helpful in clinical differentiation of MSA-P from PD. Up to the present, however, only a minor study sample has been reported.

In this study we sought to improve our knowledge about impaired thermoregulation of distal extremities in patients suffering from MSA and PD. Furthermore, the coexistence and possible interrelations for the different subtypes of autonomic dysfunction were assessed in a large cohort of MSA, PD and control patients.

In the study 112 MSA, 500 PD and 129 control patients underwent a standard cooling - rewarming procedure, termed ice test (IT). Additionally the subdivision of the patients received

peripheral and autonomic nervous systems clinically evaluated. The evaluation included electroneurography and autonomic function tests of heart rate variability, sympathetic skin response and lying-to-standing orthostatic test.

The results of this study indicate that the prevalence of pathological IT, the frequencies of gradually increasing temperature decrement and the mean temperature decrement in pathological IT were higher in the MSA group compared with the PD and the control groups, the differences were insufficient for statistical significance. The presence of pathological IT was significantly associated with older patient's age in the PD and the control study groups ($p < 0.05$). This association was absent for the MSA patients, who themselves were significantly younger ($p = 0.001$). Significant correlation between the presence of pathological IT and the autonomic symptom of OH was determined ($p < 0.001$).

To conclude, defective thermoregulation of distal extremities is potentially more severe in the MSA patients. So-called ice test however does not differentiate MSA-P and PD reliably. The dysfunction of preganglionic sympathetic neurons might be involved in impaired response to cooling for MSA. The results suggest a pathophysiological affinity between pathological IT and OH, which could be defective sympathetic neurovascular regulation. Specific autonomic testing including IT may help in the differentiation of MSA-P and PD.

I. INTRODUCTION

1. Multiple System Atrophy

1.1. Clinical Presentation

Multiple system atrophy (MSA) is a sporadic, adult-onset and relatively rapidly progressive neurodegenerative disorder. MSA clinically is characterized by prominent autonomic dysfunction with combinations of parkinsonian features (MSA-P), cerebellar ataxia (MSA-C) and possible corticospinal symptoms [1].

MSA-P is determined in 60% of patients in the western hemisphere, whereas MSA-C constitutes 40% of patients. The approximately reverse distribution is observed in the eastern hemisphere [2, 3].

Parkinsonism in MSA-P is characterized by progressive akinesia and rigidity. The motor findings are sometimes asymmetrical. Resting tremor is less common than in Parkinson‘s disease (PD). However irregular postural and action tremor with superimposed jerks can be seen in as many as 50% of patients with MSA [4]. Postural stability is impaired early after the onset of disease. Recurrent falls at disease onset are however rather unusual in MSA. Progressive degeneration of the striatum accounts for the poor response or lack of response to levodopa. Nevertheless, a transient response to levodopa may be observed in approximately 40% of patients during early disease stages [5]. This is sometimes accompanied by drug-induced involuntary movements, such as head–neck dystonia, an involuntary muscle contraction resulting in abnormal twisting postures [6].

The cerebellar disorder of MSA-C is composed of gait ataxia, limb kinetic ataxia, and scanning dysarthria, as well as cerebellar oculomotor disturbances. Spontaneous, gaze-evoked, or positional downbeat nystagmus may be present.

Generalized hyperreflexia with or without a Babinski sign, may occur in 30 to 50% of cases [4].

In addition, features of abnormal spine posture (camptocormia), disproportionate antecollis, severe lateral flexion of the spine (Pisa syndrome) and hand or foot dystonia are associated with the motor presentation of MSA in 16 to 42% of patients [7]. Dysphonia, dysarthria, drooling (saliva loss) and dysphagia are defining symptoms of advanced stage disease [1].

Autonomic failure develops in almost all patients with MSA [8]. Early impotence is typical in men. Urinary incontinence or incomplete bladder emptying, often early after the disease onset or even as a presenting symptom, are frequent. Orthostatic hypotension (OH) is present in approximately 70% of MSA patients being main feature of cardiovascular autonomic failure in clinically established MSA [4]. Symptoms of OH include lightheadedness, dizziness, postural instability, vertigo and blurred vision. OH can manifest as recurrent syncope, but it may also be asymptomatic. When associated with clinical symptoms OH indicates a decrease in cerebral perfusion. Falls with injuries may also result. OH often deteriorates the general condition of MSA patients and may contribute to morbidity, disability and even death, because of the potential risk of substantial injury [9, 10]. Postprandial hypotension and supine and nocturnal hypertension accompany orthostatic hypotension in half of patients with MSA [11]. Respiratory problems are common in MSA. Inspiratory stridor develops in nearly 50% of patients at some time. It is more frequent in advanced disease [7].

Other autonomic disturbances in MSA include constipation [12], pupillomotor abnormalities, vasomotor failure with diminished sweating [13, 14] and disturbed neurovascular thermoregulation of distal extremities [15] with a possible cold hand sign [14].

1.2. Historical Approach

The concept of MSA as a unifying entity comprising several clinical syndromes has a long history. The first cases of MSA were presented as olivopontocerebellar atrophy in 1900. The Shy-Drager syndrome with autonomic failure including OH and striatonigral degeneration featuring parkinsonism was described in 1960. These cardinal presentations of MSA - striatonigral degeneration, olivopontocerebellar atrophy and Shy–Drager syndrome - were regarded as distinct entities until Graham and Oppenheimer recognized their substantial clinicopathological overlap and proposed MSA as an umbrella term in 1969 [16].

Ubiquitinpositive glial cytoplasmic inclusions (GCI) were first reported in 1989 [17] and subsequently confirmed as a cellular marker of MSA regardless of the phenotypic presentation. Alpha-synuclein immunostaining of GCI was first described in 1998. Following this discovery, MSA has been regarded as α-synucleinopathy along with PD, Lewy body dementia, and pure autonomic failure. Quinn proposed the first set of diagnostic criteria and distinguished the motor subtypes MSA-P and MSA-C, although there was unintended overlap. A consensus conference was convened in 1998 that proposed clinical guidelines exclusively for the diagnosis of MSA. The latter was divided into MSA-P and MSA-C depending on the dominant motor presentation at the time of examination [18]. Based on improvements of early diagnosis by defining warning signs and sensitive neuroimaging indices, the consensus guidelines were revised in 2008 [19]. A Unified MSA Rating Scale quantifying disease severity has been established and validated in the meantime by the European MSA Study Group [20]. Animal models have become available as preclinical test beds for translational neuroprotection and neuroregeneration studies [21]. The first clinical trials have been conducted by two independent consortia using minocycline [22] and riluzole [23]. Other international networks have been established in the last few years including North American MSA Study Group [24], Japanese MSA Consortium, and Chinese MSA Study Group. Globalization of MSA research has led not only to the clinical trials, mentioned above, but also to the first genetic breakthrough by identifying variants in the α-synuclein gene and their association with increased disease risk in a large population of MSA patients [25].

Table I. Timeline for definitions of MSA.

Definition	Time	Authors	Subject
Olivopontocerebellar atrophy (OPCA)	1900	Dejerine and Thomas	Introduction of the term olivopontocerebellar atrophy
Orthostatic hypotension	1925	Bradbury and Eggleston	Introduction of autonomic failure as a clinical syndrome
Shy-Drager syndrome (SDS)	1960	Shy and Drager	Origin of this term as a neuropathologic entity with parkinsonism and autonomic failure with OH
Striatonigral degeneration (SND)	1960	Van der Eecken et al	Description of SND
Multiple system atrophy	1969	Graham and Oppenheimer	Introduction of the term MSA, which represents SDS, SND, and OPCA as one entity
Glial cytoplasmic inclusions (GCI)	1989	Papp et al, Matsuo et al	Discovery of GCI as hallmark of MSA
Alpha-synuclein inclusion	1998	Spillantini et al, Wakabayashi et al	Alpha-synuclein immunostaining as a sensitive marker of MSA
MSA classification	1996-1999	Consensus Committee	Classification of MSA based on clinical domains and features and neuropathology
Unified MSA Rating Scale (UMSARS)	2003	European MSA Study Group	Unified MSA Rating Scale as a standard to define MSA symptoms
Second consensus for definition of MSA	2008	Consensus Committee	New definition of MSA with simplified criteria

1.3. Epidemiologic Features

The estimated prevalence of MSA is reported to be between 3.4-4.9 cases per 100000 of population. The estimated mean incidence is 0.6-0.7 cases per 100000 persons per year. Hence MSA meets orphan disease status [26, 27]. The reported prevalence increases to 7.8 per 100000 among persons older than 40 years of age [28]. Regional reports on the MSA occurrence state, that in the European Union the prevalence reaches 4-5 cases per 100000 persons. The incidence rate is about 0.6 cases per 100000 persons per year [28]. In the United Kingdom the overall prevalence of MSA, including all probable and possible cases, is 3.3 per 100000 of population [4]. In Iceland the annual incidence is 0.6 per 100000 and prevalence is 3.1 per 100000 [4]. In Japan the prevalence is 13.1 per 100000 individuals [2]. The mean annual incidence is 0.68 [3].

1.4. Etiology and Neuropathophysiology

We lack a complete understanding of the etiopathologic factors for MSA. Similar to other sporadic neurodegenerative diseases, a complex interaction of genetic and environmental mechanisms seems very likely [29]. Mercury, methanol, carbon tetrachloride, carbon disulfide and cyanide have been associated with MSA-like parkinsonism in a small series of cases [30]. Manganese intoxication reported in miners can produce clinical, neuroimaging, and pathological findings that may overlap with those of MSA [30]. The few controlled studies available in the literature have attempted to associate the increased risk of MSA with occupational and daily habits like farming using pesticides, working exposure to solvents, plastic monomers, additives and metals [31, 32, 33]. However, the increased risk of MSA related to occupational toxins has not been confirmed by any previous study [34]. Nicotine use and alcohol consumption are less common among the MSA patients than in healthy controls [1].

MSA is considered to be a sporadic disease. Nevertheless, rare familial cases have been reported indicating that genetic factors have an etiologic role in some families [35, 36]. In a few European and Japanese pedigrees, MSA has been transmitted in an autosomal dominant or recessive inheritance pattern [37, 38]. In addition, some studies suggest that spinocerebellar ataxia type 1 and other forms of spinocerebellar ataxias as well as fragile X–associated tremor/ataxia syndrome might be associated with MSA-like presentations [39, 40, 41, 42]. Mutations, duplications, and triplications of α-synuclein gene *SNCA* may cause familial PD with characteristics similar to MSA in some affected persons [43]. Two single-nucleotide polymorphisms of the *SNCA* locus showed a significant association with MSA in a large series of European patients [25]. This association was confirmed in follow-up replication studies [44], but not in the preliminary analysis of the first genome-wide association study of MSA [45, 46].

Genes coding for apolipoprotein E, dopamine β-hydroxylase, ubiquitin C-terminal hydrolase-1, fragile X mental retardation 1, and leucine-rich kinase 2 showed no association with MSA [47]. In contrast, polymorphisms of several genes involved in inflammatory processes have been associated with an elevated MSA risk. These include genes coding for interleukin-1A,

interleukin-1B, interleukin-8, intercellular adhesion molecule-1 and tumor necrosis factor, and alpha-1-antichymotrypsin [48, 49, 50, 51, 52]. Homozygosity for interleukin-1A allele 2 increases the risk to develop MSA as much as five-fold. The alpha-1-antichymotrypsin AA genotype is associated with a significantly earlier onset and faster disease progression [51]. A previous study showed that MSA is also associated with polymorphisms of genes involved in oxidative stress [53].

