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Cardiac autonomic control during sleep in patients with Multiple System Atrophy: a pilot study on the impact of CPAP therapy

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Table of contents

Abstract

Objectives

To characterize sleep-stage autonomic regulation in multiple system atrophy (MSA) using cardiorespiratory coupling (K2) and symbolic dynamics (0V%, 2UV%, 2LV%) from ECG tracings in video-polysomnography (v-PSG), to compare patients with and without sleep-disordered breathing (SDB), and to assess acute autonomic effects of CPAP during the second night in patients with SDB.

Methods

We retrospectively analyzed v-PSG recordings from MSA patients who underwent v-PSG over two consecutive nights at the sleep unit of Pitié Salpêtrière Hospital, Paris, France, between June 2016 and October 2022.

HR, K2, and symbolic dynamics were computed from Wake, NREM, and REM segments. First, HRV indices were compared in patients with and without SDB. Secondary analyses stratified SDB+ into OSA, isolated stridor, and OSA+stridor.). Thirdly, Night 1 vs Night 2 changes were evaluated in patients treated with CPAP during the second night and compared with a no-CPAP subgroup.

Results

At baseline (Night 1), SDB+ patients showed a markedly impaired autonomic profile versus noSDB: K2 was lower across Wake, NREM, and REM (all $p < 0.001$), 2LV% was reduced in each state ($p = 0.012$, < 0.001, 0.003), and 0V% was selectively higher in REM ($p = 0.005$), with no significant differences in HR.

Across SDB phenotypes, K2 differed between groups in all phases (Wake $p = 0.026$; NREM $p = 0.012$; REM $p = 0.021$), and 2LV% differed in NREM and REM ($p = 0.011$; $p = 0.021$), while 0V% and 2UV% showed no phase-wise group differences.

Under CPAP in Night 2 SDB+ patients showed a coherent autonomic shift: HR decreased in NREM and REM (both $p = 0.02$), 0V% declined in Wake and REM ($p = 0.02$ and < 0.001), 2UV% and 2LV% increased in Wake and REM (2UV% p

= 0.04 and 0.03; 2LV% p = 0.01 and 0.02), and K2 increased in Wake and REM (p = 0.05 and 0.04) while remaining unchanged in NREM (p = 0.84). The no-CPAP subgroup showed no consistent night-to-night autonomic changes.

1 Introduction

1.1 Multiple System Atrophy: facing the beast

1.1.1 Definition and pathophysiology

Multiple System Atrophy (MSA) is a sporadic, adult-onset neurodegenerative disease characterized by rapid progression and an invariably fatal outcome. [1] Although classified within the group of α-synucleinopathies, alongside Parkinson's Disease (PD) and Dementia with Lewy Bodies (DLB), MSA is distinguished by a unique pathological hallmark: the accumulation of α-synuclein aggregates predominantly in oligodendrocytes, the support cells of the central nervous system (CNS). [2] This defines it as a primary oligodendrogliopathy, clearly setting it apart from other synucleinopathies on a pathological basis, where the accumulation is seen mainly in neurons.

From an epidemiological standpoint, MSA is considered a rare disease, with an estimated incidence of 0.6-0.7 cases per 100,000 person-years [3] and a prevalence of 3.4-4.9 cases per 100,000. [4] The prognosis is poor, with a median survival ranging from 6 to 10 years from symptom onset. [5] This aggressive clinical course has a devastating impact on patients' quality of life and imposes an enormous care burden on families and caregivers. The complex clinical picture and the symptomatic overlap with other parkinsonian syndromes, particularly PD, make the diagnosis of MSA a relevant challenge, especially in the early stages. Compounding this diagnostic uncertainty is the absence of therapies capable of modifying the course of the disease. Most clinical trials of neuroprotective drugs have failed [6], likely because the diagnosis is made when the neuropathological damage is already at an advanced stage. In this context, the identification of objective, sensitive, and specific biomarkers capable of facilitating an early and accurate diagnosis represents an unmet clinical need.

The neuropathological hallmark that defines MSA is the widespread presence of glial cytoplasmic inclusions (GCIs) within oligodendrocytes, composed of filamentous aggregates of α-synuclein in an abnormal, hyperphosphorylated, and

insoluble conformation. [7] The primarily glial, rather than neuronal, location of these protein aggregates is what makes MSA a unique pathological entity. The topographical distribution of these lesions and the consequent neurodegeneration closely correlate with the clinical phenotype: predominant damage in the striatonigral system (SND) underlies MSA with predominant parkinsonism (MSA-P), while atrophy of the olivopontocerebellar system (OPCA) characterizes MSA with predominant cerebellar features (MSA-C). Since a positive correlation between the density of GCIs and the degree of neuronal loss has been shown [8], the accumulation of GCIs is likely to be an important factor in neurodegeneration in MSA. Nevertheless, the cascade of events that leads to progressive neurodegeneration remains unclear. Much attention has been caught by the phosphoprotein-25α (p25α), a physiological constituent of myelin: in MSA patients, it has been shown to relocate from the myelin sheath to the cell body of oligodendrocytes, where it accumulates leading to by oligodendrocyte swelling. [9] The next step is an abnormal uptake or overexpression of α-synuclein by oligodendroglia [10,11], and then its aggregation α-synuclein, stimulated by p25α [12]; eventually, this process will lead to the formation of α-synuclein GCIs. How neuronal death is promoted by oligodendroglial pathology remains to be clarified, but probable secondary events include reduced neurotrophic support mediated by reduced glial cell line-derived neurotrophic factor (GDNF) expression, autophagy disruption and mitochondrial failure[13] ; also neuroinflammation, with astroglial and microglial activation and cytotoxic cytokine release, reasonably play a role. As already proposed for other α-synucleinopathies, toxic α-synuclein species may then spread in a prion-like fashion to other functionally connected brain areas leading to the multisystem neuronal involvement that is typical of MSA. [14]

