UNIVERSITÀ
DI PAVIA

PhD IN BIOMEDICAL SCIENCES

DEPARTMENT OF BRAIN AND BEHAVIORAL SCIENCES

UNIT OF NEUROPHYSIOLOGY

External anal sphincter electromyographic patterns in multiple system atrophy: implications for diagnosis, clinical correlations, and novel insights into prognosis

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a.a. 2021/2022

TABLE OF CONTENTS

1 INTRODUCTION

1.1 MSA: neuropathology, phenomenology, and diagnostic criteria

Multiple system atrophy (MSA) is a sporadic, progressive, adult-onset α-synucleinopathy clinically featured by autonomic failure associated with poorly levodopa-responsive parkinsonism and cerebellar syndrome in various combinations [1]. Anatomical sites and load of the insoluble a-synuclein aggregates, forming glial cytoplasmic inclusions in the oligodendrocytes, determine the type and severity of autonomic disturbances along with the predominant motor phenotype [2]. Early-onset and severe autonomic symptoms are associated with poor prognosis in MSA patients [3–9].

Urogenital dysfunction is a common manifestation of dysautonomia in MSA and often appears earlier than cardiovascular abnormalities, such as orthostatic hypotension [10, 11]. Urinary symptoms of MSA patients stem from detrusor hyperreflexia and urethral sphincter weakness, which cause urgency and incontinence, or from impaired detrusor contraction and detrusor–sphincter dyssynergia, which result in increased post-void residual volume [12]. A widespread neurodegeneration of areas subserving autonomic control accounts for the multifaceted pathophysiology of urogenital dysfunction [13]. Neuronal loss in the basal ganglia, which physiologically send inhibitory input to the pontine micturition center, leads to detrusor hyperreflexia. Loss of preganglionic parasympathetic neurons in the intermediolateral cell columns of the sacral spinal cord in combination with degeneration of the dorsal pons and lateral medullary reticular formation are responsible for the inability to voluntarily initiate voiding and incomplete bladder emptying due to detrusor failure. Loss of anterior horn cells in Onuf's nucleus, which is located between the second and forth sacral segments of the spinal cord, causes denervation of the pelvic floor muscles, in particular the striated muscles of urethral and anal sphincters, resulting in sphincter weakness and consequent urinary incontinence [14].

An early MSA diagnosis is critical to prevent potentially life-threatening complications, to allow the possible recruitment of patients in disease-modifying clinical trials, and to avoid futile urological surgical interventions. For instance, it was previously found that about 40% of men underwent prostatic or bladder neck surgery for outflow obstruction symptoms before being diagnosed as MSA [12]. However, especially in the early disease stage, the differential diagnosis can be tricky, as shown by the evidence that movement disorders specialists perform a correct antemortem clinical diagnosis in about 50% of pathologically proven MSA patients [15]. In particular, the differentiation of MSA from Parkinson's disease (PD) is hampered by the occurrence of autonomic dysfunction symptoms in both conditions, although with different levels of severity [16].

Due to the suboptimal accuracy of the second consensus criteria for MSA diagnosis [17], a Study Group of the International Parkinson and Movement Disorder Society has recently developed novel diagnostic criteria [18]. While the criteria for neuropathologically established MSA did not change, a new category of clinically established MSA was designed to maximise specificity with acceptable sensitivity. In particular, brain magnetic resonance imaging markers suggestive of MSA are required for

this diagnostic level. In parallel, the category of clinically probable MSA aimed to increase sensitivity without any significant reduction in specificity. A research category of possible prodromal MSA was introduced to identify patients in the earliest stages when criteria for clinically established or clinically probable MSA are not satisfied.

1.2 Methodology of EAS EMG: acquisition and analysis

Neurophysiological assessment has proven to be of great relevance in exploring distinctive clinical features of MSA patients [19, 20]. Notably, electromyography (EMG) of the external anal sphincter (EAS) was proposed as an ancillary investigation in the past diagnostic flow chart of MSA, since it can provide useful clues for the differential diagnosis [21]. Indeed, bearing in mind that EAS is a striated muscle of the pelvic floor that is innervated by motor neurons of Onuf's nucleus via the pudendal nerve, the EMG observation of EAS denervation and reinnervation may indirectly corroborate the histopathological findings of Onuf's nucleus degeneration, therefore supporting MSA diagnosis.

The electrophysiological procedure requires that the patient assumes the lateral position on the bed examination, keeping hips and knees flexed. A concentric needle electrode is inserted into the four quadrants of the EAS under audio guidance, while the ground electrode is placed on a limb, typically the thigh contralateral to the side-lying position. Given that the recording site and type of muscle activation influence motor unit action potential (MUAP) parameters of the EAS, both the superficial and deep muscle layers as well as both the tonic and phasic EAS activity should be explored to achieve a comprehensive MUAP characterization. Indeed, MUAPs recorded from the superficial layer of the EAS and low-threshold MUAPs, constituting its basal activity, have a lower amplitude and shorter duration as compared to MUAPs from the deep layer and high-threshold MUAPs recruited after voluntary or reflex activation [22]. The superficial layer is assessed by means of perpendicular insertion of the needle electrode, 1 cm laterally to the anal orifice at a depth of 3–6 mm, whereas the deep layer is reached at the anal orifice by inserting the needle electrode at an angle of 30º to a depth of 15–25 mm [22, 23]. The basal tonic activity is observed at rest with the subject fully relaxed, otherwise from the voluntary or reflex phasic activation, which is recorded by asking the patient to squeeze glutes or to cough, respectively. It is noteworthy that at least ten MUAPs from different EAS sites should be assessed to draw appropriate conclusions from the electrophysiological examination, since denervation and reinnervation can affect only a proportion of MUAPs [12].

Several EMG findings can be evaluated to identify the presence and severity of neurogenic damage of the EAS. Pathological spontaneous activity (i.e., fibrillation potentials, positive sharp waves, or complex repetitive discharges) can be challenging to discriminate from EAS basal tonic activity, thus it is often not taken into account [24, 25]. Nevertheless, a careful analysis of the characteristic shape and firing rate enables the detection of pathological spontaneous activity that may represent a valuable electrophysiological finding, especially when not associated with abnormalities of MUAP parameters, therefore suggesting an initial disease stage of ongoing active denervation without significant chronic

reinnervation.

Rather than amplitude, percentage of polyphasic potentials and mainly duration were found to represent the most reliable MUAP parameters to discriminate neurogenic abnormalities in MSA patients [24–28]. Measurement of MUAP parameters greatly depends on the method of acquisition and analysis. On the one hand, the automated techniques (i.e., manual-MUAP and multi-MUAP analysis) allow a quick and thorough collection of a sizeable sample of EAS MUAPs, but fail to detect unstable complex MUAPs and do not include satellite potentials, if not followed by manual revision [25, 29]. Available studies on MSA patients did not always include satellite potentials when calculating MUAP duration, and this evidence may be a relevant source of heterogeneity. Although there is no consensus on this matter, several authors argued that EAS EMG examination should encompass these late components of MUAPs, as satellite potentials may be an expression of neurogenic damage per se, deriving from increased temporal dispersion in collateral branches of regenerating axons [24, 30, 31]. On the other hand, single-MUAP analysis is time-consuming, examiner-dependent, and biased towards the highest-amplitude MUAPs using a 'trigger and delay line'. However, the single-MUAP technique requires a manual revision to ensure a correct placement of markers and thereby enables an accurate calculation of MUAP duration, including satellite potentials [25]. Despite these methodological discrepancies, which can explain some disagreement among studies, the different approaches of acquisition and analysis of MUAPs were shown to detect EAS neuropathic changes at similar rates [32–34]. Whatever the technique for MUAP analysis used, it is crucial that each EMG laboratory collects its own data from healthy controls or, at least, considers normative values reported in the literature accurately replicating the electrophysiological methodology [29].

There is a variety of assessment methods of MUAP recruitment, although only few authors investigated this electrophysiological parameter in MSA patients. The subjective grading of interference pattern was replaced over time by the calculation of the ratio of simple phase and simple-mix phase or by the measurement of amplitude and phase pattern during maximal voluntary contraction [35–38]. Alternatively, the mean number of MUAPs per insertion site, alone or together with other quantitative parameters, represents another objective evaluation of recruitment that was explored in MSA cohorts [39].

An appropriate clinical setting is crucial for a proper interpretation of the electrophysiological findings. Indeed, when EAS EMG abnormalities are detected, the diagnostic accuracy towards Onuf's nucleus degeneration increases after excluding alternative causes of neurogenic damage, such as pudendal nerve entrapment or cauda equina syndrome [40]. However, in the case of comorbidities potentially associated with neurogenic alterations (e.g., history of pelvic surgery or diabetes mellitus), EAS EMG is of value when providing normal electrophysiological findings, since it makes MSA diagnosis less likely [41].