MSA is regarded as an oligodendroglial α-synucleinopathy. Other known α-synucleinopathies - Parkinson's disease, dementia with Lewy bodies and pure autonomic failure - are characterized by neuronal α-synuclein aggregates, generally known as Lewy bodies [1, 10].

The unifying histopathological hallmark of MSA is the appearance of α-synuclein positive GCI in affected brain regions including striatonigral and/or olivopontocerebellar systems. Incidence of GCI is well correlated with disease severity and duration in the early stages of the disease [54, 55].

The mechanisms underlying MSA still remain elusive. However, the evidence from preclinical models and postmortem studies suggests that it is a primary oligodendrogliopathy [56, 57]. Early myelin alterations in MSA brain have been demonstrated by the presence of altered myelin basic protein p25α, an important stabilizer of myelin integrity [57]. The p25α protein has been shown to colocalize with α-synuclein-positive CGI and to accumulate abnormally in MSA oligodendrocytes [58, 59, 60, 61]. Relocalization of p25α into the oligodendroglial soma appears to precede α-synuclein aggregation [62]. This is followed by oligodendrocyte swelling and abnormal uptake or overexpression of α-synuclein by oligodendroglia [63, 64]. The interaction between p25α and α-synuclein promotes phosphorylation and aggregation of α-synuclein into insoluble oligomers first and glial cytoplasmic inclusions later on. The formation of GCI interferes with neuronal support and has activation effect on quiescent microglial cells. Further on progressively dysfunctional oligodendrocytes release misfolded α-synuclein into the

extracellular space and this misfolded α-synuclein may be taken up by neighboring neurons to form neuronal cytoplasmic inclusions. The processes of neuroinflammation, loss of oligodendroglial neurotrophic support, and neuronal dysfunction due to α-synuclein inclusions together may synergistically promote neuronal death and subsequent reactive astrogliosis. Toxic α-synuclein species may spread in a prion-like fashion to other functionally connected brain areas, leading to the multisystemic neuronal impairment [65].

1.5. Diagnostic Considerations

The diagnosis of MSA is based largely on history and neurological examination. The consensus guidelines specify three degrees of certainty: definite, probable, and possible [1, 19]. Diagnosis of definite multiple-system atrophy requires neuropathological evidence of widespread α-synuclein – positive GCI with presence of neuronal multisystem changes: striatonigral degeneration or olivopontocerebellar atrophy [1, 19].

Probable MSA is defined as sporadic, adult-onset and relatively rapidly progressive disorder having prominent autonomic dysfunction with combinations of parkinsonian features (MSA-P) or cerebellar ataxia (MSA-C). Autonomic dysfunction required for probable MSA must include urinary incontinence and/or severe OH, defined as a blood pressure decrease of 30 mmHg systolic or 15 mmHg diastolic within 3 minutes after active standing from the recumbent position (Table II).

A diagnosis of possible MSA requires the presence of a sporadic, progressive, adult-onset disorder with predominant parkinsonism or cerebellar ataxia, which has at least one feature suggestive of autonomic dysfunction and at least one of supportive features (warning signs) (Table II).

Table II. Diagnostic Approach to MSA.

Definite MSA
Neuropathological findings during postmortem examination with the evidence of: ▪Widespread and abundant cerebral α-synuclein–positive GCI ▪Neuronal changes: striatonigral degeration or olivopontocerebellar atrophy
Probable MSA
Sporadic, progressive disease in adults (onset after 30 years of age) characterized by autonomic failure, including urinary incontinence (with erectile dysfunction in men), or an orthostatic decrease in blood pressure by at least 30 mmHg systolic or 15 mmHg diastolic within 3 min of standing, plus one of the following: ▪Slowness, rigidity, and postural instability with poor response to levodopa (MSA-P) ▪Gait ataxia, uncoordinated limb movements, action tremor, and nystagmus (MSA-C)
Possible MSA
A sporadic, progressive, adult-onset disease characterized by the following: ▪Parkinsonism (slowness of movements, rigidity, and tendency to fall) or a cerebellar syndrome (wide-based gait, uncoordinated limb movements, action tremor, and nystagmus) ▪At least one feature suggesting autonomic dysfunction (otherwise unexplained urinary urgency or frequency, incomplete bladder emptying, erectile dysfunction in men, or a substantial orthostatic blood pressure decline that does not meet the level required for probable MSA) At least one of the following additional features: ▪Possible MSA-P or MSA-C: Babinski sign with hyperreflexia, stridor ▪Possible MSA-P: rapidly progressive parkinsonism; poor response to levodopa; recurrent falls within 3 years after the onset of motor symptoms; cerebellar features (wide-based gait; cerebellar dysarthria; uncoordinated limb movements; or spontaneous, gaze-evoked, or positional downbeat nystagmus); recurrent choking within 5 years after the onset of motor symptoms; atrophy on MRI of the putamen, middle cerebellar peduncle, pons, or cerebellum; hypometabolism on FDG-PET in the putamen, brain stem, or cerebellum ▪Possible MSA-C: parkinsonism; atrophy on MRI of the putamen, middle cerebellar peduncle, or pons; hypometabolism on FDG-PET in the putamen; presynaptic nigrostriatal dopaminergic denervation on PET

Features supporting and not supporting the diagnosis of MSA
▪Supporting (warning signs): head–neck dystonia; disproportionate antecollis; bent spine (forward, lateral, or both); contractures of the hands or feet; inspiratory sighs; severe dysphonia; severe dysarthria; new or increased snoring; cold hands and feet; emotional incontinence (pathologic laughter or crying); jerky, irregular postural or action tremor ▪Not supporting: classic "pill-rolling" rest tremor, clinically significant neuropathy, hallucinations not induced by drugs, onset after 75 years of age, family history of ataxia or parkinsonism, dementia (in accordance with DSM-IV criteria), white-matter lesions suggesting multiple sclerosis

1.6. Prognosis

Patients with MSA have a poor prognosis. Patients develop the disease when being usually older than 40 years (average 52-55 years) and they experience fast progression. The disease progresses by worsening of motor and non-motor symptoms during an average period of 10 years, with more rapid progression at the onset [66]. Approximately 50% of patients require walking aids within 3 years after the onset of motor symptoms [2]. 60% require a wheelchair after 5 years [67]. The median time before the patient is bedridden is 6 to 8 years [2]. Median survivals of 6.2-9.5 years from the onset of first symptoms have been reported [26]. There are reports of both aggressive variants with disease duration of less than 3 years [26] and more benign cases with prolonged survival [68]. Older age at onset [2, 67] a parkinsonian phenotype [65] and early development of severe autonomic failure [2, 69, 70] are negative prognostic factors, whereas a cerebellar phenotype and later onset of autonomic failure [68] predict slower disease progression. The overall striatonigral cell loss is associated with the severity of the disease at the time of death. Bronchopneumonia (48%) and sudden death (21%) are common terminal conditions in MSA. Sudden death often occurs at night as a result of either acute bilateral vocal-cord paralysis or acute disruption of the brain-stem cardiorespiratory drive. Urinary dysfunction in MSA often leads to lower urinary tract infections. More than 50% of patients with MSA suffer from recurrent lower urinary tract infections and a substantial number die of related complications [8].

1.7. Paraclinical Examination

When diagnosing MSA, paraclinical tests are of great importance to reject possible alternative causes of a neurological disorder. Cranial computerized tomography (CT) is useful to rule out MSA-mimicking brain lesions. CT could be normal in MSA. In about half of the patients however there may be atrophy of the brainstem, cerebellum, and rarely cerebral cortex. In general, sensitivity and specificity of CT are fairly low. All patients with suspected MSA should be investigated by magnetic resonance imaging (MRI) of the strength ≥ 1.5 Tesla. In T2-weighted sequences atrophy of putamen, middle cerebellar peduncle, pons, or cerebellum, a so-called “rim sign” (hyperintense signal of the dorsolateral border of putamen) and putaminal hypointensity could be seen in MSA-P brain. In MSA-C atrophy of putamen, middle cerebellar peduncle or pons and “hot-cross-bun sign” (cruciform hyperintensity in the pons) are possibly present. "Rim sign" and "hot-cross-bun sign" have high specificity but low sensitivity for the diagnosis of MSA [71].

Positron emission tomography (PET) with appropriate radiotracers is proved to be useful in the differentiation of the patients with MSA from PD and controls [72]. Fluorodeoxyglucose-PET (FDG-PET) has substantial specificity to differentiate patients with parkinsonism as PD, MSA, progressive supranuclear palsy (PSP) or corticobasal degeneration (CBD) [73].

Cardiac sympathetic innervation visualized using myocardial 123I-metaiodobenzylguanidine (MIBG) scintigraphy [74, 75, 76] or F-dopamine PET [77] may help differentiating MSA-P from PD [78]. Normal cardiac radiotracer uptake indicates the presence of spared cardiac postganglionic sympathetic fibers in MSA, while most PD patients – even those without symptomatic dysautonomia – show a reduced cardiac postganglionic sympathetic innervation. The tests have however suboptimal diagnostic accuracy. Some PD patients may show normal cardiac MIBG uptake [79] whereas sympathetic cardiac denervation can occur in MSA as well [80].

Demonstration of severe autonomic failure in different domains of autonomic regulation supports a presumptive diagnosis of MSA. Investigation of bladder function is necessary in patients with suspected MSA [81]. Urodynamic examination may show vesical detrusor overactivity, absence of detrusor–sphincter coordination, bladder atony usual in MSA. Videourodynamics can provide additional anatomical information in particular cases. Post-void residual volume needs to be determined by ultrasound examination. This test is recommended for patients reporting voiding difficulties. Postvoiding residual urine volume >100 ml is an important urological symptom in MSA [81]. Beside severe OH in orthostatic testing, which is described in previous paragraph, nocturnal hypertension in 24-hours blood-pressure monitoring, impaired cardiovascular autonomic reflexes in Valsalva maneuver and reduced heart rate variability are characteristic cardiovascular autonomic abnormalities for the patients with suspected MSA [1].

Rapid eye movement (REM) sleep behavior disorder during polysomnography is of prognostic value in case of early premotor MSA. This test however has no specificity differentiating MSA-P and PD [82].

Impaired stimulation of growth hormone by clonidine or arginine has been reported to differentiate both MSA-P from PD and possibly MSA-C from idiopathic, late-onset cerebellar ataxia [19]. However the tests have suboptimal diagnostic accuracy and to date the available diagnostic protocols have not entered routine clinical practice.

In autonomic domain of thermoregulation thermoregulatory sweat test may reveal rapidly progressive failure of whole-body sweating, thus potentially differentiating MSA-P and PD [13]. Normal findings in quantitative sudomotor axon reflex test indicate sparing of skin postganglionic sympathetic innervation in MSA and could differentiate MSA-P from PD [79].