The nature of MSA as a primary oligodendrogliopathy offers a crucial perspective for interpreting the severity and precocity of autonomic dysfunction. Since oligodendrocytes are essential for maintaining the myelin sheath and for the trophic support of axons in the CNS, their primary dysfunction can lead to widespread secondary axonal damage. The degeneration of central autonomic nuclei, which underlies dysautonomia in MSA [15, 16], might therefore not stem from primary neuronal death, as occurs in other pathologies, but from a sort of "death by

abandonment": the functional failure of surrounding oligodendrocytes would deprive the axons of autonomic pathways of their vital support, leading to functional disconnection and, ultimately, retrograde degeneration. This mechanism could explain why autonomic dysfunction in MSA is so pervasive and severe compared to that observed in PD, where the pathology is primarily neuronal. MSA thus emerges as a unique pathological model for the study of centrally originated autonomic dysfunction.

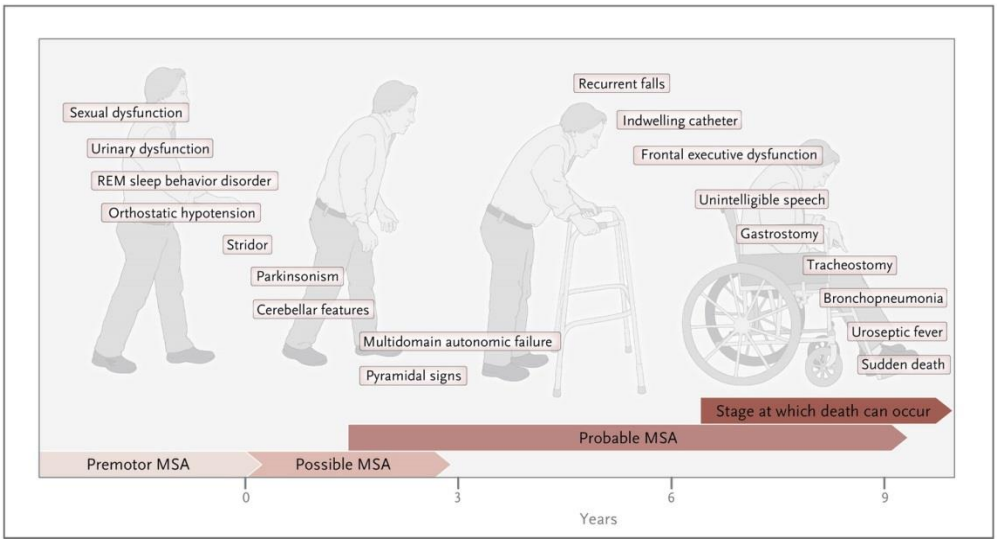

Figure 1: History of disease in MSA patients

Fanciulli A. & Wenning, G.K. 2015, "Multiple-system atrophy", The New England journal of medicine, vol. 372, no. 14, pp. 1375.

1.1.2 Clinical subtypes and diagnostic criteria

The clinical presentation of MSA patients is heterogeneous but can be traced back to two predominant motor phenotypes, reflecting the areas of the CNS primarily affected by neurodegeneration. MSA-P is the most common form in Europe [17, 5] and North America [18] and manifests with a typically symmetric, akinetic-rigid parkinsonism that shows a poor or only transient response to Levodopa therapy. Postural instability, with consequent falls, is an early and disabling symptom. MSA-C is considered the most frequent variant in Japan. [19, 20] It is dominated by a

cerebellar syndrome that includes gait and limb ataxia, ataxic dysarthria, and eye movement abnormalities, such as hypermetric saccades.

Beyond the motor dichotomy, autonomic nervous system dysfunction represents the true core feature of the pathology, a unifying clinical element mandatory for diagnosis according to the most recent criteria. The presence of specific motor and non-motor "red flags" such as cranio-cervical dystonia (particularly disproportionate antecollis), Pisa syndrome, pyramidal signs, REM sleep behavior disorder (RBD), and laryngeal stridor, are of fundamental importance in guiding the differential diagnosis and supporting the clinical suspicion of MSA [21].

The progression of the clinical picture is generally fast, although variable from patient to patient: in the earlier phases, postural instability and gait disorder may lead to the need of walking aids; they will eventually require a wheelchair by 5 years of disease and be bed ridden by 7-8 years[20, 22, 1] .The mean survival is 6 to 10 years from onset [5, 23], with only few patients having a slowly progressive disease and surviving more than 10 years [24]. Both sexes are equally affected.

The main causes of death are urogenital or respiratory infections (generally aspiration pneumonias), respiratory insufficiency, commonly due the afore mentioned vocal cord paralysis, but sudden death is also possible, perhaps due to acute disruption of the brain-stem cardiorespiratory drive [1].

Given the poor life expectancy, a careful counselling to the patient and the family by the treating physician is needed in the early phases. Negative prognostic factors include older age at onset, parkinsonian phenotype, severe autonomic dysfunction early in the disease [20, 25, 22, 1.]