Some of the skepticism towards a systematic application of EAS EMG in MSA derives from the belief that this investigation may require particular expertise and could be poorly tolerated by some

individuals [23, 26, 42]. In keeping with other authors [23], we believe, however, that EAS EMG can be easily performed in any neurophysiology laboratory without causing patients too much discomfort.

1.3 Usefulness of EAS EMG for MSA diagnosis

It is well known that MSA patients show a high prevalence of EAS EMG abnormalities, which range from 62% to 93% in studies assessing MSA cohorts without any control group [11, 40, 43–47]. Similar findings were also reported in comparison with healthy subjects [25, 38, 48, 49]. Whether this instrumental assessment can help in the differential diagnosis of MSA is instead a controversial topic. Nonetheless, the majority of authors share the view that EAS EMG can be of value for MSA diagnosis, although there is debate about which electrophysiological parameter should preferably be used. The evidence of prolonged values of duration and increased rates of polyphasic potentials in MSA allowed its discrimination from other causes of cardiovascular dysfunction or cerebellar ataxia, such as diabetes mellitus and spinocerebellar ataxia [37, 48], and mostly from PD [12, 24–28, 35, 36, 38, 49, 50–54]. By contrast, two studies found that pathological spontaneous activity or reduced recruitment pattern were the only electrophysiological parameters to ensure a MSA diagnosis [39, 42].

There are inconsistencies among definitions of neurogenic abnormalities and cut-off values of MUAP parameters to discriminate MSA from mimic disorders, above all PD. A mean MUAP duration > 10 ms is the most common parameter supporting MSA diagnosis [11, 24, 25, 27, 38, 40, 43, 45, 49, 52, 55]. However, other authors established different or supplementary criteria of neurogenic damage: more than 20% of MUAPs with duration > 10 ms [12, 27, 38, 45, 49], more than 30% of MUAPs with duration > 10 ms and mean number of MUAP phases > four [25], mean MUAP duration > 9 ms [12], more than 40% of polyphasic potentials [25, 38], average amplitude > 1 mV or more than 27% of polyphasic potentials [52], or proportion of satellite potentials > 13% [38]. Valldeoriola et al. defined EAS EMG abnormalities in the presence of more than 50% of MUAP with duration > 12 ms and more than five phases [50]. Lee et al. classified EMG findings as abnormal when at least two of the following parameters were detected: pathological spontaneous activity, polyphasic potentials, reduced interference pattern, or increased MUAP duration [35]. Paviour et al. defined mean MUAP duration > 10 ms along with more than 20% of polyphasic potentials as criteria for neurogenic alterations [54].

Several studies showed high accuracy of MUAP parameters in the differential diagnosis of MSA. MUAP duration was found to differentiate MSA from PD with sensitivity ranging from 71% to 93% and with specificity varying from 65% to 100% [26, 28, 36, 53]. Cut-off values of MUAP duration having the best diagnostic accuracy differ among studies, since they vary from 10.9 ms to 14 ms [26, 28, 36, 53]. Some authors noted that a higher threshold for MUAP duration raises the specificity but lowers its sensitivity substantially [54]. For instance, Tison et al. found a specificity of 100% but a sensitivity of 55% with MUAP duration > 16 ms, implying that this finding is exclusively observed in MSA patients, although almost half of MSA cases are missed [26]. In parallel, sensitivity of 100% corresponded to a threshold value of 12 ms, consequently no MSA patients had a mean MUAP duration

below 12 ms [26]. Differently from other authors, Yamamoto et al. reported that MUAP duration > 10 ms allowed the identification of MSA with a lower sensitivity of 35%, but a comparable specificity of 90% [27]. Of note, the percentage of more than 20% of MUAPs with duration > 10 ms was not a significant criterion for the differential diagnosis [27]. Rate of satellite potentials, number of phases, and prevalence of polyphasic potentials were observed to be other useful EMG parameters for the differential diagnosis between MSA and PD, with sensitivity of 73–80% and specificity of 65–93% [28, 51]. In this regard, Rodi et al. found that no MSA patients had a proportion of polyphasic potentials below 50%, while no PD patient showed a percentage of polyphasic MUAPs above 60% [51]. Studies that defined neurogenic damage taking into account simultaneously different electrophysiological features confirmed the diagnostic value of EAS EMG, finding a sensitivity of 86–96% and a specificity of 67–99% [35].

The notion that EAS EMG would be highly specific but poorly sensitive also arises from reports supporting the time dependency of Onuf's nucleus involvement in MSA. Indeed, Yamamoto et al. argued that the lower sensitivity values described in their study were probably due to the shorter disease duration of the recruited patients [27]. In the early disease stage, a normal EAS EMG would not rule out a MSA diagnosis, since neurogenic changes were detected in 52% of MSA patients one year after symptom onset, and then increased to 83% by the fifth year [45]. Similarly, Stocchi et al. showed normal EAS EMG findings in individuals with less severity and shorter duration of disease, but those same subjects developed electrophysiological abnormalities within two years after the baseline evaluation [52]. Furthermore, correlation with disease duration may concern the severity other than the prevalence of neurogenic alterations, given that – for example – MUAP duration was found to increase over time in MSA patients [46]. Accordingly, it was proposed that an abnormal EAS EMG in the early disease stage firmly supports a MSA diagnosis, while alternative diagnoses should be considered in the event of a normal neurophysiological evaluation more than five years after symptom onset [56]. Although the absence of EAS EMG abnormalities in the advanced disease stage is very unlikely in pathologically proven MSA patients [54], this eventuality cannot be ruled out considering pathology data of Onuf's nucleus preservation in a few cases [57, 58]. In addition, the observation of EAS EMG alterations in a long-standing disease complicates the diagnostic interpretation, due to an increase of MUAP duration and a higher rate of neurogenic abnormalities in the mid- and late-stage of PD [25, 38]. In contrast, other authors showed the lack of correlation between neurophysiological data and MSA stage, therefore supporting the diagnostic value of EAS EMG regardless of disease duration [26, 53, 54].

1.4 Evidence against the diagnostic value of EAS EMG in MSA

The diagnostic criticism of EAS EMG is mainly fueled by the above mentioned reports of non-negligible rates of false negative and false positive patients, contributing to assign a marginal role to this instrumental technique. The suggestion of EAS EMG abnormalities in patients with alternative diagnoses was also corroborated by the pathology evidence of Onuf's nucleus degeneration in a few

cases of PD and progressive supranuclear palsy (PSP) [59, 60]. Still, some authors found that PD patients could be misdiagnosed as MSA in the event of neurogenic changes at EAS EMG, supporting its negative rather than positive predictive value for differential diagnosis [41]. The prevailing dissenting opinions therefore cast doubt on whether the observation of EAS EMG findings discriminates MSA from other degenerative parkinsonisms. In this regard, a large overlap of MUAP parameters was shown between MSA and PD groups [42, 55, 61, 62]. At first glance, these findings could result from the recruitment of patients in the advanced disease stage and from the exclusion of satellite potentials for calculation of MUAP duration causing an underestimation of neurogenic abnormalities [42, 61]. However, similar results were also found in the early disease stage and after inclusion of satellite potentials for MUAP analysis [55, 62]. Moreover, some authors described that EAS EMG failed to discriminate between MSA and PSP, since both patients' cohorts showed neurogenic changes to a similar extent [50, 53, 62]. The study by Gilad et al. even questioned the presence of EMG abnormalities in MSA because of the lack of significant differences of MUAP parameters between MSA patients and healthy controls [39].

1.5 Clinical correlations of EAS EMG findings in MSA

Even though motor neurons innervating urethral and anal sphincters have a different location within Onuf's nucleus, both cell groups are involved throughout the disease course [63]. Therefore, the detection of neurogenic changes at EAS EMG may be a neurophysiological correlate of several symptoms of both bladder and bowel dysfunction in MSA patients. Nonetheless, the lack of correlation of urinary symptoms with the presence and severity of EAS electrophysiological abnormalities would not be surprising, given that Onuf's nucleus degeneration is only one of the underlying multiple pathophysiological mechanisms in MSA. For example, a combination of detrusor overactivity and urethral sphincter denervation accounts for urinary incontinence in MSA patients, thus sphincter etiology may be a mere contributory factor [50]. The notion that sphincter denervation does not univocally lead to urinary symptoms was suggested by Wenning et al., who described two MSA patients with abnormal EAS EMG but no urinary symptoms [40]. Still, the same authors pointed out that all patients with urinary incontinence showed neurogenic abnormalities, while an individual with urgency in the absence of incontinence had only borderline EAS EMG findings. Stocchi et al. found that, in the early disease stage, only 77% of MSA patients with urinary urgency and abnormal urodynamic examination showed neurogenic changes at EAS EMG [52]. However, all remaining patients complaining of urinary symptoms with a normal electrophysiological evaluation developed neurogenic abnormalities within two years from the baseline evaluation. Tison et al. reported a slight, but not significant increase of the mean MUAP duration with longer duration of urinary symptoms [26]. Similar assumptions could be made for bowel disturbances. Indeed, fecal incontinence is reported in MSA patients regardless of the observation of marked neurogenic changes in EAS MUAPs [57]. A recent study showed that rates of EAS neurogenic abnormalities did not depend on the presence of defecation disorders as well [38]. The argument that Onuf's nucleus degeneration is not necessarily associated with

bladder and bowel disturbances was also supported in PSP patients, who denied urinary or fecal incontinence despite having EAS neurogenic impairment in some cases [50]. Conversely, PD patients can manifest urinary incontinence even without showing sphincter denervation, due to involvement of a different pathway of the autonomic nervous system [50].