1.8. Neuropathophysiology of Dysautonomia in MSA

The neuropathology of autonomic dysfunction in MSA has received considerable attention. Neurodegenerative changes of the dorsal nucleus of the vagus nerve and nucleus ambiguus have been determined [83]. Furthermore, some selected brainstem nuclei appear to be affected as well [84, 85, 86, 87]. Cholinergic cell loss at the mesopontine level (pedunculopontine tegmental nuclei/laterodorsal tegmental nuclei involved in the sleep control) has also been reported in MSA [87]. The hypothalamus is affected [88]. Few studies demonstrated a loss of arginine-vasopressin neurons in the posterior portion of the paraventricular nucleus and in the suprachiasmatic nucleus [84], and a loss of hypocretin/orexin neurons of the posterolateral hypothalamus [89]. All these cell populations are involved in circadian regulation of endocrine and autonomic functions. At lower levels of the autonomic nervous system, lesions are observed typically in sympathetic preganglionic neurons in the intermediolateral column of the thoracolumbar spinal cord that have been linked to OH [90]. Alpha-synuclein aggregates have been also reported in sympathetic ganglia of MSA brains [90, 91, 92]. Urogenital and sexual disorders have been related to neuropathological changes in parasympathetic preganglionic neurons of the spinal cord including Onuf's nucleus [93, 94, 95].

1.9. Neuroanatomical Basics of Human Thermoregulation

Hypothalamus plays a central role for thermoregulatory responses in human body. The anterior hypothalamus contains both warm- and cold-sensitive neurons. Warm-sensitive neurons outnumber cold-sensitive neurons by a 3:1 ratio. Increased core temperatures are associated with increased firing rates of warm-sensitive neurons; cold-sensitive neurons increase their firing rates when core temperatures fall [96]. Afferent pathways for thermal receptors in the skin begin in the dorsal roots and ascend predominantly in the spinothalamic tracts. Thermal receptors in deep tissues such as the abdominal viscera may course through the vagus and splanchnic nerves before entering the central nervous system. Both skin and deep thermal receptor pathways terminate in the anterior and posterior hypothalamic areas. The posterior hypothalamus integrates signals from the skin, deep tissues and anterior hypothalamus, further the posterior hypothalamus triggers autonomic responses appropriate for temperature correction [97].

The intermediolateral and intermediomedial cell columns of spinal segments T1 to L3 of the spinal cord are the origin of preganglionic sympathetic outflow involved in autonomic temperature regulation. These preganglionic neurons receive input from cell groups in the hypothalamus and brainstem, including the rostral ventrolateral medulla, rostral ventromedial medulla, caudal raphe nuclei, lateral and posterior hypothalamus, periaqueductal gray and preoptic area [98]. The axons of preganglionic neurons form the white rami communicantes that pass to the sympathetic trunk. Preganglionic axons may synapse with postganglionic neurons in the sympathetic ganglia and rejoin the spinal nerves as gray rami communicantes. Preganglionic axons may also ascend or descend the sympathetic trunk before making synapses on postganglionic neurons. Other preganglionic fibers course through the sympathetic ganglia and form the splanchnic nerves that synapse in the prevertebral ganglia [97].

Acetylcholine is the neurotransmitter released at preganglionic synapses on postganglionic neurons. Acetylcholine is also released by the postganglionic sympathetic innervation of sweat glands; norepinephrine is released by the vast majority of postganglionic nerves that innervate blood vessels. Other neurotransmitters, particularly peptides, may be released in combination with either acetylcholine or norepinephrine [97].

2. Differential Diagnostics of MSA-P and PD

Due to overlapping motor presentations it can be very challenging to distinguish MSA-P from PD. The cardinal MSA autonomic symptoms - OH and urinary incontinence - are of limited assistance while these symptoms are common among the PD patients as well [99]. Neuroimaging modalities, like MIBG scintigraphy, do not always provide a certain differentiation between these two diseases as well [100, 101, 102].

In PD, alike MSA, there can be failure or dysregulation in some different autonomic domains. Autonomic abnormalities for PD patients may also include constipation, heat or cold intolerance, postprandial hypotension and relative nocturnal hypertension [103, 104]. Pathophysiologically PD involves a peripheral catecholaminergic lesion with the loss of postganglionic sympathetic noradrenergic neurons and parasympathetic cholinergic denervation [105, 106].

Contemporary studies agree that ordinary autonomic testing can hardly help differentiating MSA-P and PD with autonomic failure [106]. The role of specific and sophisticated evaluation of different autonomic domains remains rather controversial [13, 102].

As reported in previous publications, disturbed neurovascular thermoregulation of distal extremities belongs to MSA autonomic features [14, 15]. Hand skin temperature and its response to cooling in subjects with probable MSA-P and PD were compared determining significant differences regarding hand temperature before and after cooling, as well as kinetics of natural rewarming [14, 15]. To date only a minor study sample has been reported. The prevalence of disturbed neurovascular thermoregulation in sizeable patient cohorts remained obscure.

The methodology for testing hand skin temperature and its response to cooling has been established in some specialized medical centers and routinely used examining patients with parkinsonian syndromes. This is a retrospective study, which was conducted on the patients from a specialized neurological acute care hospital, recruited from August, 1997 until July, 2015.

II. PATIENTS AND METHODS

1. Objectives

To improve our knowledge about impaired thermoregulation of distal extremities in MSA-P and PD.

To assess the coexistence and possible interrelations for the different subtypes of autonomic dysfunction in a large cohort of MSA-P, PD and control patients.

2. Patients

Study cohort comprises 577 patients diagnosed with PD according to the UK PD Society Brain Bank diagnostic criteria (PD group) [107], 130 probable MSA-P patients (MSA-P group) [19] and 150 patients having insufficient clinical evidence of a possible α-synucleinopathy or other neurodegenerative disease (control group). The complaints of the control patients after a thorough clinical assessment were addressed to a cerebrovascular disease, neurological complications of diabetes or functional disorders. In the follow-up the controls developed no signs of a neurodegenerative disease. Demographical and anamnestic data of the patients are presented in Table 1. Over the course of the follow-up in the study groups the overlapping of parkinsonian syndromes could be largely excluded.

All the patients were recruited in the Parkinson's Disease Clinic Ortenau, Germany. The retrospective recruitment extended from August, 1997 until July, 2015. This study was carried out in accordance with the approved guidelines of the university research ethics committee.

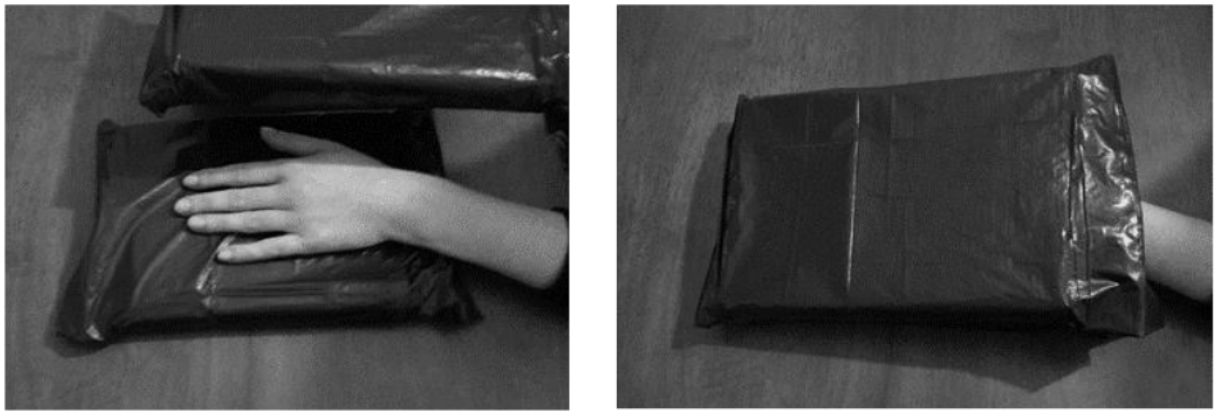
	MSA group	PD group	Control group	p-value
Age, Years	$67.07\pm7.78_a$	$70.37\pm8.97_b$	$74.84\pm6.92_b$	0.001
Age at onset, Years	63.51±7.856	65.11±9.755	-	> 0.05
Symptoms duration, Years	4.56±2.643	6.26±4.911	-	0.001
UPDRS motor	42.56± 13.645	36.54 ±11.611	-	<0.001
UPDRS total	68.37± 22.247	57.37± 17.856	-	<0.001
Male sex	67 (59.82%)	296 (59.20%)	88 (68.22%)	> 0.05
Arterial hypertension	$52\ (46.51\%)_a$	$284\ (56.97\%)_a$	$89\ (68.67\%)_b$	0.001
Diabetes	$11\ (9.82\%)_a$	$47\ (9.42\%)_a$	$27\ (20.93\%)_b$	0.001
Hypothyroidism	14 (12.61%)	82 (16.40%)	17 (13.18%)	> 0.05

Table 1. Demographical data and clinical findings. Data represented as Mean ± Standard Deviation. Unequal subscript letters indicate significant differences between groups.

3. Methods

At the beginning of the retrospective recruitment there was no specific disability rating scale for the MSA patients available. Instead we have used Unified Parkinson's Disease Rating Scale (UPDRS) for the MSA and the PD study groups.

All patients underwent a standard cooling - rewarming procedure, termed ice test (IT). Hand skin temperature was initially measured at rest with a digital thermometer (Quigg TS 402) adhered to the palmar surface of the third finger in patient's left hand. Then the hand was cooled between two standardly prepared ice packs for 5 minutes (Picture 1).



Picture 1. Performing the IT.

Directly after removal from the ice packs, hand temperature was measured again and the hand was left over to rewarm naturally. The measurements were repeated consequently on the 7th, 9th, 11th, 13th and 15th minute of the IT. Some percentage of the patients did not tolerate IT because of painful, unpleasant sensations related to cooling. In such a case the IT was discontinued annotating initial hand temperature (Table 2). All the measurements were performed in a specialized room with controlled temperature (20°C) and neutral humidity. Patients were tested in the morning while they were fasting and had not taken their morning medications.

	MSA group	PD group	Control group	p-value
Ice test completed	112	500	129	-
Ice test discontinued	18 (13.85%)	77 (13.34%)	21 (14.0%)	0.91
Pathological ice test, temperature decrement:				
≥ 0 °C	66 (58.93%)	260 (52.0%)	73 (56.59%)	> 0.05
≥ 1 °C	50 (44.64%)	205 (41.0%)	55 (42.64%)	> 0.05
≥ 2 °C	43 (38.39%)	172 (34.40%)	42 (32.56%)	> 0.05
≥ 3°C	39 (34.82%)	137 (27.40%)	37 (28.68%)	> 0.05
≥ 4 °C	29 (25.89%)	107 (21.40%)	32 (24.81%)	> 0.05
≥ 5 °C	25 (22.32%)	87 (17.40%)	25 (19.38%)	> 0.05
≥ 6 °C	25 (22.32%)	69 (13.80%)	15 (11.63%)	> 0.05
≥ 7 °C	17 (15.18%)	48 (9.60%)	11 (8.53%)	> 0.05
≥ 8 °C	11 (9.82%)	31 (6.20%)	7 (5.43%)	> 0.05
≥ 9 °C	6 (5.36%)	16 (3.20%)	4 (3.10%)	> 0.05
≥ 10 °C	3 (2.68%)	13 (2.60%)	3 (2.33%)	> 0.05
≥ 11 °C	1 (0.89%)	9 (1.80%)	2 (1.55%)	> 0.05
Temperature decrement in pathological IT, ºC	4.33±3.30	4.05±3.28	3.98±3.25	1.0
Time to full rewarming, min	7.91±2.11	7.34±2.18	7.25±2.37	0.40
Temperature increment rate, ºC/min	1.68±0.854	1.79±0.91	1.82±1.01	0.60

Table 2. The prevalence of pathological IT, the frequencies of gradually increasing temperature decrement and evaluation parameters of natural rewarming in the three study groups. Data represented as Mean ± Standard Deviation.