The clinical diagnosis of MSA has historically been burdened by high uncertainty, with diagnostic error rates reaching 21-38% in clinicopathological studies. To address this need for greater accuracy, the Movement Disorder Society (MDS) published a revision of the diagnostic criteria in 2022 [26], representing a substantial evolution from the second consensus of 2008 [27]. The new MDS criteria introduce four levels of diagnostic certainty:

- **Neuropathologically established MSA:** The diagnosis of certainty, which requires autopsy confirmation with evidence of widespread GCIs and neurodegeneration in the striatonigral or olivopontocerebellar systems.

- **Clinically established MSA:** A new category designed to maximize specificity. It requires the presence of autonomic dysfunction (urogenital, with post-void residual >100 mL, or defined neurogenic orthostatic hypotension - nOH) in combination with either poorly Levodopa-responsive parkinsonism OR a cerebellar syndrome. These must be associated with at least two supportive clinical features ("red flags") and at least one MRI marker.

- **Clinically probable MSA:** This category aims to balance sensitivity and specificity. It requires the presence of at least two of the three core features (autonomic dysfunction, parkinsonism, cerebellar syndrome) associated with at least one supportive clinical feature.

- **Possible prodromal MSA:** A category created for research purposes to identify patients in the very early stages of the disease. It requires the presence of isolated autonomic dysfunction or video-polysomnography (V-PSG) confirmed RBD, in association with subtle motor signs that do not meet the criteria for the other categories.

A fundamental innovation of the MDS 2022 criteria is the mandatory role of neuroimaging biomarkers for the diagnosis of clinically established MSA. Signs such as atrophy of the putamen, pons, or middle cerebellar peduncles, and the "hot cross bun" sign at the pontine level are now considered not just supportive, but essential for achieving the highest degree of clinical certainty.

Concerning sleep disorders, RBD is considered as a research biomarker for MSA diagnosis due to its different weighing for the diagnosis of MSA-P, while stridor is distinctive of both motor subtypes of MSA compared to their mimics and, as such, is considered a supportive non-motor feature.

1.1.3 Autonomic Dysfunction: The Core of Pathology in MSA

Autonomic nervous system dysfunction is not one of many symptoms of MSA but represents its clinical and pathophysiological core, to the extent that it is an indispensable requirement for diagnosis according to the latest criteria. The most relevant and well-defined manifestations are nOH and urogenital dysfunction. nOH is defined as a sustained drop in systolic blood pressure of at least 20 mmHg and/or diastolic blood pressure of at least 10 mmHg within 3 minutes of standing up. A key element that distinguishes the neurogenic form from other causes of hypotension is the inadequate compensatory response of the heart rate, which increases minimally or not at all. This is conventionally defined as a ratio of heart rate increase to systolic blood pressure drop, $\Delta HR/\Delta SBP$, of less than 0.5 bpm/mmHg [28]. Concurrently, urogenital dysfunction, manifesting as urge incontinence or, more specifically, difficulty in emptying with a post-void residual volume greater than 100 mL, is another fundamental diagnostic criterion.

The centrality of dysautonomia in MSA becomes strikingly clear when compared with PD. Although PD patients can also develop autonomic dysfunction, it is generally later, less severe, and, most importantly, reflects a different pathogenetic mechanism. Myocardial scintigraphy with 123I-metaiodobenzylguanidine (MIBG), a tracer of post-ganglionic sympathetic innervation, shows typically a reduced uptake in PD, indicating peripheral cardiac denervation. In contrast, in MSA, MIBG scintigraphy is generally normal, testifying that the lesion is not at the level of peripheral nerve endings but upstream, at the pre-ganglionic level, i.e., in the central nervous system[29]. This pathophysiological distinction not only has crucial diagnostic value but also fully justifies the use of techniques that investigate central autonomic function, such as heart rate variability (HRV) analysis, as a privileged research tool for studying MSA.

The neuroanatomical basis of the severe dysautonomia observed in MSA lies in the neuronal degeneration and reactive gliosis that selectively affect CNS structures responsible for autonomic control. The neurodegenerative process extensively involves brainstem nuclei, such as the nucleus of the solitary tract, the dorsal motor nucleus of the vagus, the nucleus ambiguus, and the ventrolateral reticular

formation, as well as the intermediolateral cell columns of the spinal cord, where pre-ganglionic sympathetic neurons reside [30]. Neuronal loss in these areas disrupts the efferent pathways that regulate cardiovascular, respiratory, urogenital, and gastrointestinal function. There is a direct correlation between the degeneration of specific nuclei and the onset of particular symptoms; for example, damage to the nucleus ambiguus and the raphe nuclei has been implicated not only in cardiorespiratory dysfunction but also in the pathogenesis of laryngeal stridor and the increased risk of sudden nocturnal death, one of the most feared complications of the disease.

1.1.4 Therapeutic approach

Up to this moment, only symptomatic treatments are available for MSA.

In MSA-P patients, the parkinsonian syndrome is refractive to L-Dopa treatment by definition. Nevertheless, a transient beneficial response to L-Dopa is seen in up to 40% of patients [17]. An abrupt withdrawal in patients with no evident response occasionally irreversible worsening of motor disturbs, sometimes in a permanent way; thus, in patients with no collateral effects, L-Dopa therapy should be maintained. Dopamine-agonists may give relief in case of dystonia, but normally show no considerable motor benefit.