In contrast with the aforementioned line of thought, several authors substantiated the hypothesis of a correlation of bladder and bowel dysfunction with EAS EMG alterations in MSA. Indeed, sphincter denervation may directly determine urinary incontinence in patients without urodynamic evidence of overactivity or low compliance of the detrusor muscle [45], and could be linked to a decrease in anal squeeze pressure, resulting in fecal incontinence [49]. Pramstaller et al. found urinary urgency or incontinence in all MSA patients with pathological EAS EMG, but only in one of the four subjects with a normal electrophysiological examination [43]. Other studies showed that virtually all MSA patients with genitourinary symptoms had EAS denervation and reinnervation [11, 12, 24]. Yamamoto et al. also noted a higher incontinence severity in the presence of EAS abnormalities [45]. Moreover, it was argued that the correlation between the degree of neurogenic changes and the extent of post-void residual volume could be a non-causal finding, reflecting a parallel degeneration of sacral parasympathetic neurons and Onuf's nucleus [45, 47].

EAS neurogenic alterations were observed irrespective of the detection of postural hypotension [38, 45]. Likewise, several studies did not report a parallelism between EAS neurogenic damage in MSA patients and motor impairment [35, 47, 53]. Conversely, in a single study the prevalence of EAS neurogenic abnormalities was found to increase with the severity of posture and gait disturbances [45]. According to most literature, it could be therefore speculated that the degenerative process in MSA may involve several neuronal areas to varying degrees or at different disease stages, leading to a heterogeneous progression of symptoms.

2 STUDY RATIONALE AND AIMS

Several authors have suggested that EAS EMG may help in the differential diagnosis between MSA and PD, particularly within the first five years of symptom onset. Indeed, in contrast with normal findings in PD patients, most subjects with MSA show EAS neurogenic abnormalities that are considered to provide indirect evidence of Onuf's nucleus degeneration, which is a pathological hallmark of MSA. Nevertheless, the diagnostic value of EAS EMG has been a matter of debate for three decades due to controversial reports. Accordingly, current diagnostic criteria do not acknowledge EAS EMG as part of the instrumental toolbox, even though it can provide additional clues supporting an MSA diagnosis. Literature on the clinical correlations of EAS EMG abnormalities is even more discordant, and the usefulness of EAS EMG for MSA prognosis is unknown. Discrepancies among authors derive from the lack of a standardized electrophysiological methodology [31], from differences in sample size, age and disease duration of MSA patients and control subjects, and from the absence of healthy controls in several studies.

In the present study, we identified a novel electrophysiological classification consisting of four EAS EMG patterns, aiming to explore their usefulness in the differential diagnosis between MSA and PD, their correlations with clinical features and cardiovascular autonomic function, and their role as potential prognostic predictors of survival in MSA.

3 MATERIALS AND METHODS

3.1 Study design

In this retrospective study, we enrolled 72 patients with clinically established MSA and 21 with PD admitted to the Casimiro Mondino Foundation in Pavia (Italy) between January 2003 and July 2019. The local Ethics Committee approved the study and all patients gave their written informed consent. MSA and PD were diagnosed in accordance with the Movement Disorder Society criteria [18, 64]. Diagnosis of MSA or PD was confirmed at follow-up evaluations. We applied the following exclusion criteria: history of lumbosacral radiculopathy, lumbar spinal stenosis, pelvic irradiation, lumbar spine or pelvic surgery; diagnosis of diabetes mellitus, polyneuropathy, pudendal nerve entrapment or traumatic injuries, cauda equina or conus medullaris syndrome; presence of severe hemorrhoids or previous hemorrhoidectomy; other concomitant causes of autonomic dysfunction.

This study included a cross-sectional analysis and a longitudinal evaluation. For the cross-sectional investigation, we analyzed clinical and instrumental data collected during hospital stays. All MSA and PD patients underwent brain magnetic resonance imaging (MRI), EAS EMG examination, and extensive clinical evaluation, including an acute challenge test with levodopa 250 mg after an overnight withdrawal of dopaminergic drugs. In the MSA cohort, 61 patients performed cardiovascular reflex tests, and a subgroup of 56 subjects underwent 24-hour blood pressure monitoring. For the longitudinal evaluation, MSA patients or their caregivers were contacted by phone, allowing us to ascertain the survival times of 49 subjects with MSA who had died by July 2021. Clear causes of death were not identifiable in most patients, so this information was not taken into account for further analyses.

The recent coronavirus disease 2019 (COVID-19) outbreak did not allow us to conduct an additional prospective study, which could be of value to confirm findings deriving from the retrospective investigation.

3.2 Clinical assessment

All MSA and PD patients were evaluated by a movement disorder specialist. Motor impairment was assessed by means of the motor section of the Unified Multiple System Atrophy Rating Scale (UMSARS II) and the motor section of the Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS III), respectively. Levodopa equivalent daily dose (LEDD) was also calculated. The parkinsonian (MSA-P) and the cerebellar (MSA-C) variants of MSA were identified on the basis of the predominant motor phenotype, and we also established the symptom type at disease onset (i.e., urogenital, orthostatic, or motor disturbances) and the presence of urogenital symptoms or fecal incontinence at the time of hospital admission. Urogenital symptoms referred to storage disturbances (i.e., urinary urgency or incontinence), voiding disorders (i.e., incomplete bladder emptying or urinary retention), and erectile dysfunction in males, while orthostatic symptoms meant disturbances deriving

from orthostatic hypotension, such as dizziness, blurred vision, fatigue, weakness, or nausea only on standing. Motor disturbances referred to symptoms due to parkinsonian syndrome or cerebellar ataxia.

3.3 EMG investigation

EAS EMG in the MSA and PD patients was performed by a neurologist with expertise in clinical neurophysiology, who was blinded to the diagnosis and to other clinical or instrumental findings. The EMG examination was carried out using a Synergy SYN5-C EMG machine (Viasys Healthcare, Old Woking, Surrey, UK). Since bladder and rectal filling can influence the basal tonic activity of the EAS [22, 33], EMG was performed after bladder and bowel emptying. Patients were asked to lie on their left side, with their hips and knees flexed, while their right thigh was electrically grounded. A 28-gauge concentric needle electrode was inserted under audio guidance into the four quadrants of the EAS. For the superficial layer, the needle electrode was inserted, perpendicularly, 1 cm laterally to the anal orifice to a depth of 3–6 mm; and for the deep layer, it was inserted at the anal orifice at an angle of 30º to a depth of 15–25 mm [22, 23]. The following EMG parameters were evaluated: presence of pathological spontaneous activity (i.e., fibrillation potentials, positive sharp waves, or complex repetitive discharges); duration of MUAPs; and spatial recruitment of MUAPs. The bandpass filter was set at 3 Hz–10 kHz. With regard to the sensitivity settings, we used a gain of 50 μV/division and a sweep speed of 20 ms/division to assess spontaneous activity, and a gain of 100 μV/division and a sweep speed of 5 ms/division to analyze MUAPs. Given that pathological spontaneous activity can be difficult to distinguish from EAS basal tonic discharge [24, 25], we evaluated it after asking patients to simulate defecation. Moreover, the characteristic shape and firing rate of pathological spontaneous activity can facilitate its detection, in the event of persistent activity of some MUAPs in patients who are unable to fully relax their EAS. We performed single-MUAP analysis by means of the 'trigger and delay line' technique, which makes it possible to identify MUAPs with the highest amplitude values [65]. In particular, we analyzed MUAPs triggered during either basal tonic activity or a state of constant slight voluntary activation, in keeping with other authors [22, 23, 29, 34, 39]. Despite the automatic positioning of cursors by the EMG software, manual revision was needed in order to ensure the correct placement of markers, delete duplicated MUAPs, and include satellite potentials (also known as late components of MUAPs) in the calculation of MUAP duration. This latter parameter has been shown to allow early and reliable discrimination of EAS neurogenic abnormalities in MSA [24–26]. For each patient, the duration of 20 MUAPs was compared to reference values obtained from 40 age-matched healthy subjects (23 men; mean age ± standard deviation: 61.3 ± 10.5 years; age range: 48–76 years). Normative values of mean duration ± standard deviation were 6.8 ± 1.4 ms (range: 3.2–10.5). MUAPs were categorized as 'neurogenic' when their mean duration exceeded the 97.5th percentile of normal range, corresponding to 10.2 ms, which is in line with the cut-off proposed by most previous studies [24–26, 35, 39, 42, 43, 52, 62]. Spatial recruitment of MUAPs was measured as the mean number of MUAPs per insertion site during basal tonic activity and after voluntary activation. In agreement with other

findings from multi-MUAP analysis [39], at least three MUAPs per insertion site could be detected in our group of healthy subjects; thus, recruitment was classified as ‘reduced’ when a mean number of less than three MUAPs per insertion site was identified.