Once the hand temperature at the end of 15-minute IT was lower than the initial temperature at rest, IT was stated to be pathological in terms of defective neurovascular regulation. As a result temperature decrement (initial minus endpoint temperature) was noted. Time to complete rewarming was noted for the patients who after the cooling recovered back to the initial hand temperature earlier than the end of the IT. Considering kinetics of natural rewarming temperature increment rate (endpoint temperature minus temperature directly after the cooling per time to complete rewarming) was calculated (Table 2).

Furthermore, the subdivision of the patients received peripheral and autonomic nervous systems evaluated (Table 3).

	MSA group	PD group	Control group	p-value
Orthostatic hypotension	12 out of 18 (66.67%$_a$)	38 out of 116 (32.76%$_b$)	4 out of 15 (26.67%$_b$)	0.015
Suppressed SSR	64 out of 80 (80.00%$_a$)	144 out of 219 (65.75%$_b$)	34 out of 54 (62.96%$_b$)	0.04
Reduced heart rate variability	67 out of 74 (90.54%)	106 out of 124 (85.48%)	23 out of 30 (76.67%)	> 0.05
Peripheral neuropathy	33 out of 76 (43.42%)	146 out of 344 (42.44%)	54 out of 110 (49.09%)	> 0.05

Table 3. Autonomic disturbances and peripheral neuropathy in the three study groups. Unequal subscript letters indicate significant differences between groups.

The evaluation included autonomic function test of heart rate variability, based on time-domain analysis of directly measured heart periods [108, 109], which are considered as measurements predominantly reflecting parasympathetic modulation of the heart [108].

Electrophysiologic sympathetic skin response (SSR) test was deployed to assess the reflex activity of sympathetic sudomotor pathways [110, 111, 112, 113, 114]. This test has been used in various peripheral and central neurologic disorders [115, 116, 117, 118, 119, 120]. Studies have been performed on PD and MSA patients [121], showing marked qualitative and quantitative SSR abnormalities, including suppression of SSR amplitudes (suppressed SSR) or even loss of response in these diseases [122]. SSR is a multisynaptic reflex that assesses the function of the sympathetic sudomotor pathways [110, 111, 112, 113]. The SSR afferent component depends on

the type of stimulus. At a central level, different structures, such as the reticular formation, limbic cortex, and posterior hypothalamus, seem to be involved. The efferent pathway consists of preganglionic and postganglionic sympathetic neurons and peripheral C fibers, innervating the sweat glands and acting as terminal efferents of the reflex [122, 123, 124]. In this study SSR was elicited by electrical stimulation of the median nerve and simultaneously recorded on the palm, corresponding to the previously described methodology [121].

To evaluate orthostasis, lying-to-standing orthostatic test was performed according to the international consensus on the definition and diagnosis of OH [125]. Patients laid down 10 min and systolic blood pressures (SBP) were measured three times within. After active standing SBP was measured immediately and consequently at each minute while patients stand motionless for 10 min. The severity of OH was expressed in the maximal drop of SBP after standing up from the supine position as the mean of the three measurements.

Electroneurography was deployed in detection of axonal peripheral neuropathy [126].

4. Statistical Analysis

Statistical analyses were performed using the statistical software IBM SPSS Statistics 23 (Armonk, NY: IBM Corp. Software). Data are presented as Mean (M) ± Standard Deviation (SD).

After testing for normality continuous variables were compared with the Student’s t-test and ANOVA for independent or related groups. For the skewed data nonparametric criteria including Mann-Whitney U-test, Kruskal-Wallis 1-way ANOVA or Friedman's test were used when appropriate to compare quantitative samples.

The correlations or associations among the investigated parameters were evaluated by using Pearson's correlation coefficient (r) or Contingency coefficient. To test hypotheses about independence the chi-square testing was applied. For pairwise comparison of proportions z-test was used. The level of statistical significance was set to 0.05.

III. RESULTS

The mean age of the MSA patients was 67.07±7.78 years (Mean ± Standard Deviation). The patients in the MSA study group were significantly younger ($p = 0.001$) than the PD and the control group patients (70.37±8.97 and 74.84±6.92 years, respectively).

The medical history of arterial hypertension, diabetes and medicated hypothyroidism were similarly common in the MSA and the PD study groups (Table 1).

PD patients had median Hoehn and Yahr stage 3; staging histogram for the PD patients is presented in Figure 1. Likewise, Hoehn and Yahr staging was applied for 41 patients in the MSA study group (Figure 2).

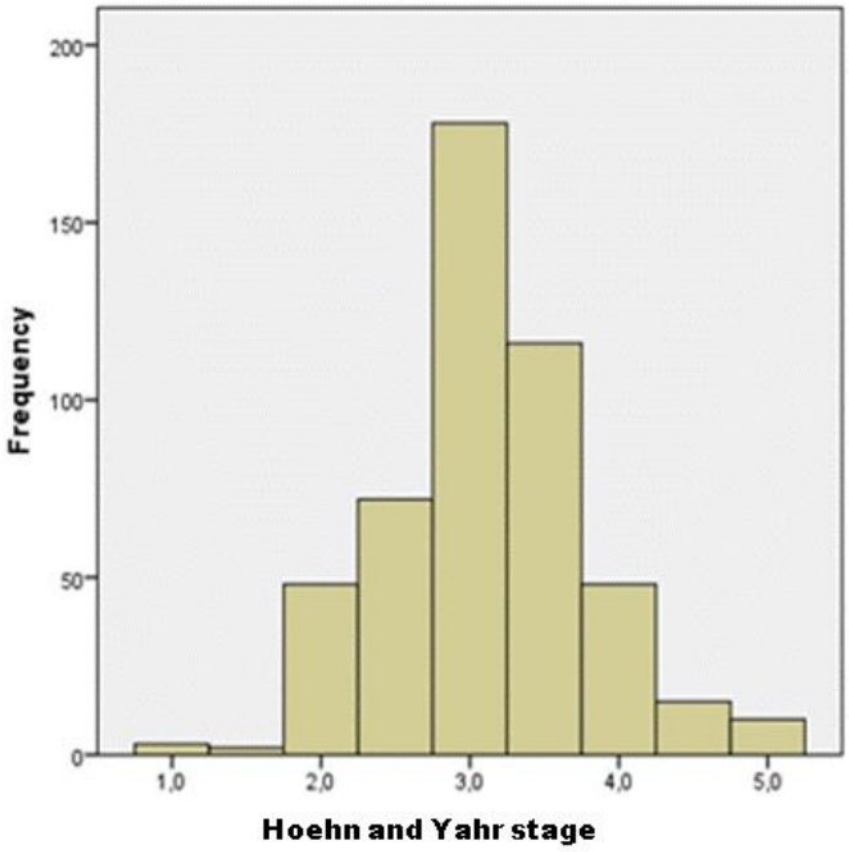


Figure 1. Hoehn and Yahr staging histogram for the PD study group.

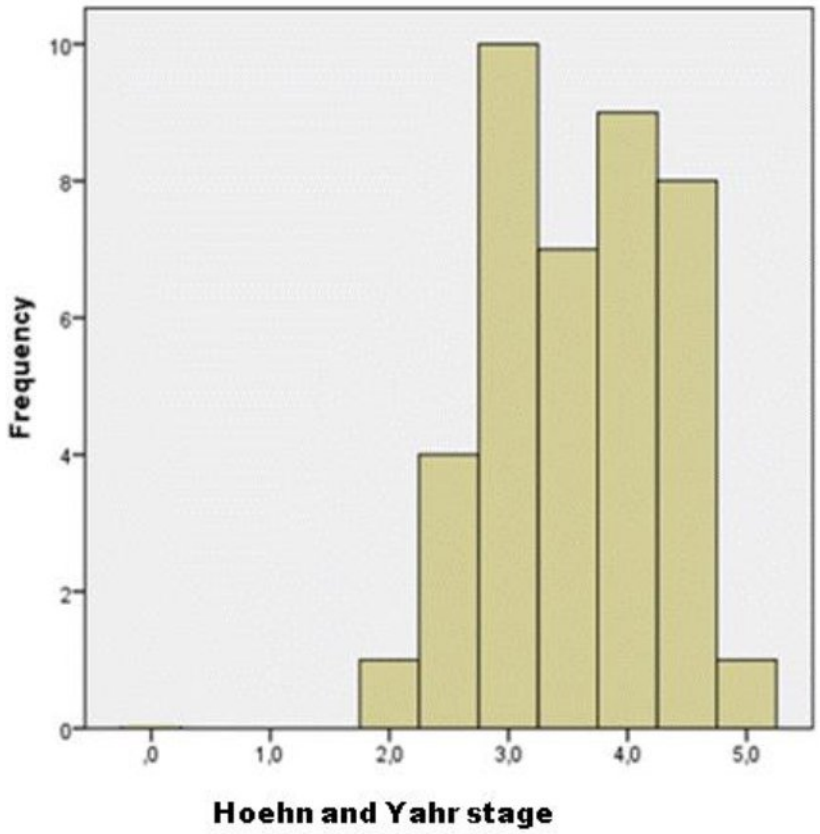


Figure 2. Hoehn and Yahr staging histogram for the MSA study group.

The MSA patients had higher UPDRS motor and total scores than the PD patients ($p < 0.001$).

In the PD group the history of L-dopa medication was available for 275 patients. 28 out of 275 patients did not receive L-dopa, for the rest the mean L-dopa daily dose was 463.25±184.20 mg. Similarly, the history of L-dopa medication was available for 88 MSA patients. 9 patients received no L-dopa, for the rest 79 patients the mean L-dopa daily dose was 651.66± 293.61 mg ($p < 0.001$, Figure 3). For PD and MSA study groups L-dopa consumption histograms are shown in Figures 4 and 5.

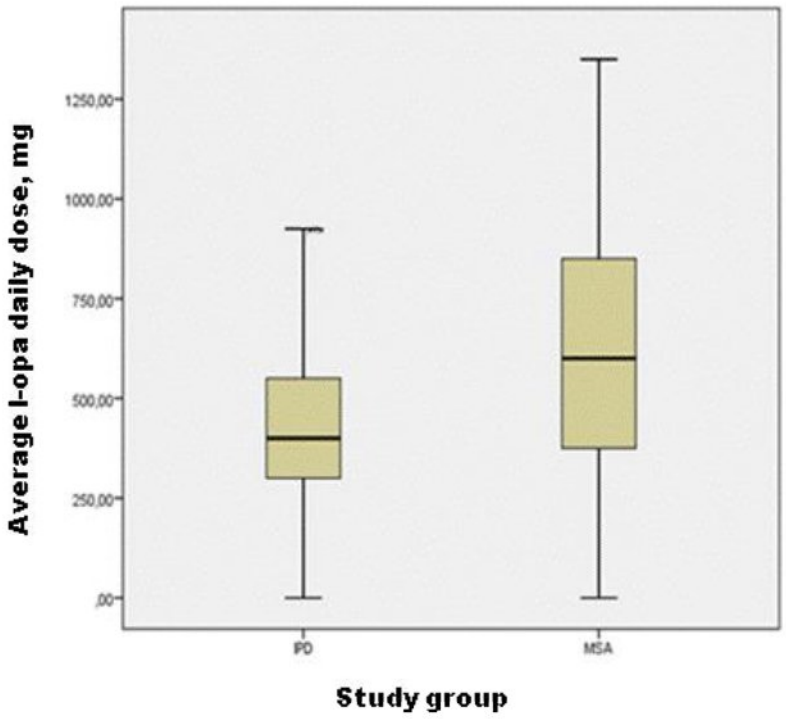


Figure 3. Mean L-dopa daily dose in the PD and the MSA study groups, $p < 0.001$.