The approach to cardiovascular dysautonomia depends on the characteristics of the patient and the possible coexistence of both OH and hypertensive manifestations. In case of symptomatic OH, the first steps are represented by avoiding excessive dopaminergic or antihypertensive therapy and any other possible behaviour (like rapid postural changes, excessive efforts or straining, hot temperatures, etc.) that could trigger OH. The patients should learn counter-pressure manoeuvres (such as leg crossing, clenching the fists, etc.) to contrast the pressure drop as soon as presyncopal symptoms occur and, when possible, use abdominal binders or tight stockings. The pharmacological approach to OH includes fludrocortisone, midodrine [31] and droxidopa [32, 33.] As for supine hypertension, the use of short acting calcium antagonists (e.g. Nifedipine) or Clonidine before sleep [34] is advisable. An phase III trial testing a norepinephrine reuptake inhibitor (Ampreloxetin) is currently ongoing at the time of the writing.

We are currently living a period of rapid technological progress and evolving comprehension of the pathogenesis of neurodegenerative mechanisms, that are likely to influence the way we diagnose and cure MSA. The main aim of research in the last years has been finding compounds with neuroprotective or disease modifying properties, namely able to prevent neuronal death and to slow down, or even stop, the molecular and clinical progression of the disease. Unfortunately, the great majority of drugs that showed promising results in laboratory setting led to disappointing clinical trials. Despite promising results coming from the laboratories, the evident and discouraging inability to translate putative neuroprotective compounds into strategic therapeutic agents still lacks explanation, but it is probably the demonstration of the presence of structural mistakes in the design and the realisation of clinical trials, rather than a mere inefficacy of all the compounds that have been tested. Traditionally, physicians diagnose MSA when motor symptoms appear, and treat them with symptomatic therapies. Nevertheless, as previously anticipated, most of the non-motor symptoms can be detected way before the motor involvement becomes evident: some longitudinal studies on high-risk individuals (i.e. suffering from RBD or Pure Autonomic Failure) demonstrate latencies to phenoconversion (namely the development of the classic motor clinical picture) longer than 10 years [35, 36]. That would represent a period of possible clinical detectability but still partial neurodegeneration: certainly, a better moment for the administration of neuroprotective or disease modifying drugs than the first phases of motor disease, like in the previous clinical trials. This is the rational of the previously described MDS criteria for possible prodromal MSA [26].

1.2 MSA and sleep disorders: frequency, diagnosis and treatment

1.2.1 Sleep disorders in MSA

Sleep disorders are a common non-motor manifestation in all a-synucleinopathies, and they include REM sleep behaviour disorder (RBD), restless legs syndrome (RLS), periodic limb movements (PLMs), or sleep-disordered breathing (SDB).

Neuropathological studies have documented that 98% of patients with video-polysomnography (vPSG)-proven RBD and a motor or cognitive impairment have an underlying synucleinopathy [37]. Therefore, demonstration of RBD can help to distinguish MSA from non-synucleinopathy neurodegeneration such as PSP but cannot be used to distinguish MSA from Lewy body disorders. RBD can present before MSA onset; a multicentre prospective study demonstrated that approximately 10% of patients with idiopathic RBD who develop neurodegenerative disease after 4 to 5 years, were diagnosed as clinically probable MSA [38]. Other sleep abnormalities are also common in MSA. They include general disruption of the sleep architecture, upper airway dysfunction (apnoea and stridor), loss of REM atonia, and PLM during sleep. MSA patients have generally more severe loss of REM atonia compared to patients with PD and idiopathic RBD [39, 40]
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SDB can manifest with inspiratory stridor and obstructive sleep apnoea mainly due to upper-airway obstruction, as well as with central sleep apnoea, abnormal breathing patterns (including Cheyne–Strokes breathing), and irregular breathing, caused by impaired autonomic control of respiration [41].

According to the last consensus definition, stridor is a "*strained, high-pitched, harsh respiratory sound, mainly inspiratory, caused by laryngeal dysfunction leading to narrowing of the rima glottidis*" [42], possibly occurring both during sleep and in wakefulness. When physiological breathing occurs, the vocal cords abduct or open actively during inspiration. Patients with MSA develop stridor due to impaired vocal fold motion, such as vocal fold abduction restriction, or paradoxical vocal fold adduction during inspiration. Inspiratory stridor is found in about 15 to

40% of MSA patients [43, 44, 45, 46], but has been only rarely documented in other degenerative parkinsonian disorders suggesting high specificity, although controlled studies are lacking and it is therefore considered a red flag towards the diagnosis of this disease [21]. Consequently, stridor is mentioned in the updated consensus diagnostic criteria as a supportive clinical feature, necessary for the diagnosis of clinically established or clinically probable MSA [26]. Stridor can manifest at any stage of the disease [44, 45, 47], more frequently becoming evident in the advanced stages [48, 43], both as a diurnal or nocturnal symptom [21]. Stridor onset within the first 3 years from disease onset was present in 16% of patients with MSA, suggesting low sensitivity in early stages [47]. However, stridor has also been reported as presenting [49, 50] or even the sole symptom of MSA [51, 52]. Previous reports suggest an association of stridor with reduced life expectancy [53] in particular when presenting in the early phases of the disease [47], or a possible role in predicting sudden nocturnal death [54, 55]. The exact underlying mechanism of sudden nocturnal death in MSA is unresolved and currently considered of multifactorial origin [56]. A recent study also confirms that patients with early onset of stridor showed not only a shorter survival but also a more severe and more rapid disease progression [57].