On the basis of the presence and severity of the underlying neurogenic damage, we identified and defined four EAS EMG patterns:

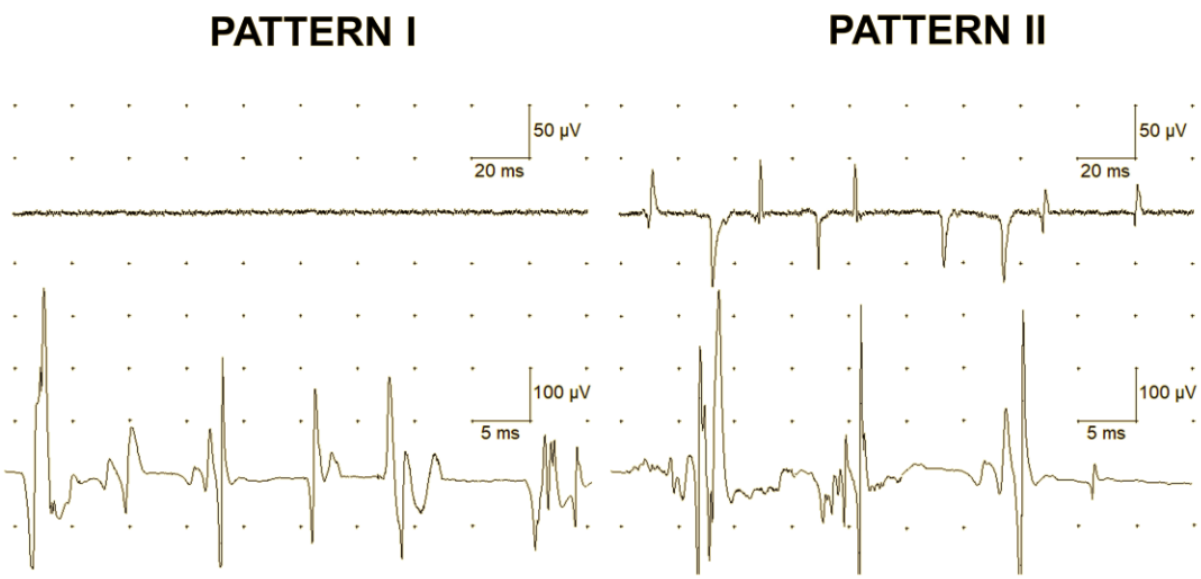
I. no pathological spontaneous activity, normal duration and normal spatial recruitment of MUAPs (normal findings);

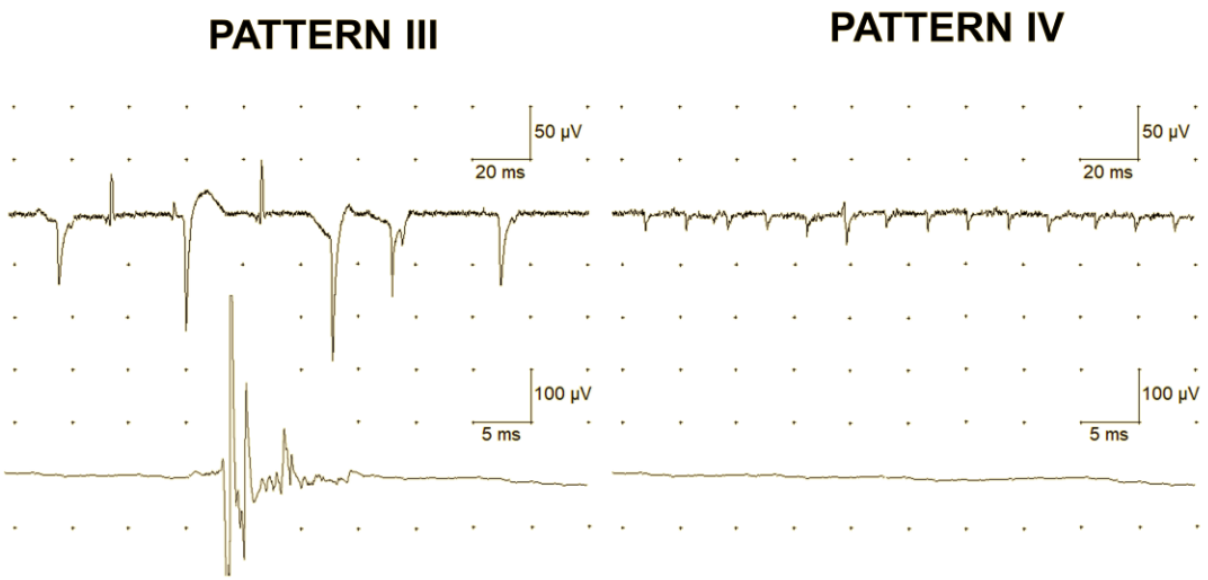
II. neurogenic MUAPs, normal spatial recruitment of MUAPs, with or without pathological spontaneous activity (mild neurogenic damage);

III. neurogenic MUAPs, reduced spatial recruitment of MUAPs, with or without pathological spontaneous activity (moderate neurogenic damage);

IV. absent recruitment of MUAPs, with or without pathological spontaneous activity (severe neurogenic damage).

Illustrative traces of EAS EMG patterns are reported below: the upper traces show the presence or absence of pathological spontaneous activity, while the lower traces show MUAP parameters (i.e., duration and spatial recruitment).





A random sample of 20 patients was reassessed by a second clinical neurophysiologist, again in a blinded fashion. In these subjects, the two examiners were in complete agreement with regard to the assignment of EAS EMG patterns.

3.4 Cardiovascular reflex tests and 24-hour blood pressure monitoring

Orthostatic changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were assessed by means of the head-up tilt test (HUTT) and were expressed as 'delta' values, namely differences between supine and orthostatic values. The following HUTT parameters were evaluated: 3-minute and maximum SBP delta; 3-minute and maximum DBP delta; 3-minute and maximum HR delta. Blood pressure was measured continuously (beat-to-beat) through a finger cuff system (Finometer® Pro, Finapres Medical Systems, Amsterdam, Netherlands). Several acquisition hardware modules embedded in a single device (Task Force® Monitor, CNSystems Medizintechnik GmbH, Graz, Austria) allowed an automatic correction of blood pressure values, by means of a contralateral oscillometric system, and HR measurement through electrocardiogram chest leads. Patients lay on the tilt-table in the supine position for 10 minutes, before being passively tilted to a 60° upright position in which they remained for 10 minutes.

After the HUTT, cardiovagal function was evaluated, according to standard procedures, by means of the Valsalva maneuver, a deep breathing test, and active standing, to obtain the following parameters: Valsalva ratio; deep breathing index; and 30:15 ratio [66]. The Valsalva ratio was defined as the ratio between the longest R-R interval during phase IV and the shortest R-R interval during phase II of the Valsalva maneuver. The deep breathing index was calculated as the mean of the difference between the maximum and minimum HR within each of six breathing cycles during the deep breathing test. The 30:15 ratio was the ratio between the longest R-R interval at around the 30th beat and the shortest R-R

interval at around the 10th beat during active standing.

Blood pressure was measured automatically every 15 minutes in the daytime and every 30 minutes at night by means of an oscillometric device (Ultralite™ 90217A Monitor, Spacelabs Healthcare, Snoqualmie, Washington, USA). The nocturnal blood pressure profile was classified as 'dipping' when nocturnal mean values dropped more than 10%, 'non-dipping' if nocturnal mean values dropped less than 10%, and as 'reverse dipping' when nocturnal mean blood pressure exceeded the diurnal values [67].