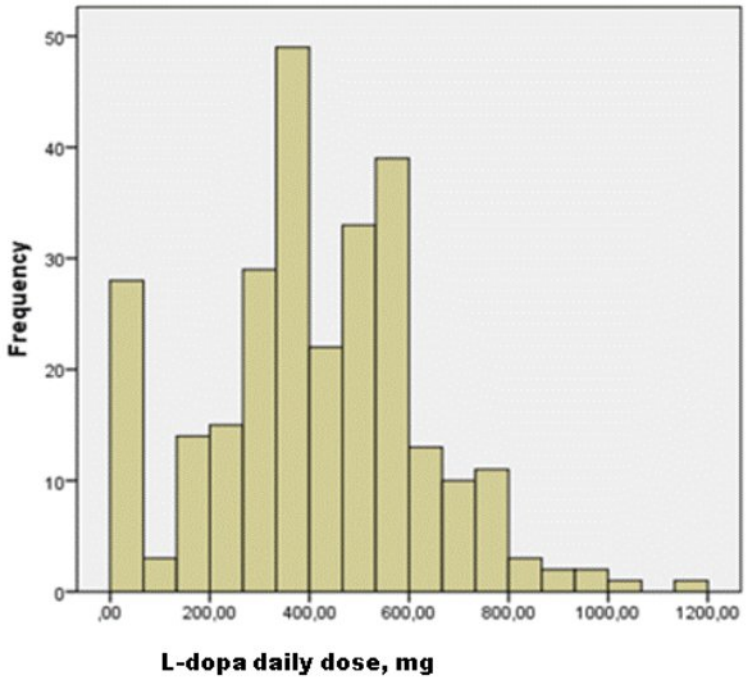


Figure 4. L-dopa consumption histogram for the PD study group.

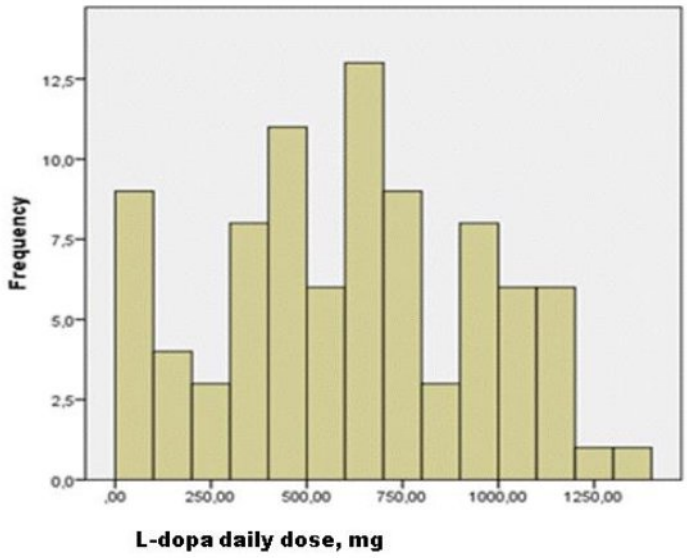


Figure 5. L-dopa consumption histogram for the MSA study group.

Principally used dopamine agonists in the MSA and the PD study groups are presented in Table 4.

	MSA group	PD group	p-value
Pramipexole	13 (11.61%)	137 (27.45%)	<0.001
Piribedil	4 (3.57%)	66 (13.23%)	<0.001
Ropinirole	10 (8.93%)	130 (26.05%)	<0.001
Rotigotine	6 (5.36%)	23 (4.61%)	<0.001
Cabergoline	3 (2.68%)	-	-
Anticholinergics	2 (1.79%)	19 (3.80%)	<0.001

Table 4. Dopamine agonists used in the MSA and the PD study groups.

Initial hand skin temperature histograms for the three study groups are shown in Figure 6 A, B and C. The probability distributions of initial hand skin temperature in MSA, PD and control study groups are presented in the Appendix.

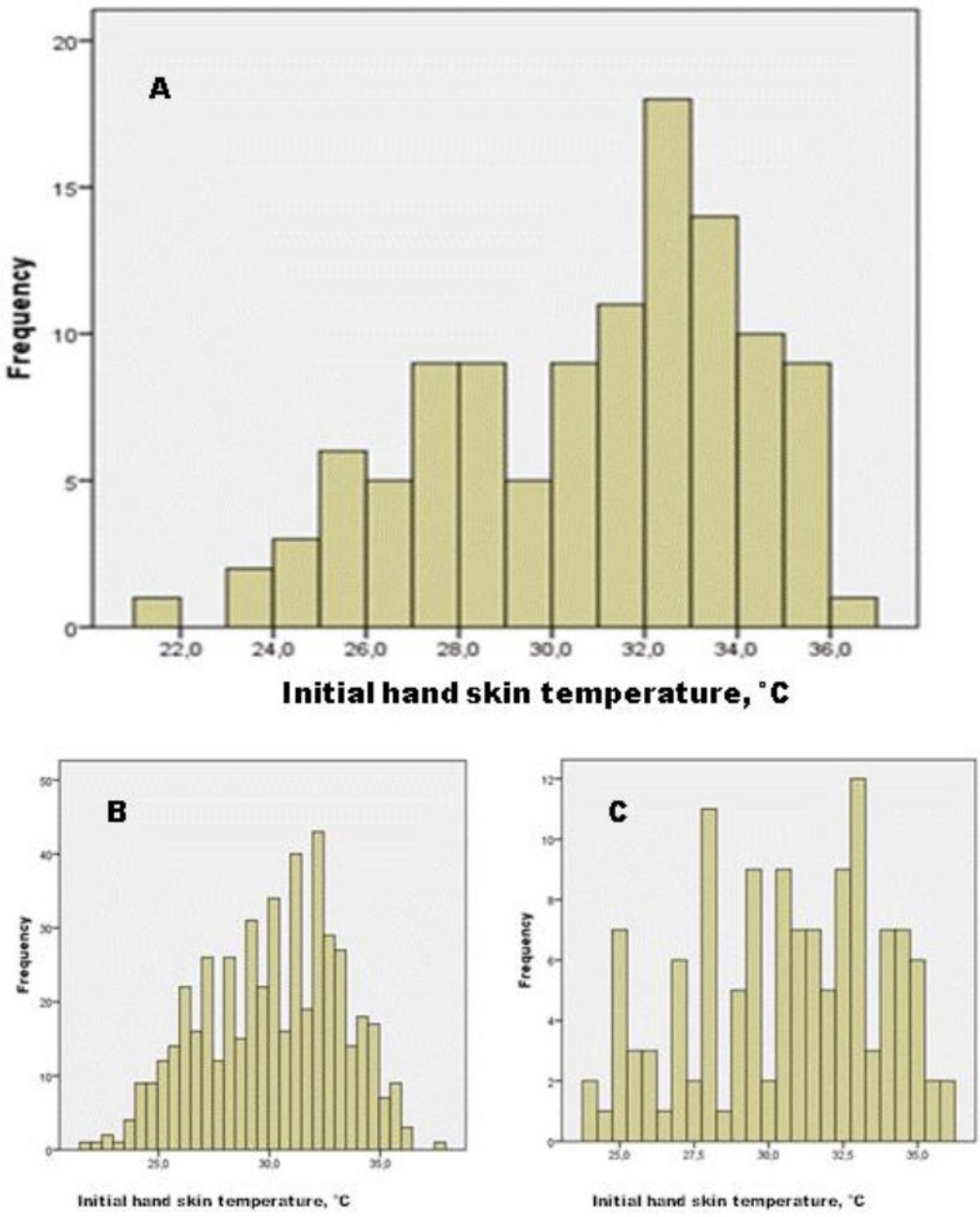


Figure 6 A, B and C. Initial hand skin temperature histograms in MSA, PD and control study groups respectively.

In MSA, PD and control study groups the hand skin temperatures measured at rest (30.75±3.35; 30.11±3.09 and 30.59±3.13°C, respectively, p = 0.072, Figure 7), immediately after the cooling (13.87±7.64; 13.86±6.66 and 13.96±7.18°C, respectively, p = 0.99) and subsequently at each measurement during natural rewarming did not differ significantly (Table 5, Figure 8).

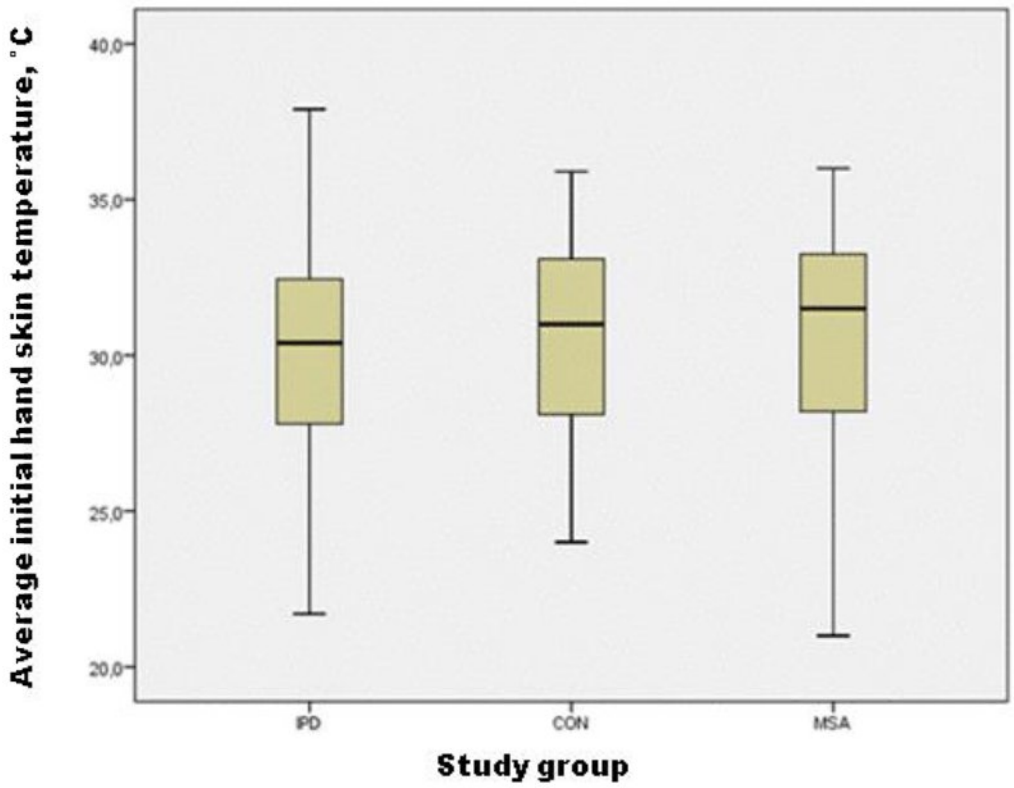


Figure 7. Initial hand skin temperature means for the three study groups, p = 0.072.