Obstructive sleep apnoea (OSA) is another common disturbance in MSA patients, present in up to two third of patients [58, 56]. It was not found to be an independent factor associated with mortality[59] and contrary to what is commonly observed in the general population, it has not been linked to a higher BMI [60]. Previous reports agree that obstructive apnoeas and stridor are not always coincidental, but estimating the frequency of their coexistence in other cohorts is frequently difficult due to the variability of the definition on sleep apnoea syndrome depending on different values AHI [58, 61, 62] .

Post-mortem studies suggest that breathing disorders are the consequence of the extension of the neurodegenerative process to pontomedullary brainstem respiratory nuclei [63], and that sudden death may be linked to depletion of serotonergic neurons in the ventromedullary medulla [64]. A recent neuroimaging study found higher grey matter density in the cerebellum and lower density in the striatum in MSA patients with stridor compared to MSA patients without it [65].This

finding is in line with striatal lesions triggering dystonia and the hypothesis that stridor has a dystonic nature.

1.2.2 Diagnostic tools to detect sleep and breathing disorders

The clinical diagnosis of sleep-related breathing disorders in MSA and especially stridor remains challenging. The presence of a night-time witness is typically necessary to suspect stridor because patients may be unaware of it. In daily clinical practice, physicians can suspect the presence of laryngeal stridor when caregivers report a high-pitched breathing sound that was emitted by the patient during sleep or while awake. The imitation of stridor by the physician can be useful for the patient and caregiver to recognize the presence of stridor, however its distinction from snoring can be sometimes not straightforward. In such cases, physicians may encourage the patient and caregiver to record sounds when an episode of suspected stridor occurs.

Polysomnography including audio is not necessary if the physician has already diagnosed stridor [42], but it is generally needed to make a precise and comprehensive diagnosis of sleep disturbs, including apnoeas, RBD and PLMs. Increased apnoea/hypopnea index is commonly observed in MSA patients on vPSG, but not clearly increased compared with other neurodegenerative conditions [39].

Laryngopharyngeal dysfunction should be assessed in all MSA patients, being associated with decreased life expectancy and quality of life [53] and, in case of clinical history of stridor, laryngoscopy should be considered to exclude mechanical lesions or functional vocal cord abnormalities related to other neurological disorders. Irregular arytenoid cartilage movements were observed on flexible endoscopic evaluation of swallowing in 91% of patients with MSA (of whom, 44% showed clinically overt laryngeal dysfunction with stridor) [66] and could be an interesting diagnostic marker towards the diagnosis of MSA.

1.2.3 Therapeutic options for sleep disordered breathing in MSA

CPAP can be effective in abolishing laryngeal stridor in MSA patients [67, 68] and can be a useful long-term treatment, particularly in patients with lower motor disability at CPAP therapy start [69, 61]. It is generally a well-tolerated treatment, and the most

frequent causes of discontinuation are pulmonary infection, respiratory insufficiency of undetermined origin, and CPAP intolerance associated with discomfort caused by the CPAP mask [70]. Since CPAP is a non-invasive treatment, it should be the first choice when approaching laryngeal inspiratory stridor in MSA patients, with initial inspiratory pressure levels of 5–10 mmHg. CPAP is also the first line therapy in case of both isolated OSA and a combination of OSA and stridor, since stabilizes the upper airway and maintains its patency. However, in rare cases, it may be contraindicated due to possible worsening of apnoea syndrome in case of floppy epiglottitis [71]; in those cases, the use of an oral appliance should be considered [72]. As for other breathing disturbs, CPAP is only partially effective on central apnoeas, and ineffective on hypoventilation. An advantage of CPAP is the possible diversity of its settings. A previously proposed criterium of decision is the combination of stridor with other disturbs: stridor as an independent issue can be treated with a fixed CPAP, the combination of stridor and OSA with auto adjusting CPAP and the combination of stridor and CSA with a fixed CPAP first, and if not tolerated or unable to control CSA, with auto-served ventilation [62, 73].

In a previous analysis on a large cohort of patients, our group demonstrated the immediate objective benefits on sleep and respiratory measures with CPAP therapy when treating SDB in MSA patients [74].

A minimal-invasive option to treat laryngeal inspiratory stridor in MSA may be botulinum toxin injection into the adductor muscle complex [75], but with a risk of worsening dysphagia. Single case reports have reported the possible use of posterior cordotomy and arytenoidectomy [76, 77, 78].

When non-invasive measures are not able to abolish stridor, tracheostomy should be considered [79]. Tracheostomy is furthermore recommended when, in addition to stridor, vocal fold motion is significantly restricted and the vocal folds fixed in a paramedian position [69]. In contrast, tracheostomy could potentiate dysphagia, with aspiration necessitating the use of a cuffed cannula, resulting in cessation of speech, which could potentially worsen the quality of life of these patients. Moreover, the possibility of a lethal exacerbation of CSA has been suggested as well after tracheostomy [80].

Although both CPAP and tracheostomy can be used as symptomatic treatment for stridor, it is still uncertain whether they improve survival in MSA [42]. A recent study suggests that tracheostomy could be associated to longer survival in patients with stridor if compared to both CPAP and no treatment [81], but the specific clinical and PSG characteristics of patients who may potentially benefit from either CPAP or tracheostomy have not been described. There is insufficient evidence for the efficacy and safety of unilateral vocal cord adductor injections with botulinum toxin or minimally invasive vocal cord surgery.