3.5 Statistical analysis

MSA and PD patients were compared using the χ^2 test, *t* test, or Wilcoxon rank-sum test, as appropriate. Correlation analyses of EAS EMG patterns in the MSA cohort were performed by means of the Spearman's correlation coefficient. The Bonferroni correction for multiple comparisons was applied. Nominal logistic regression analysis and log-likelihood χ^2 test were performed to test whether EAS EMG patterns were significant diagnostic predictors. Receiver operating characteristic (ROC) analyses provided area under the curve (AUC), sensitivity, specificity, positive predictive and negative predictive values. Odds ratio (OR) and related 95% confidence interval (CI) values were then calculated for each EAS EMG pattern. Survival was defined as the time from symptom onset to death. First, differences in survival based on EAS EMG patterns were explored by means of the Kruskal-Wallis test and the post-hoc Dunn's test. Second, survival analysis was carried out using Kaplan-Meier curves and EAS EMG patterns were compared using the log-rank test. Statistical significance was set at $p < 0.05$. JMP Pro 14.0 software (SAS Institute Inc., USA) was used for statistical analyses.

4 RESULTS

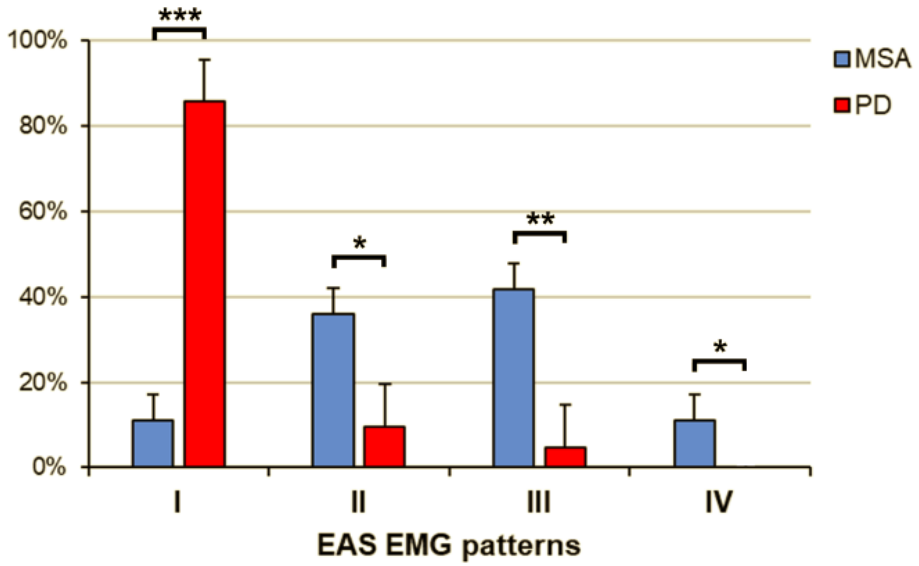
4.1 Comparisons between MSA and PD patients

Demographic, clinical, and instrumental features in MSA and PD patients are listed below. Data are reported as number of patients (%) or mean ± standard deviation. In particular, none of the subjects with MSA had a significant improvement at levodopa challenge test, defined as a greater than 30% reduction in the MDS-UPDRS III score. As requested to fulfil the clinically established level for MSA diagnosis, these patients showed at least one MRI marker, namely atrophy and hypointensity of the posterior putamen and slit-like putaminal hyperintensity, 'hot cross bun' sign, atrophy and/or hyperintensity of the middle cerebellar peduncles, atrophy of the pons and/or the cerebellum, on DP-T2- and T2*-weighted sequences [18].

			MSA	PD
Gender, male			35 (48.6)	13 (61.9)
Age at symptom onset, years			63.9 ± 9.7	59.5 ± 11.4
Age at EMG, years			68.3 ± 9.6	64.9 ± 10.0
Disease duration at EMG, years			4.4 ± 2.4	5.4 ± 4.1
LEDD, mg			601.2 ± 447.1	681.4 ± 305.3
MDS-UPDRS III, score			-	25.6 ± 9.5
UMSARS II, score			19.9 ± 5.6	-
Phenotype	**MSA-P**		60 (83.3)	-
	MSA-C		12 (16.7)	-
Symptom at onset	**Urogenital**		38 (52.8)	-
	Orthostatic		11 (15.3)	-
	Motor		23 (31.9)	-
Cardiovascular reflex tests	**HUTT**	**3-min delta SBP**	28.3 ± 12.4	-
		3-min delta DBP	14.2 ± 8.9	-
		3-min delta HR	-2.4 ± 9.3	-
		Max delta SBP	35.4 ± 14.7	-
		Max delta DBP	23.3 ± 10.4	-
		Max delta HR	-5.8 ± 10.0	-
	Valsalva ratio		1.21 ± 0.23	-
	Deep breathing index		7.13 ± 4.34	-
	30:15 ratio		1.01 ± 0.05	-
Nocturnal blood pressure profiles	**Dipping**		5 (8.9%)	-
	Non-dipping		31 (55.4%)	-
	Reverse dipping		20 (35.7%)	-

The MSA and PD patients did not differ with regard to gender ($p = 0.328$), age at symptom onset ($p = 0.073$), age and disease duration at EMG ($p = 0.181$ and $p = 0.067$, respectively), or LEDD ($p = 0.133$). Conversely, the distribution of EAS EMG patterns was found to differ between the two cohorts ($p < 0.001$): normal EAS findings (i.e., pattern I) were more frequent in subjects with PD as compared with MSA patients (85.7% vs. 11.1%, respectively, $p < 0.001$), whereas each of the abnormal EAS EMG patterns was more frequent in the MSA group (pattern II: 36.1% in MSA vs. 9.5% in PD, $p = 0.011$; pattern III: 41.7% in MSA vs. 4.8% in PD, $p = 0.002$; pattern IV: 11.1% in MSA vs. no PD patient, $p = 0.028$).

Frequency distribution of EAS EMG patterns in MSA and PD patients is depicted below. Vertical error bars represent standard errors. Horizontal bars indicate significant differences detected with the χ^2 test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

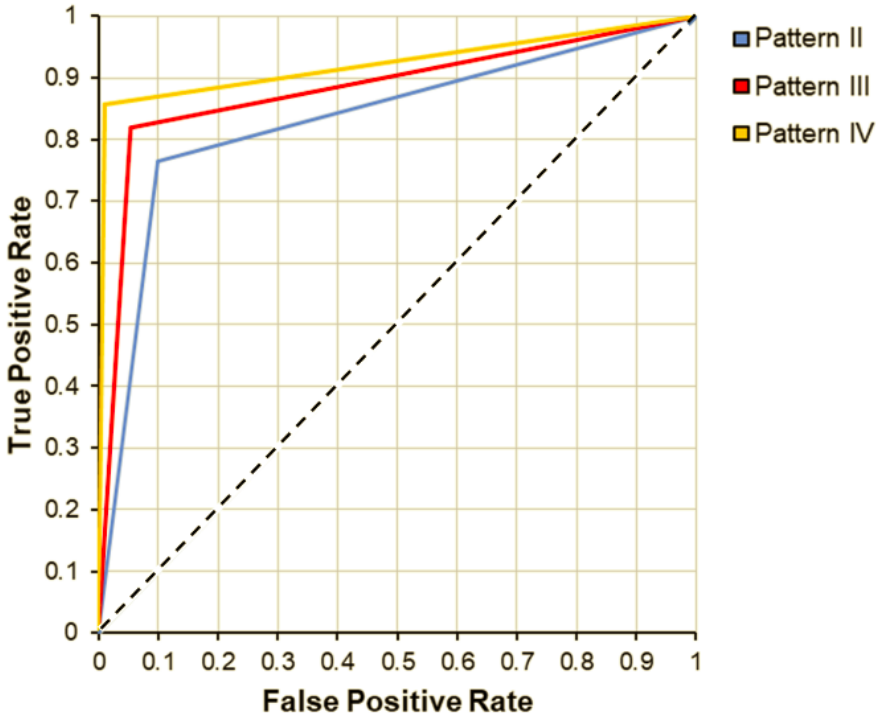


4.2 Accuracy of abnormal EAS EMG patterns for MSA diagnosis

The presence of an abnormal EAS EMG pattern (i.e., patterns II, III or IV) correlated with MSA diagnosis ($R^2 = 0.43$, $p < 0.001$), with an AUC of 0.87, sensitivity of 88.9%, specificity of 85.7%, positive predictive value of 95.5%, negative predictive value of 69.2%, and OR of 48.0 (95% CI: 11.5–199.8). In particular, the likelihood of an MSA diagnosis paralleled the severity of EAS EMG impairment. Pattern II was a diagnostic predictor of MSA ($R^2 = 0.32$, $p < 0.001$), with an OR of 29.3 (95% CI: 5.6–54.1). Pattern III correlated with MSA diagnosis ($R^2 = 0.35$, $p < 0.001$), and showed an OR of 67.5 (95% CI: 7.8–104.9), higher than that shown by pattern II. Finally, pattern IV predicted MSA diagnosis ($R^2 = 0.44$, $p < 0.001$), with an OR of 103.7 (95% CI: 23.8–219.7).