	MSA group		PD group		Control group	
	Mean, °C	Std. Dev., °C	Mean, °C	Std. Dev., °C	Mean, °C	Std. Dev., °C
Initially	30.75	3.35	30.11	3.09	30.59	3.13 *
5 min.	13.86	7.64	13.86	6.66	13.96	7.18 *
7 min.	19.78	8.10	19.91	7.36	20.40	7.91 *
9 min.	23.83	7.13	23.87	6.69	24.43	7.11 *
11 min.	26.22	6.33	26.14	5.99	26.69	6.25 *
13 min.	27.80	5.83	27.66	5.37	28.06	5.46 *
15 min.	28.71	5.22	28.62	4.98	28.82	5.05 *
5 min., %	44.80	23.57	45.52	19.94	44.81	20.86 *
7 min., %	63.78	24.10	65.48	21.31	65.60	22.11 *
9 min., %	77.02	20.45	78.71	18.77	79.14	19.38 *
11 min., %	85.03	17.86	86.43	16.35	86.74	16.35 *
13 min., %	90.31	16.31	91.61	14.16	91.43	13.73 *
15 min., %	93.33	14.11	94.93	12.93	94.03	12.51 *

Table 5. Hand skin temperatures initially at rest and at each measurement of the IT. The percentage temperature values at each point after the cooling. Std. Dev. Standard Deviation.

* denotes $p > 0.05$.

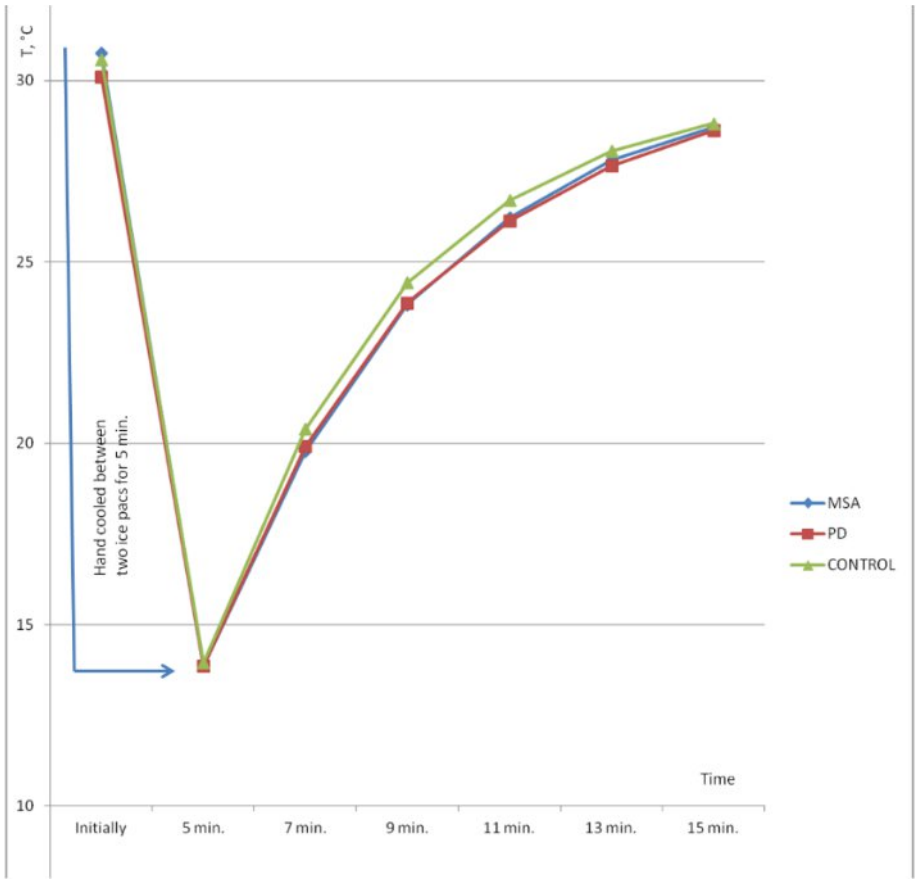


Figure 8. Hand temperature means in the course of IT.

Despite the absence of consistent group difference, to evaluate the kinetics of cooling and rewarming accurately, hand temperature at each measurement of the IT was expressed as the percentage of initial resting hand temperature. The percentage values at each point after the cooling were consistently, but not significantly lower in the MSA group (Table 5, Figure 9).

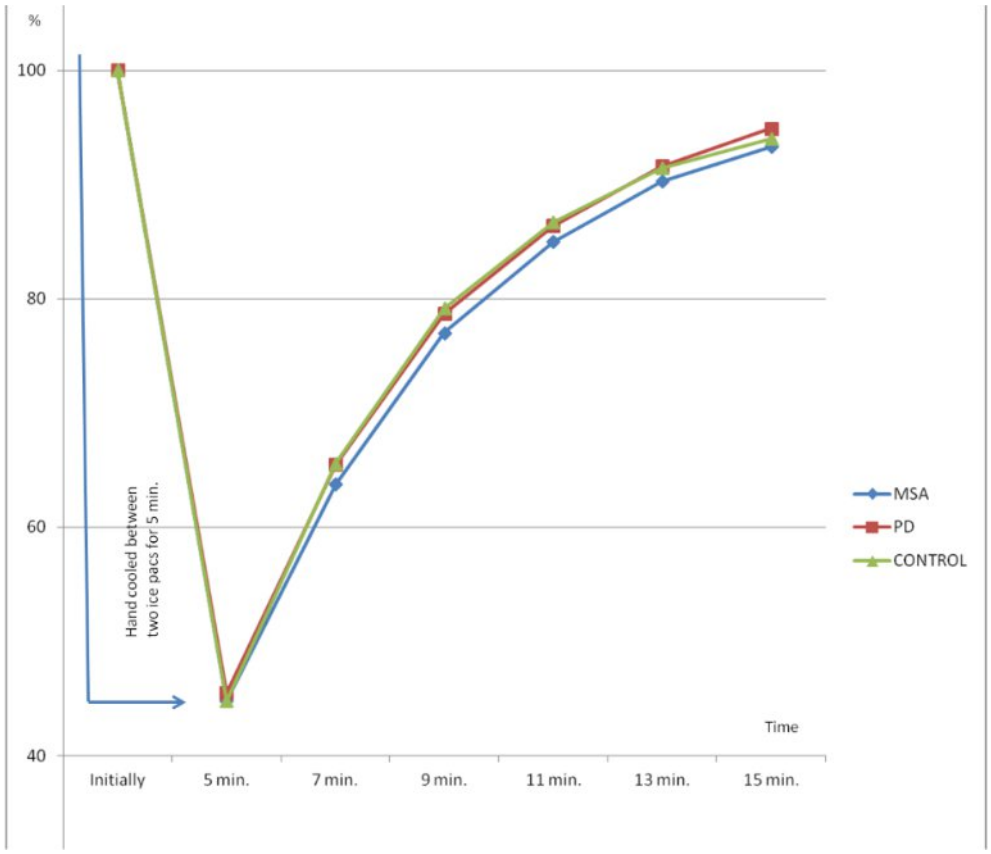


Figure 9. Hand temperature means expressed as the percentage of initial resting hand temperature.

The prevalence of pathological IT was higher in the MSA than in the PD and the control groups, the difference, however, was not statistically significant (58.93%; 52.0% and 56.59%, respectively, $p = 0.33$).

Patients in the MSA study group were significantly younger than the PD patients and the controls. To alleviate the possible influence of age, we have arranged all the study patients into three preset age-layers of younger than 65; 65 - 75; and older than 75 years of age. Further, we have compared the prevalence of pathological IT between the three study groups in the each age-layer separately and found no significant relation for the study group and the IT result ($p = 0.21$; 0.14 and 0.32, respectively).

In the study cohort the patients having a pathological IT had a significantly lower hand temperature already before the cooling compared to the patients with a non-pathological IT (29.92±3.26 and 30.73±2.90°C, respectively, $p < 0.001$). IT for the patients whose initial hand temperature was abnormally low (≤ 29.92°C) and who afterwards did not tolerate the cooling were consequently assumed to be pathologic. In such a manner the prevalence of pathologic IT in the three study groups was re-evaluated remaining not significantly higher in the MSA group (60.0%; 54.0% and 58.59%, respectively, $p = 0.24$).

Hypothesizing that potentially the pathological IT gains more specificity in the differentiation of MSA *versus* PD along with the gradually increasing temperature decrement, the frequencies of temperature decrement ≥ 1, 2, ..., 11°C in the three study groups were estimated. The frequencies of gradually increasing temperature decrement were consistently higher in the MSA group, the differences remained not sufficient for a statistical significance (Table 2). The greatest temperature decrement observed in the study cohort was 17.4°C (the PD group).

Defective thermal regulation of distal extremities could possibly occur due to conditions such as hypothyroidism, known peripheral neuropathy, diabetes and possible diabetic cutaneous microangiopathy, which are merely associated with autonomic dysfunction [14, 15, 127]. To avoid this potential confusion we have re-evaluated the prevalence of pathological IT excluding the patients with treatment for hypothyroidism and diabetes in their medical history or with detected peripheral neuropathy. Still, the results remained similar, there was no statistically significant difference for the prevalence of pathological IT in the three study groups observed (62.50%; 56.20% and 48.48%, respectively, $p=0.52$).

Neither the presence of pathological IT, nor the temperature decrement in case of pathological IT were associated with the L-dopa daily dose or the principally used dopamine agonist in the MSA and the PD study groups.

In the PD group the presence of pathological IT was related with older patient's age ($p < 0.001$), older age at onset of disease ($p < 0.001$), higher UPDRS motor ($p = 0.007$) and total scores ($p = 0.002$). Interestingly, there was no such relation in the MSA group observed. In the control group the presence of pathological IT was related to patient's age in a statistically significant manner ($p = 0.034$).

A significant association for the presence of pathologic IT and suppressed SSR in the study cohort was determined ($p = 0.003$). The rate of suppressed SSR itself was significantly higher in the MSA group compared to the PD and the control groups (80.00%; 65.75% and 62.96%, respectively, $p = 0.04$) (Table 3).

There was no statistically significant relation between the pathological IT and the history of arterial hypertension, diabetes, medicated hypothyroidism or detected peripheral neuropathy neither in the three study groups separately, nor in the study cohort observed. An association for the pathological IT and patient's gender could not be determined.

In the study cohort we observed a significant association between the presences of pathological IT and the symptom of orthostatic hypotension ($p < 0.001$). The association remained statistically significant in the PD study group ($p < 0.01$). To evaluate the influence of possible confounders (patient's age, age at onset of disease, disease duration, UPDRS motor and total scores, history of arterial hypertension, hypothyroidism, diabetes and detected peripheral neuropathy) we performed multivariate logistic regression analysis. These confounders were determined having no significant influence ($p > 0.05$).