1.3. Heart rate variability and MSA

1.3.1. Physiological Principles and Analysis Methodologies

HRV is traditionally defined as the physiological fluctuation that occurs in the time intervals between consecutive heartbeats, known as R-R intervals.[82, 83] This variability is not a random phenomenon but reflects the complex and dynamic interaction of the two branches of the autonomic nervous system (ANS), the sympathetic and the parasympathetic systems, which modulate the activity of the sinoatrial node, the natural pacemaker of the heart. In a healthy individual, these two systems are in a constant, dynamic balance, producing continuous physiological fluctuations in the time interval between consecutive heartbeats. This variability is an indicator of the inner ability of the system to adapt rapidly to internal and external stimuli. High HRV is thus generally associated with a healthy cardiovascular system and efficient autonomic regulation, while low HRV indicates compromised autonomic function and reduced adaptability [83].

HRV analysis has established itself as a powerful, non-invasive, reliable, and quantitative tool for assessing ANS activity and the sympatho-vagal balance, both in physiological and pathological conditions. However, its applications in the field of a-synucleinopathies has been sparse and with contrasting results.

1.3.1.1. Time-Domain and Frequency-Domain Analysis

Formal criteria for HRV and comparison of variables were developed by a joint task force between the European Society of Cardiology and the North American Society of Pacing and Electrophysiology in 1996 and updated in 2015. [82 83]
Standard methodologies for HRV analysis are mainly divided into two domains. The time-domain indices quantify the dispersion of R-R intervals over a given recording period. The most commonly used parameters include the standard deviation of all normal-to-normal intervals (SDNN), which is an indicator of total variability, and the root mean square of the successive differences between adjacent R-R intervals (RMSSD) and the percentage of successive intervals that differ by more than 50 ms (pNN50). These latter two are considered robust markers of short-term parasympathetic activity [84].

The frequency domain (spectral) analysis technique uses algorithms like the Fast Fourier Transform (FFT) or autoregressive (AR) models to break down the total variance of the R-R interval series into specific frequency components, quantifying the power (in ms2) within predefined bands [85].

The main bands of interest are:

- High Frequency (HF): Ranging from 0.15 to 0.4 Hz, this component is almost exclusively mediated by parasympathetic (vagal) activity and is closely linked to respiratory sinus arrhythmia, i.e., the fluctuations in heart rate synchronous with respiratory acts.
- Low Frequency (LF): Ranging from 0.04 to 0.15 Hz, this component reflects a more complex modulation, including both sympathetic and parasympathetic influences. It has been associated with baroreceptor activity [86].

The ratio of the power of the low-frequency band to that of the high-frequency band **(LF/HF ratio)** has long been used as an index of sympathovagal balance. An increase in this ratio is traditionally interpreted as a shift in the autonomic balance toward a predominance of sympathetic activity, while a decrease indicates a vagal predominance. However, this interpretation should be used with caution. The LF/HF ratio is a simplified indicator of a very complex interaction, and its validity may be limited, especially in pathological conditions where the relationships between the two branches of the ANS can be altered.

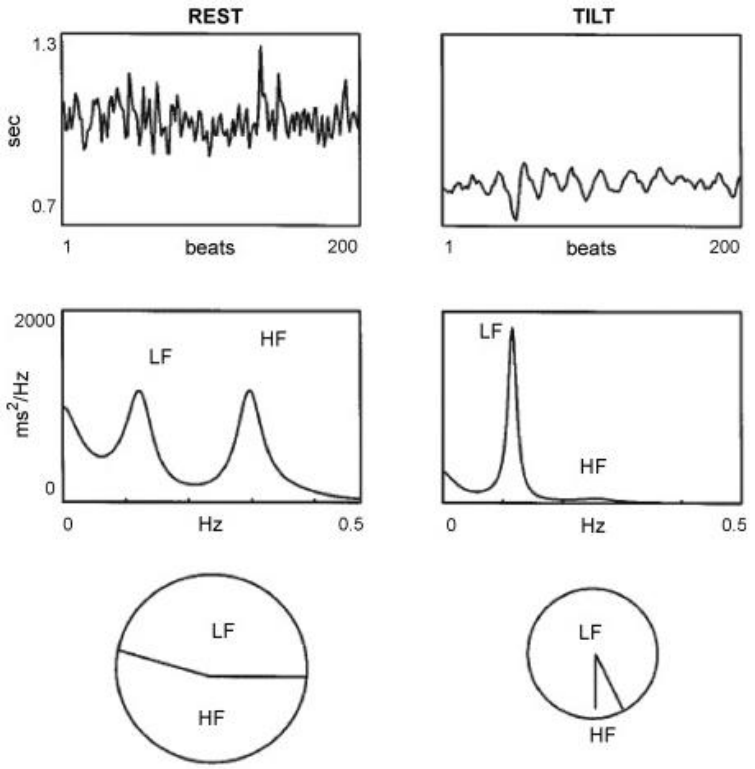

Figure 1.2: *Spectral analysis of HRV in a young subject at rest and during 90° tilt*.

The RR interval time series (i.e. tachograms) are illustrated in the top panels. The middle panels contain the autospectra which indicate the presence of two major components (LF = low frequency; HF = high frequency). During tilt, the LF component becomes largely predominant. In this example the total power (i.e. variance) is markedly reduced during tilt and consequently LF and HF powers are both decreased when expressed in absolute units (LFau, HFau). The use of normalized units (nu) clearly indicates the altered relation between LF and HF during tilt as represented by the pie charts which show the relative distribution together with the absolute power of the two components represented by the area. VAR = variance; VLF = very low frequency.