ROC curves of EAS EMG patterns for differential diagnosis between MSA and PD are shown

below. MSA diagnosis was used as positive level for logistic regression analyses. Pattern II showed an AUC of 0.83, sensitivity of 76.5%, specificity of 90.0%, positive predictive value of 92.9%, and negative predictive value of 69.2%. Pattern III showed an AUC of 0.88, sensitivity of 82.0%, specificity of 94.7%, positive predictive value of 96.8%, and negative predictive value of 74.5%. Pattern IV showed an AUC of 0.96, sensitivity of 85.6%, specificity of 99.0%, positive predictive value of 99.5%, and negative predictive value of 89.7%.



4.3 Correlation analysis of EAS EMG patterns in MSA

In the MSA cohort, EAS EMG patterns did not correlate with gender ($p = 0.556$), age at symptom onset ($p = 0.821$), age and disease duration at EMG ($p = 0.862$ and $p = 0.412$, respectively), LEDD ($p = 0.117$), UMSARS II score ($p = 0.613$), or phenotype ($p = 0.648$).

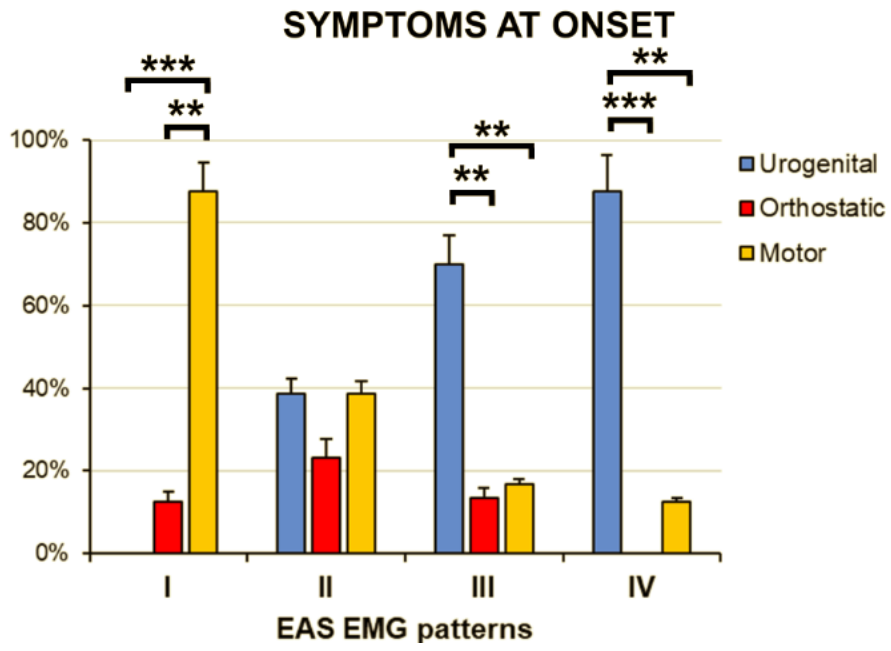
Instead, EAS EMG patterns were related to symptom type at onset in this group ($p < 0.001$). The subjects with pattern I showed a prevalence of motor onset (87.5%), as opposed to urogenital disturbances (none, $p < 0.001$) or orthostatic symptoms at onset (12.5%, $p = 0.001$). The patients with pattern II showed a more even distribution of symptoms at onset (urogenital disturbances: 38.5%; orthostatic symptoms: 23%; motor impairment: 38.5%; $p = 0.154$). Instead, subjects with patterns III and IV more frequently showed urogenital disturbances at onset (70.0% and 87.5%, respectively), while fewer patients reported orthostatic symptoms at onset (13.3% of those with pattern III, $p = 0.002$; none with pattern IV, $p < 0.001$) or a motor onset (16.7% in the subgroup with pattern III, $p = 0.003$; 12.5% of the patients with pattern IV, $p = 0.001$). The predominance of urogenital symptoms at onset in the subjects with patterns III or IV, versus the group with pattern I, was significant ($p < 0.001$ and $p = 0.002$,

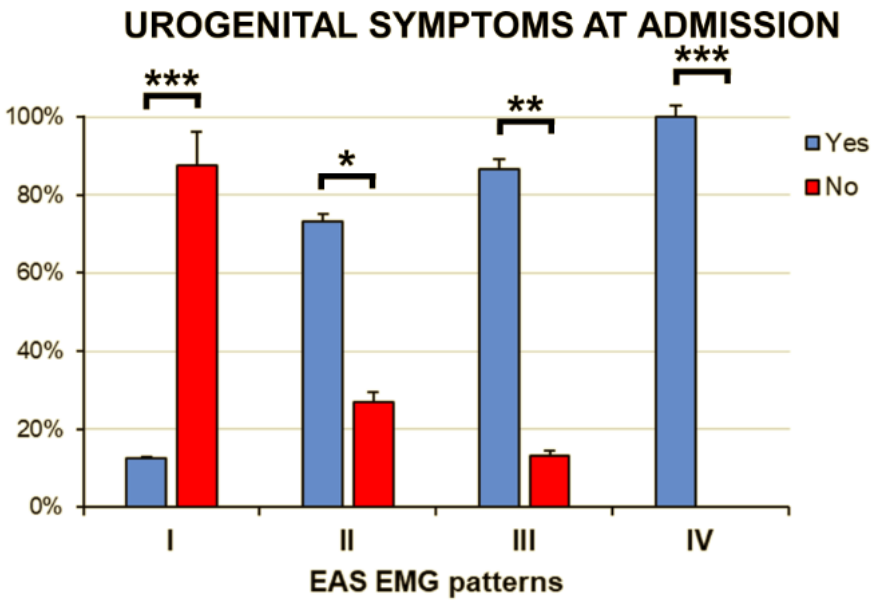
respectively).

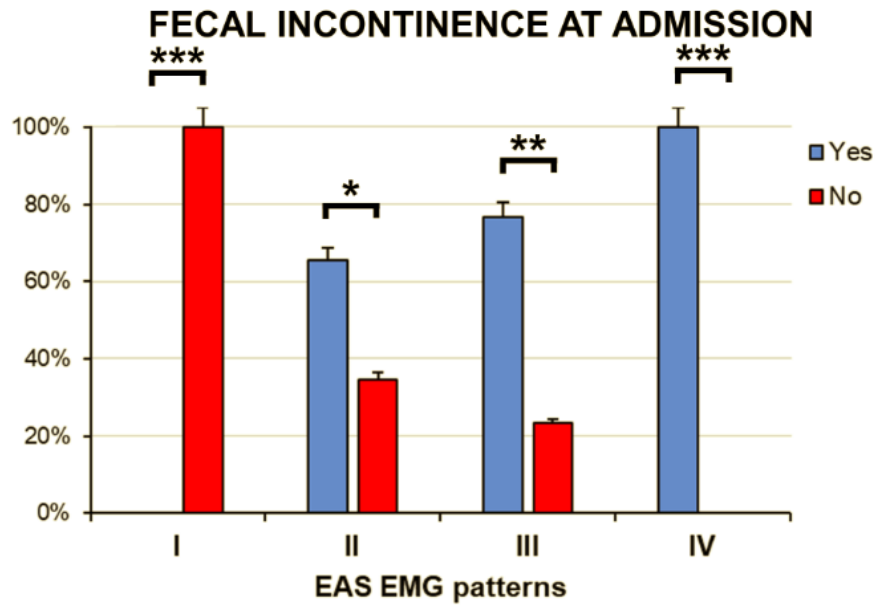
In the MSA patients, a correlation was also found between EAS EMG patterns and the prevalence of urogenital symptoms and fecal incontinence at the time of EMG. Urogenital disturbances were uncommon in subjects with pattern I (12.5%, $p < 0.001$), frequent among patients with patterns II (73.1%, $p = 0.011$) or III (86.7%, $p = 0.001$), and were always present in subjects with pattern IV ($p < 0.001$). Urogenital symptoms were preponderant in the patients with patterns II, III or IV with respect to the subjects with pattern I ($p = 0.001$, $p < 0.001$, and $p = 0.002$, respectively), and in the patients with pattern IV as compared with the subjects with pattern II ($p = 0.008$). Fecal incontinence was never reported by patients with pattern I ($p < 0.001$), whereas it was significantly present among subjects with patterns II (65.4%, $p = 0.012$) or III (76.7%, $p = 0.009$), and was always reported by patients with pattern IV ($p < 0.001$). Fecal incontinence was predominant in patients with patterns II, III or IV as compared with subjects with pattern I ($p = 0.001$, $p < 0.001$, and $p = 0.002$, respectively), and in patients with pattern IV vs. pattern II ($p = 0.008$).