In case of pathologic IT and a drop of systolic blood pressure after active standing in orthostatic test there was no statistically significant correlation for the temperature decrement and the maximal drop of systolic blood pressure determined ($p = 0.27$).

The symptom of OH was significantly more prevalent in the MSA than in the PD and the control groups (66.67%, 32.76% and 26.68%, respectively, $p = 0.015$). Male patients suffered from OH more often than female patients ($p = 0.001$). The symptom of OH was not related with the history of arterial hypertension, diabetes, hypothyroidism as well as with the detected peripheral neuropathy, reduced heart rate variability and suppressed SSR in a statistically significant manner. Neither the OH itself, nor the maximal drop of systolic blood pressure after active standing were associated with the L-dopa daily dose nor with the principally used dopamine agonist in the MSA and the PD study groups.

In the MSA group the severity of OH expressed in the maximal drop of systolic blood pressure after active standing tended to correlate with older patient's age ($p = 0.051$, $r = 0.48$) and correlated with older age at onset of disease significantly ($p = 0.029$, $r = 0.53$). For the PD patients suffering from OH the significant correlation regarding the severity of OH and patient's age, age at onset of disease or disease duration could not be determined.

In the MSA and the PD groups the presence of suppressed SSR was associated with the detected peripheral axonal neuropathy significantly ($p = 0.044$ and 0.018, respectively). Such a correlation however was absent in the control study group.

Presence of peripheral axonal neuropathy was not associated with the L-dopa daily dose in the MSA ($p = 0.18$) and the PD ($p = 0.92$) study groups significantly (Figures 10 and 11).

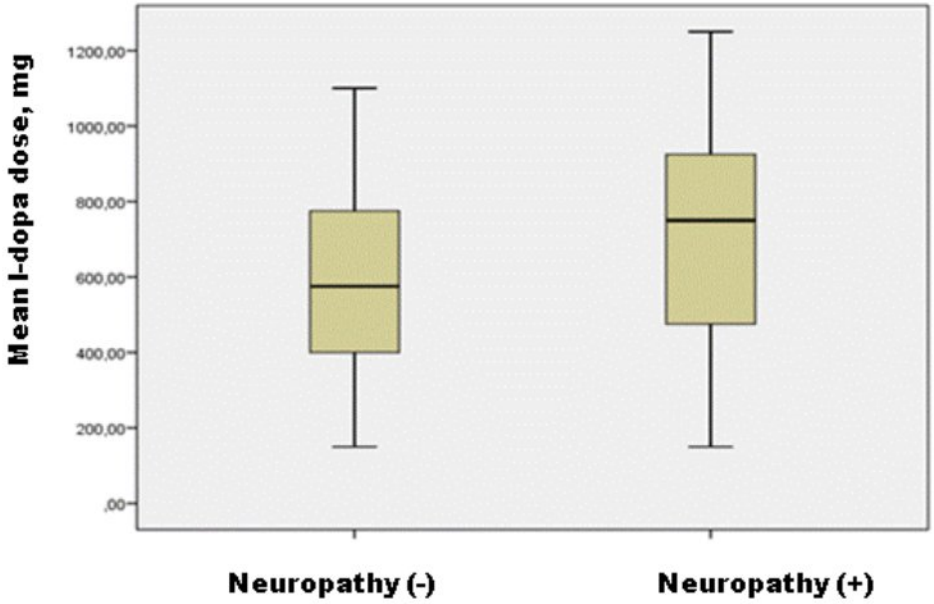


Figure 10. Mean L-dopa daily dose for the MSA patients without and with the detected peripheral axonal neuropathy, p = 0.18.

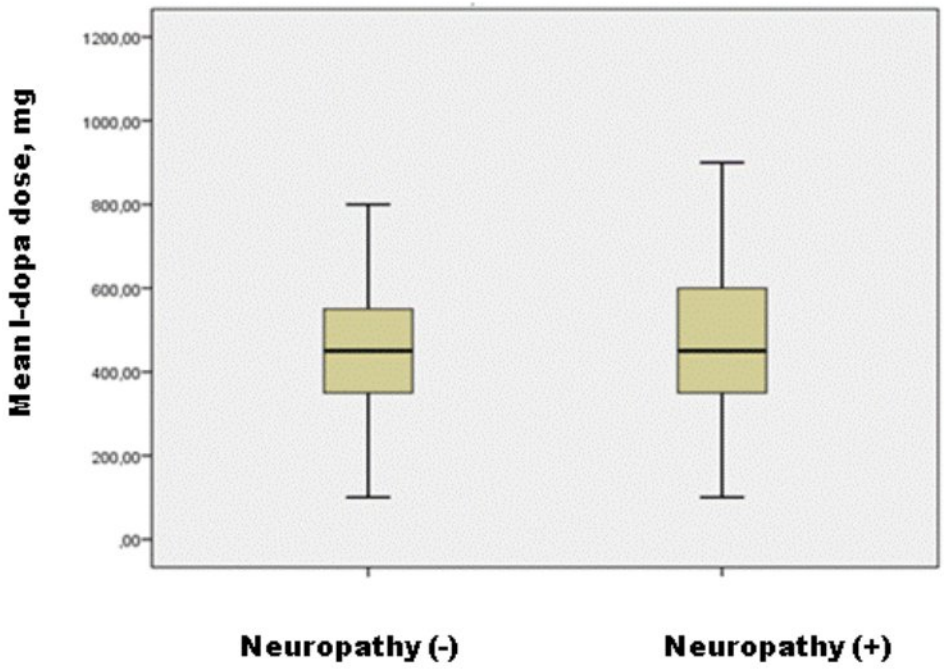


Figure 11. Mean L-dopa daily dose for the PD patients without and with the detected peripheral axonal neuropathy, p = 0.92.

The prevalence of reduced heart rate variability was higher in the MSA than in the PD and the control groups, but did not differ significantly (90.54%; 85.48% and 76.67%, respectively, $p > 0.05$) (Table 3).

IV. DISCUSSION

MSA together with PD, Lewy body dementia and pure autonomic failure form a group of neurodegenerative disorders - the α-synucleinopathies, characterized by the abnormal accumulation of misfolded α-synuclein. A definitive diagnosis of MSA requires the neuropathological findings of widespread α-synuclein positive glial cytoplasmic inclusions associated with neurodegenerative changes in striatonigral or olivopontocerebellar structures [128]. Furthermore, neurodegenerative changes affect the central autonomic nervous system, including the hypothalamus, brain-stem nuclei, dorsal nucleus of the vagus nerve, nucleus ambiguus, Onuf's nucleus and sympathetic preganglionic neurons in the intermediolateral column of thoracolumbar spinal cord [1, 129, 130]. Autonomic dysfunction in PD though seems to be caused mainly by the postganglionic lesion [13, 131].

Due to overlapping motor presentations it can be very challenging to distinguish MSA from PD. The cardinal MSA autonomic symptoms - OH and urinary incontinence - are of limited assistance while these symptoms are common among the PD patients as well [99]. Neuroimaging modalities, like MIBG scintigraphy, do not always provide a certain differentiation between these two diseases [100, 101, 102].

The results reveal the disturbed neurovascular thermoregulation of distal extremities as shown by the pathological IT to be highly prevalent for the MSA and the PD patients. The prevalence of pathological IT was slightly increased in the MSA group compared with the PD and the control groups. The frequencies of gradually increasing temperature decrement were consistently higher

in the MSA group. The mean temperature decrement in pathological IT was also higher for the MSA patients, although there were no statistically significant differences observed. This study shows that despite the substantial overlap the autonomic feature of defective thermoregulation is more severe in the MSA patients. The underlying pathophysiology remains rather incompletely understood.

The results of this work however disagree with the previously published study [14], which observed significantly lower initial hand skin temperature, greater reduction of hand temperature after cooling and significantly prolonged rewarming for the MSA patients compared with the PD patients and the controls. The previous study reported only a minor study sample though.

Having in mind the observed pattern of natural rewarming (Figures 8 and 9), which was quite similar in the three study groups during 10 minutes after the cooling, extending temperature observation should be considered to increase the specificity of pathological IT. To unify the conditions in the three study groups, the IT was performed on the left hand for all the patients. In patients with motor asymmetry the association between the presence of pathological IT and more affected side seems to be probable and should be further investigated to get more insight into the pathology causing impaired thermoregulation. Furthermore, a considerable percentage of the patients did not tolerate IT because of painful, unpleasant sensations related to cooling. It could be beneficial to quantify the patients' painful sensations experienced in the course of the IT using, for example, the Numeric Pain Rating Scale [132].

At the beginning of the retrospective recruitment for this study there was no specific disability rating scale to rate the MSA patients available. Instead we have used UPDRS for the MSA and the PD study groups. The major disadvantage of UPDRS is that this scale imperfectly reflects the complex motor impairment of MSA. On the other hand, thereby we have achieved the uniform comparison between the MSA and the PD patients.

The presence of pathological IT was significantly associated with older patient's age in the PD and the control study groups. This was not the case for the MSA patients, who themselves were significantly younger than the patients of the PD and the control groups. Defective thermoregulation appears to be an intrinsic factor to MSA. Furthermore it should be considered in aging patients with PD and in aging population comprehensively.

Patients in the MSA study group were significantly younger than the PD patients and the controls. We have tried to alleviate the possible influence of age to the comparison of the study groups by analyzing the prevalence of pathological IT in the preset age-layers. However, younger age and more rapid deterioration constitute some particular feature for the MSA.

In the study cohort pathologic IT was significantly associated with the suppressed SSR. It is reported that SSR to electric stimulation are mediated through the efferent pathway consisting of preganglionic and postganglionic sympathetic neurons. The amplitudes of responses are suppressed or responses could be even absent when disturbance in efferent pathway occurs [122, 123]. Based on the results we can hypothesize that preganglionic sympathetic neurons might be involved in the pathological mechanisms responsible for impaired response to cooling in MSA.

This is the first study to determine a significant association between the presence of pathological IT and the symptom of OH. The results suggest a pathophysiological affinity between these two types of autonomic dysfunction, which again could be a defective sympathetic neurovascular regulation. We did not find any other numeric values of temperature decrement in pathological IT and maximal drop of systolic blood pressure after active standing from supine position, therewith these two pathophysiologically related symptoms could correlate each other stronger than with the contemporary consensus values of temperature decrement ≥ 0 °C and drop of systolic blood pressure ≥ 20 mmHg.

The severity of OH expressed in maximal drop of systolic blood pressure tended to correlate with older patient's age and correlated with older age at onset of disease significantly in the MSA group. In compliance with the recent publications for the PD patients suffering from OH no significant correlation between the severity of OH and patient's age, age at onset of disease or disease duration has been determined [10, 133]. In the PD group the severity of OH seems to show no significant advance with the disease duration.

OH and suppressed SSR were significantly more prevalent in the MSA group. Abnormalities in different domains of autonomic regulation, like pathologic IT and reduced heart rate variability, were more common among the MSA patients than in the PD and the control study groups, thus adding more evidence on the multisystemic nature of underlying neuropathology.

To sum up, the abnormal IT provides supplementary information on the extent of autonomic failure. Widespread and well documented dysautonomia should increase specificity in the distinction of MSA-P and PD. Accurate *antemortem* diagnosis of MSA remains of great importance defining patient's prognosis or enrolling in particular clinical trials.