From: Montano, N. et al. "Heart rate variability explored in the frequency domain: a tool to investigate the link between heart and behavior." Neuroscience and biobehavioral reviews vol. 33,2 (2009): 71-80

1.3.1.2. Beyond Linear Analysis: Symbolic Dynamics

While time and frequency-domain analyses provide valuable linear measures, they may not fully capture the complex, non-linear interactions governing heart rate, particularly in the presence of SDB . Symbolic dynamics is a non-linear method

that offers complementary insights by transforming the RR interval time series into a sequence of symbols to analyze its underlying dynamical properties [87, 88]. This approach deliberately simplifies the signal to focus on patterns of change. Symbolic analysis is based on: (1) the transformation of time series into a sequence of symbols; (2) the construction of patterns using these symbols (ie, words); (3) the reduction of the number of patterns into four families; (4) the evaluation of their rate of occurrence expressed as percentages.

The most used method involves analyzing short sequences of three consecutive heartbeats, called "words." These three-beat patterns are then classified into categories based on their variability:

- **Zero Variation (0V):** Patterns where all three symbols are identical. An increase in 0V patterns is associated with sympathetic predominance.
- **One Variation (1V):** Patterns where two consecutive symbols are the same, while the third is different. This pattern is also linked to sympathetic modulation.
- **Two Variations (2V):** Patterns where all three symbols are different. This category is often subdivided to provide more detail:
    - **Two Like Variations (2LV):** patterns with two like variations (the three symbols form an ascending or descending ramp). This pattern is considered a marker of parasympathetic activity.
    - **Two Unlike Variations (2UV):** pattern with two unlike variations (the three symbols are organized forming a peak or a valley. This pattern is also associated with parasympathetic modulation.

By evaluating the rate of occurrence of these patterns, symbolic analysis provides a quantitative assessment of the sympathovagal balance. This non-linear tool is particularly useful for studying short-term HRV instabilities that may precede cardiac events and can be applied to very short data sequences where traditional spectral analysis might be unreliable.

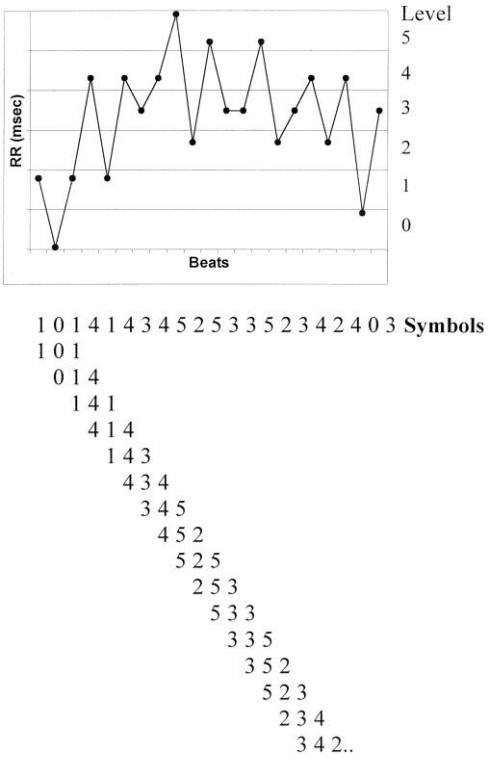

Figure 1.3. Synthetic illustration of symbolic analysis method. RR series was uniformly spread on 6 levels (from 0 to 5); each level was identified by symbol (number), and patterns of length of 3 symbols were constructed.

From: Guzzetti, S. et al. "Symbolic dynamics of heart rate variability: a probe to investigate cardiac autonomic modulation." Circulation vol. 112,4 (2005): 465-70.

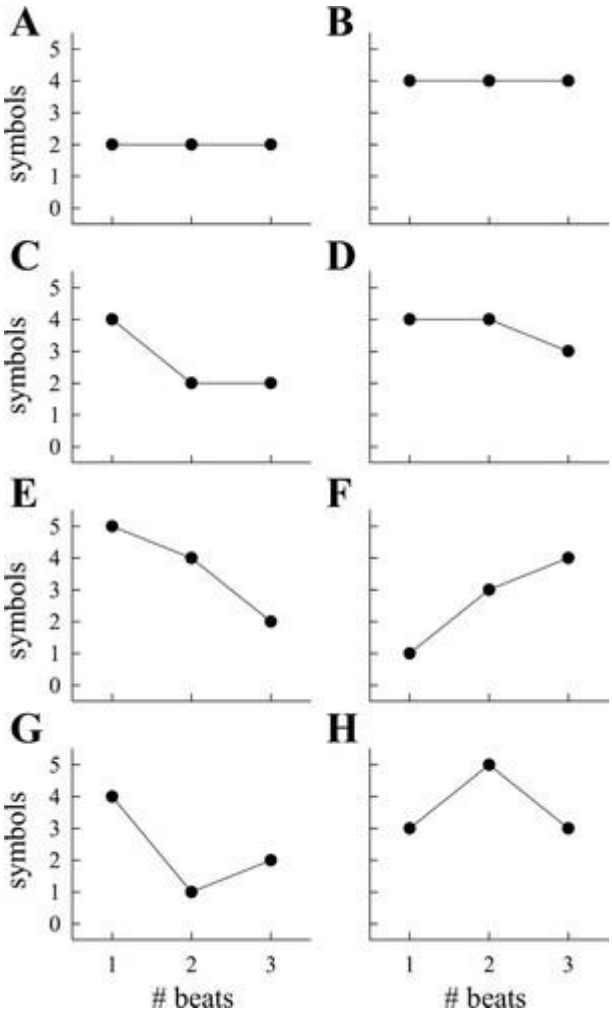

Figure 1.4. Representative examples of patterns with no variations (0V, A and B), patterns with 1 variation (1V, C and D), patterns with 2 like variations (2LV, E and F), and patterns with 2 unlike variations (2UV, G and H).