In the MSA group, EAS EMG patterns did not correlate with parameters deriving from cardiovascular reflex tests, namely 3-minute and maximum SBP delta ($p = 0.391$ and $p = 0.350$, respectively), 3-minute and maximum DBP delta ($p = 0.286$ and $p = 0.532$, respectively), 3-minute and maximum HR delta ($p = 0.658$ and $p = 0.733$, respectively), Valsalva ratio ($p = 0.325$), deep breathing index ($p = 0.590$), and 30:15 ratio ($p = 0.626$). Moreover, EAS EMG patterns were not associated with nocturnal blood pressure profiles ($p = 0.232$).

Frequency distribution, by EAS EMG patterns, of symptom types at disease onset or at hospital admission in MSA patients are depicted below. Vertical error bars represent standard errors. Horizontal bars indicate significant differences detected with the χ^2 test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).





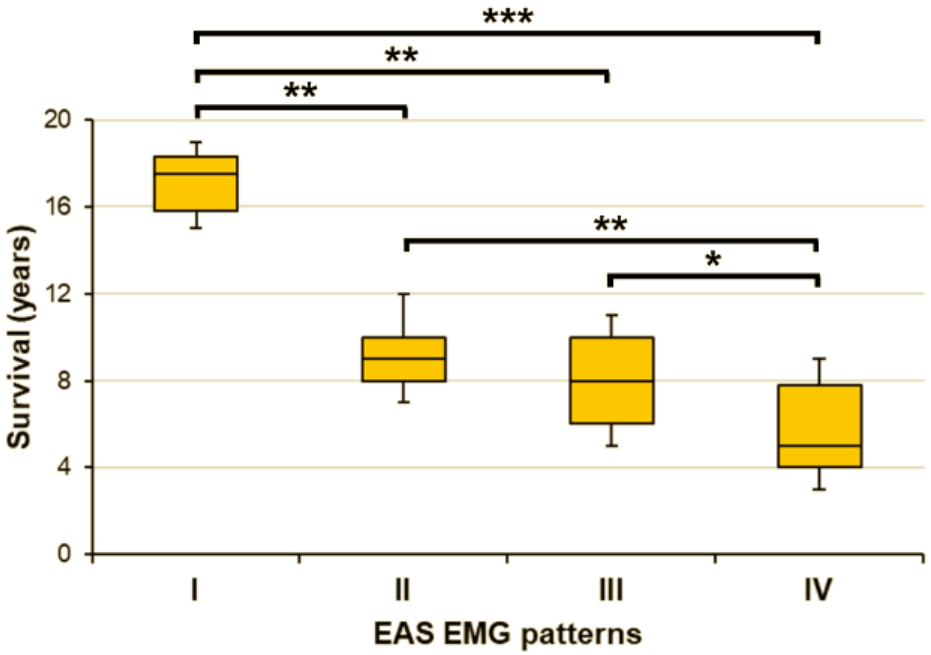


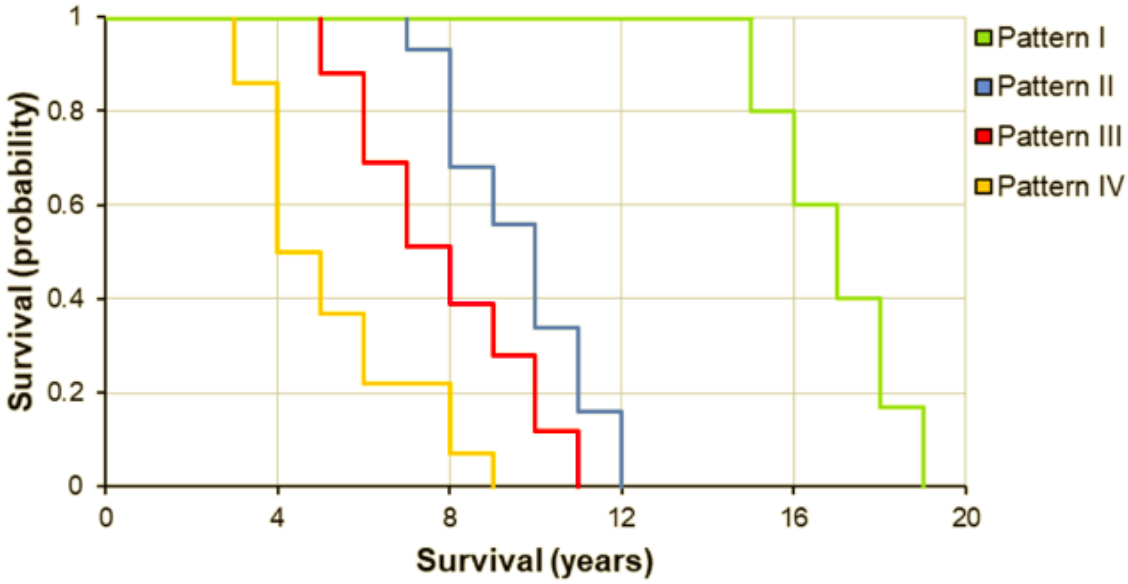
4.4 Survival analysis by EAS EMG patterns in MSA

The following survival times, indicated as median values, were recorded in the members of the MSA cohort who died during follow-up: 17.5 years (range: 15–19) in the 6 patients with pattern I; 9 years (range: 7–12) in the 16 subjects with pattern II; 8 years (range: 5–11) in the 19 patients with pattern III; and 5 years (range: 3–9) in the 8 subjects with pattern IV. As compared with the group with pattern I, survival time was shorter in the subjects with patterns II ($p = 0.004$), III ($p = 0.002$) or IV ($p < 0.001$). The patients with pattern IV also had a worse prognosis than the subjects with patterns II ($p = 0.009$) or III ($p = 0.011$). These findings were confirmed by Kaplan-Meier analyses, which showed poorer prognoses in the patients with patterns II (vs. pattern I, $p < 0.001$), III (vs. pattern I, $p < 0.001$) or, in

particular, IV (vs. pattern I, $p < 0.001$; vs. pattern II, $p = 0.001$; vs. pattern III, $p = 0.007$).

Survival differences by EAS EMG patterns in MSA patients are shown below. In particular, horizontal bars indicate significant differences detected with the post-hoc Dunn's test after the Kruskal-Wallis test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) or Kaplan-Meier curves for each EAS EMG pattern are reported.





5 DISCUSSION

In this study, EAS EMG patterns of increasing severity were identified and the resulting novel classification was used to assess the value of EAS EMG in the differential diagnosis and prognostic stratification of MSA patients. The electrophysiological impairment of the EAS was found to parallel diagnostic accuracy and survival in MSA: the more severe the EMG alterations, the greater the likelihood of an MSA diagnosis and the worse the patient's prognosis. The pathophysiological relevance of the proposed EAS EMG patterns in MSA was corroborated by their correlation with clinical features, namely symptom type at disease onset and prevalence of urogenital symptoms and fecal incontinence. MSA patients with EAS EMG abnormalities often showed fecal incontinence and urogenital symptoms, which were also frequently present at disease onset in those with impaired MUAP recruitment. This latter finding is in keeping with the observation that the disease process could begin in the sacral spinal cord before spreading to other regions responsible for motor impairment and cardiovascular autonomic dysfunction [68]. Conversely, MSA patients without EAS EMG alterations did not have fecal incontinence, rarely showed urogenital symptoms, and commonly presented with motor disturbances at disease onset.

Previous literature on the diagnostic usefulness of EAS EMG as a means of discriminating between MSA and PD has focused mainly on the evaluation of pathological spontaneous activity, amplitude and duration of MUAPs, or percentage of polyphasic MUAPs [24–26, 35, 36, 43, 45, 51, 52, 54, 69]. Despite the suggestion to include EAS EMG in the diagnostic workup of MSA [21], some authors have questioned the value of this instrumental investigation in the differential diagnosis [39, 42, 61, 62].

Our evaluation of MUAP recruitment allowed us to reveal a broader spectrum of EAS EMG abnormalities. Only a few other studies have explored EAS MUAP recruitment in MSA. Some authors subjectively graded the MUAP interference pattern during maximal voluntary contraction, considering reduction of this parameter to be an alternative expression of Onuf's nucleus degeneration in MSA patients [35, 36]. Instead, in a small sample of subjects with MSA, Gilad and colleagues objectively assessed several recruitment parameters, such as the mean number of MUAPs per insertion site [39]. On the basis of their observation of reduced recruitment pattern in the absence of other features of neurogenic damage (e.g., increased amplitude or duration of MUAPs), these authors hypothesized degeneration of upper motor neurons rather than loss of lower motor neurons of Onuf's nucleus in MSA patients. The reduced MUAP recruitment found in our study was invariably associated with increased MUAP duration, thus reflecting either a more severe Onuf's nucleus degeneration or a combination of lower motor neuron impairment and neuronal loss in supraspinal areas (e.g., the pontine micturition and storage centers) that modulate Onuf's nucleus function [13, 70].