The considerable strengths of this study are that the data from sizeable cohort of MSA-P, PD and control patients were available for analysis and the study patients all were recruited in the neurological department, specialized for the management of parkinsonian syndromes.

Conclusions

Due to overlapping motor presentations it can be very challenging to distinguish MSA-P from PD, especially when PD also involves the autonomic nervous system.

Defective thermoregulation of distal extremities seems to be more severe in the MSA patients. So-called ice test however does not differentiate MSA-P and PD reliably.

Preganglionic sympathetic neurons might be involved in the pathological mechanisms responsible for impaired response to cooling in MSA.

The results suggest pathophysiological affinity of impaired sympathetic neurovascular regulation between pathological IT and OH.

Specific autonomic testing including IT in a dedicated center contributes differentiating MSA-P and PD.

V. APPENDIX

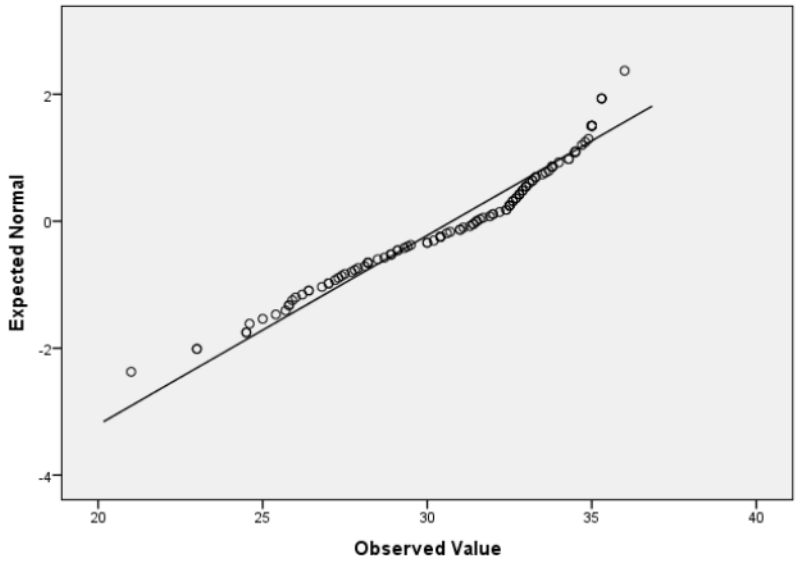


Figure 1a. Probability plot of initial hand skin temperature in MSA study group

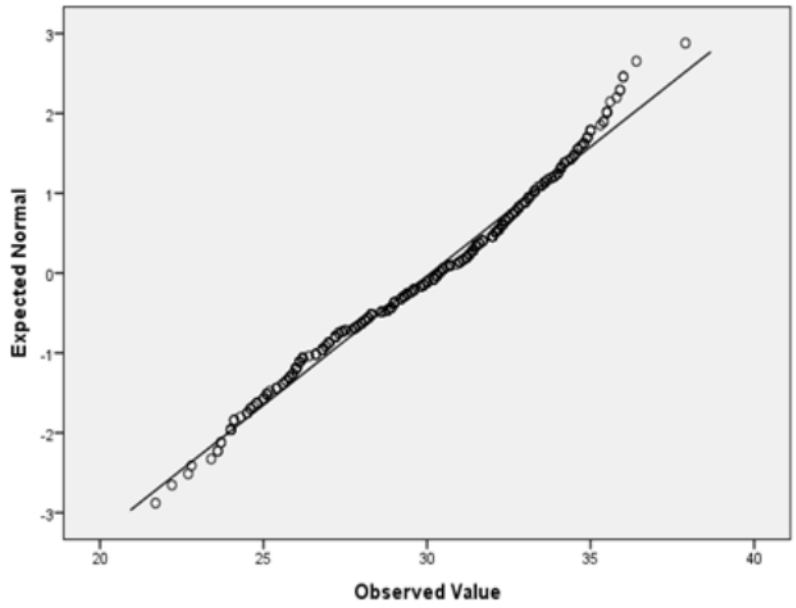


Figure 2a. Probability plot of initial hand skin temperature in PD study group.

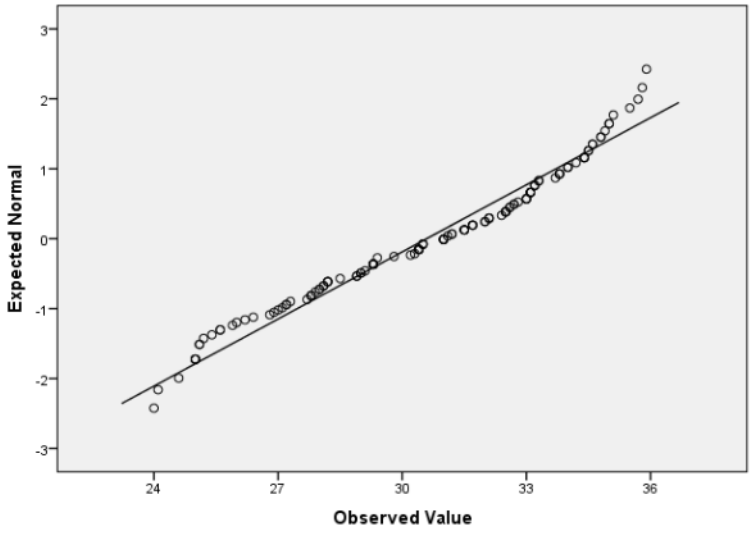


Figure 3a. Probability plot of initial hand skin temperature in control study group.

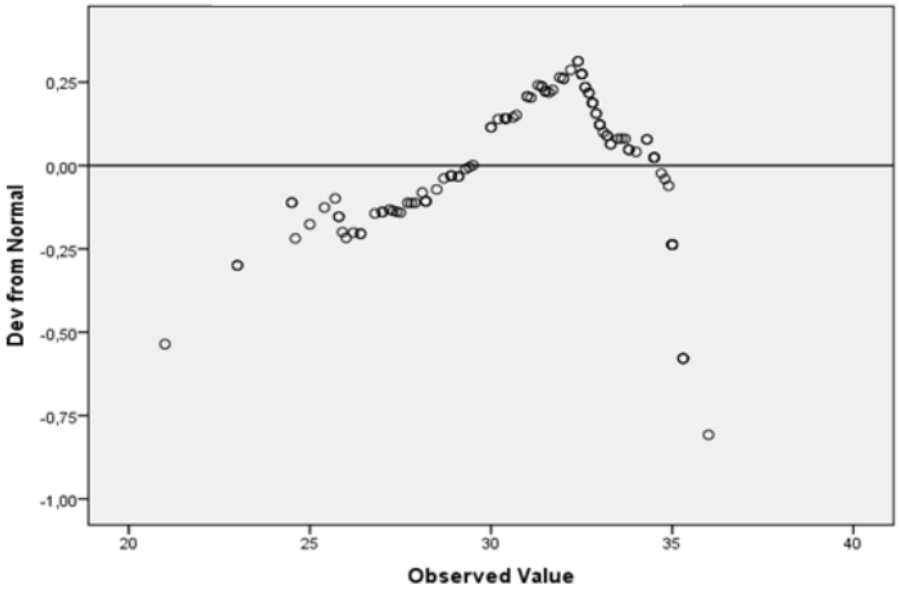


Figure 4a. Detrended probability plot of initial hand skin temperature in MSA study group

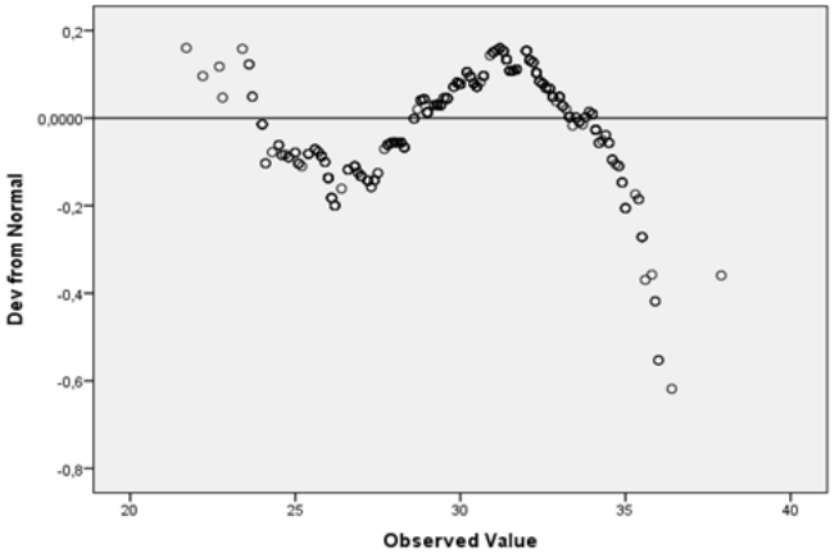


Figure 5a. Detrended probability plot of initial hand skin temperature in PD study group

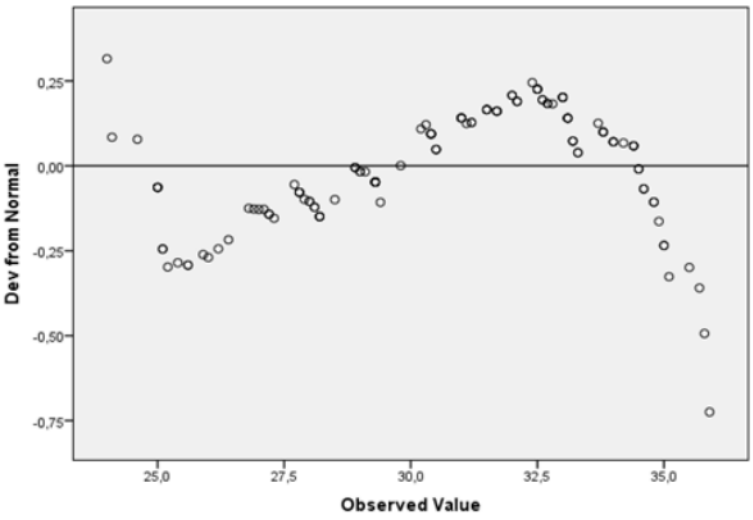


Figure 5a. Detrended probability plot of initial hand skin temperature in control study group

VI. ABBREVIATIONS

123I-metaiodobenzylguanidine (MIBG)

Analysis of variance (ANOVA)

Computerized tomography (CT)

Corticobasal degeneration (CBD)

Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV)

Fluorodeoxyglucose (FDG)

Glial cytoplasmic inclusions (GCI)

Ice test (IT)

Magnetic resonance imaging (MRI)

Mean (M)

Multiple system atrophy (MSA)

Multiple system atrophy with cerebellar features (MSA-C)

Multiple system atrophy with parkinsonian features (MSA-P)

Olivopontocerebellar atrophy (OPCA)

Orthostatic hypotension (OH)

Parkinson‘s disease (PD)

Positron emission tomography (PET)

Progressive supranuclear palsy (PSP)

Rapid eye movement (REM)

Shy-Drager syndrome (SDS)

Sympathetic skin response (SSR)

Systolic blood pressure (SBP)

Standard Deviation (SD)

Striatonigral degeneration (SND)

Unified MSA Rating Scale (UMSARS)

Unified PD Rating Scale (UPDRS)

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VIII. PUBLISHING

The results of this study were successfully published in the Journal of Neural Transmission as an original article:

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