We found a high diagnostic accuracy of EAS EMG patterns in discriminating between MSA and PD, in keeping with the sensitivity (60–80%) and specificity (93–100%) values previously reported in

studies analyzing single EMG parameters [26, 36, 51]. The finding of lower sensitivity than specificity implies that, as seen in 11.1% of our cohort and in agreement with pathology evidence [57], some MSA patients do not show EAS EMG abnormalities. It is still not known why Onuf's nucleus is preserved in a few MSA patients.

This study is the first to explore the prognostic value of EAS EMG alterations in MSA. The correlations of EAS EMG abnormalities with the prevalence of urogenital symptoms and fecal incontinence support previous clinical and urodynamic observations on the prognosis of MSA patients. Some authors have indeed shown that lower urinary tract symptoms and reduced detrusor contractility are among the strongest predictors of survival in MSA [6–9, 71]. Neurogenic urinary dysfunction was linked to recurrent lower urinary tract infections, a primary cause of death in MSA [72]. Although this link with survival has no clear explanation, urinary symptoms have been associated with loss of medullary serotonergic neurons, which contribute to micturition modulation and respiratory rhythmogenesis, and whose dysfunction can lead to nocturnal hypoxia, increasing the risk of sudden death during sleep in MSA patients [7, 73, 74].

It is interesting that, compared with the majority of the MSA cohort, the small subset of patients without EAS EMG abnormalities showed markedly longer survival. That said, survival of more than 15 years has been reported in a few pathologically confirmed MSA patients [5, 75]. In particular, subjects with longstanding disease have shown late onset of cardiovascular and urinary autonomic dysfunction [5, 76], in keeping with the association between early autonomic failure and short survival [3, 4].

Our additional finding of a lack of correlation between EAS EMG patterns and disease duration in the MSA cohort may support the diagnostic and prognostic usefulness of EAS EMG regardless of disease stage. However, in previous longitudinal evaluations of single EMG parameters, EAS neurogenic changes could not be detected in the early stages of MSA, but become evident over time [45, 49, 52]. Other authors have found EAS EMG abnormalities in the advanced stages of PD, therefore limiting the value of EAS EMG for differential diagnosis within the first years of symptom onset [25, 61].

The link between EAS EMG findings and symptom type at disease onset in MSA has not previously been investigated. Instead, some authors have explored whether EMG evidence of EAS neurogenic damage was associated with the severity of bladder and bowel symptoms in MSA patients. Motor neurons of Onuf's nucleus innervate pelvic floor muscles via the pudendal nerve, and are therefore involved in micturition, defecation, and sexual functions [77]. Accordingly, the prevalence of EAS neurogenic alterations has been found to increase with the severity of urinary incontinence, which was shown to have a 'sphincter etiology' in several MSA patients [45, 49]. However, in some subjects at least, this clinical-instrumental correlation, which is in agreement with our results, could reflect a parallel association rather than an underlying causative relationship [45]. Indeed, urinary incontinence due to detrusor overactivity or voiding symptoms in MSA may derive from involvement of other areas (e.g., the pontine micturition and storage centers) [13, 49, 70], whose degeneration can progress in

parallel with that of Onuf's nucleus. In contrast to the above findings, other authors have observed that MSA patients with EAS EMG abnormalities may not complain of lower urinary tract symptoms or fecal incontinence [52, 57]. Differences in the severity of neurogenic damage on EAS EMG could account for discrepancies in the literature.

Furthermore, our study showed that EAS EMG patterns did not correlate with disease phenotype (i.e., MSA-P or MSA-C), severity of motor impairment, or cardiovascular autonomic function in MSA patients. Of note, it is the first to have investigated these clinical-instrumental correlations using an ad hoc scale for motor symptoms in MSA (i.e., UMSARS II), as well as a thorough battery of cardiovascular reflex tests and 24-hour blood pressure monitoring. It could be speculated that the degenerative process in MSA may involve several neuronal areas to varying degrees or at different disease stages. In this sense, impairment of Onuf's nucleus might not parallel degeneration of the striatum, cerebellum, vagal nerve nuclei, or intermediolateral cell columns. Given the 'autonomic properties' of Onuf's nucleus [77], it might also be hypothesized that sacral parasympathetic centers are involved in MSA differently from the medullary parasympathetic nervous system. In keeping with our results, based on the presence and degree of EAS neurogenic changes on EMG, two studies have found no association with the severity of parkinsonism, which, however, was evaluated using the Unified Parkinson's Disease Rating Scale or the Hoehn and Yahr Scale [35, 53]. Conversely, in a single study the prevalence of EAS neurogenic abnormalities was found to increase with the severity of posture and gait disturbances, which were assessed using the corresponding item of the International Cooperative Ataxia Rating Scale [45].

Some limitations of this study have to be acknowledged. First, our findings derive from retrospective observations and the identification of symptoms at disease onset could therefore have been influenced by recall bias. Second, we cannot exclude misdiagnoses given the lack of neuropathological confirmation, which represents the gold standard for diagnosis. Third, single-MUAP analysis is time consuming and examiner dependent. That said, the automated techniques (e.g., multi-MUAP analysis) require manual revision to ensure correct placement of markers and therefore accurate calculation of MUAP duration [25, 29], which should include satellite potentials [30]. Moreover, although single-MUAP analysis with 'trigger and delay line' is biased towards the highest-amplitude MUAPs, thus preventing collection of a representative sample of EAS MUAPs [25, 65], this possible bias applied to both MSA and PD patients, and MUAP amplitude was not chosen as a parameter for categorizing EAS EMG patterns. Despite the abovementioned technical drawbacks, single-MUAP analysis is the most commonly used method and has been shown to detect EAS neuropathic changes at similar rates to other quantitative MUAP analysis techniques (i.e., manual-MUAP and multi-MUAP methods) [32–34].

6 CONCLUSIONS AND FUTURE PERSPECTIVES

The prevailing opinion in the literature supports the value of EAS EMG for the differential diagnosis of MSA. The most common caveats include the inclusion of satellite potentials for MUAP analysis and the exclusion of other potential causes of EAS neurogenic changes before speculating on Onuf's nucleus degeneration. In the appropriate clinical setting, MSA diagnosis is bolstered by the evidence of neurogenic abnormalities, namely pathological spontaneous activity, reduced MUAP recruitment, increased rate of polyphasic potentials and, particularly, prolonged MUAP duration. The diagnostic accuracy of EAS EMG is, however, limited by the observation that neurogenic findings could not be detected in the early stage of MSA, while they may be reported in the advanced stage of PD. This electrophysiological examination can therefore be implemented in the clinical practice to rule out mimic disorders, such as PD, especially in the early disease stage when the clinical picture is unclear.

Once other causes of EAS neurogenic changes have been ruled out, EAS EMG patterns can help to disentangle MSA from PD and may provide useful insights into the prognosis of MSA patients. The patterns described herein are associated with symptom type at disease onset and with prevalence of bladder and bowel disturbances. EAS EMG investigation is therefore a valuable diagnostic and prognostic tool, which should be recommended especially when the clinical picture is unclear. A normal EAS EMG pattern in subjects with MSA could identify a small subset of patients characterized by less neurodegeneration and prolonged survival. Longitudinal EMG assessments are warranted to verify whether EAS EMG patterns change over time, while neuroimaging and neuropathological studies could clarify the pathophysiological correlates of EAS EMG patterns in MSA.

The development of advanced algorithms for a precise MUAP analysis could assist the clinician in the diagnostic work-up. Future prospective studies with longer clinical and electrophysiological follow-up evaluations, larger patients' cohorts and pathological confirmation are desirable to strengthen the current diagnostic evidence, to deeply explore clinical correlations using specific instrumental examinations, and to confirm our prognostic findings in MSA.

ACKNOWLEDGMENTS

I wish to thank Dr. Enrico Alfonsi, Prof. Giuseppe Cosentino and all of my other collaborators of the Clinical Neurophysiology Unit of the IRCCS Mondino Foundation. I also thank Prof. Ioannis Ugo Isaias (Parkinson Institute of the ASST Gaetano Pini – CTO in Milan, Julius Maximilian University of Würzburg) and Prof. Vincenzo Di Stefano (University of Palermo) for their work as external reviewers.

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