From: Porta, A. et al. "Assessment of cardiac autonomic modulation during graded head-up tilt by symbolic analysis of heart rate variability." American journal of physiology. Heart and circulatory physiology vol. 293,1 (2007): H702-8.

1.3.1.3. K2 - Cardiorespiratory coupling

The HF-K2 index is a parameter used to assess cardiorespiratory coherence based on HRV analysis. A bivariate autoregressive analysis is performed to evaluate the correlation between the ECG and respiratory signals. The K2 represents the degree of synchronization between heart rate oscillations and respiratory rhythm within the HF band of HRV, which is primarily influenced by parasympathetic activity. It provides a numerical measure of cardiorespiratory coupling efficiency, ranging

from 0 (no correlation) to 1 (maximum correlation). Higher HF-K2 values indicate stronger coherence between breathing and cardiac rhythm [89], [90].

In the interest of the present study, only K2 and symbolic analysis studies have been considered.

1.3.2. Current evidence on HRV analysis in MSA patients' daytime recordings

Studies that have employed frequency-domain analysis during daytime hours or in a waking state have provided consistent results regarding the severity of autonomic dysfunction in MSA. MSA patients consistently show significantly lower Total Power, LF power, and HF power compared to healthy controls [91] [92, 93]. This can indicate a profound deterioration of both the sympathetic and parasympathetic branches of the autonomic nervous system. The results regarding the LF/HF ratio are heterogeneous. Some studies report no significant differences compared to controls , while others have found it to be reduced [94]

One study assessing HRV during tilt table testing suggested a key functional difference: while both PD and MSA patients showed reduced HF during the tilt compared to controls, while LF/HF ratio tended to decrease in PD but to increase (albeit insufficiently) in MSA, suggesting tilting causes a cardiac sympathetic dominance in patients with MSA and healthy controls, where cardiac postganglionic innervation is intact, but fails to do so in patients with PD due to postganglionic sympathetic denervation. [95]

We previously assessed HRV symbolic analyses on MSA patients from a resting ECG trace, finding no significant difference between MSA-P and MSA-C patients, as well as no correlation with neuropsychological performances [96]

1.3.3. HRV Analysis During Sleep: A Controlled Physiological Model

The analysis of HRV during nocturnal sleep offers a significant methodological advantage over daytime recordings. Sleep represents a standardized physiological state in which confounding influences related to physical activity, emotional stress, cognitive stimuli, and posture are drastically reduced. This allows for a "purer" and

more reproducible assessment of endogenous autonomic regulation. Furthermore, the different sleep stages are characterized by distinct and predictable autonomic profiles: NREM sleep is dominated by high vagal activity [97], which promotes rest and recovery, while REM sleep is characterized by sympathetic activation, with fluctuations in HR and BP [98]. The study of HRV transitions between these sleep stages provides a dynamic measure of the ability of the ANS to modulate its activity, a capacity that can be compromised in neurodegenerative diseases. [99]

Only a few studies have analysed HRV in MSA during sleep, with small sample sizes and contradictory results. Most studies analysed HRV from 24h ECG Holter registrations and did not control the results based on the presence of absence of SDB. A study from Kitae in 2001 [91] showed that MSA patients had significantly lower absolute LF and HF values during sleep. Controls exhibited a lower LF/HF ratio during sleep than waking indicating parasympathetic predominance, a circadian variation absent in MSA patients. There was no significant correlation with disease duration. A report focusing only on MSAC also showed a significant reduction of HF during night-time [100]. Another study analysed HRV from 24ECG Holter in 13 MSA patients compared to controls and PD: FD-HRV exhibited significantly lower values of total power, LF, and HF components in both PD and MSA, compared to healthy controls, while no difference was evident in the LF/HF ratio. This study confirmed that in MSA dysautonomic dysfunction is more severe and characterized by a higher degree of sympathetic derangement in respect of PD, both during daily activity and during sleep [101] .

Only one study describes HRV in MSA patients extracted from PSG traces, performed in a sleep laboratory over two consecutive nights [102]. The study involved 7 MSA patients, who presented an increased HFnu sleep compared to the controls during REM sleep and NREM sleep, and during REM sleep compared to sporadic adult-onset cerebellar ataxia patients. No difference was observed in LFnu. This increase in HFnu, reflecting parasympathetic predominance due to sympathetic dysfunction, was reported to be further supported by a decreased LF/HF ratio. Interestingly, 3 presymptomatic MSA patients exhibited similar HRV changes, whereas patients with unexplained sporadic ataxia showed no abnormalities, indicating a potential early marker for MSA.

To our knowledge, no study has performed the analysis of symbolic indices nor K2 in PSG traces of MSA patients.

The evaluation of nocturnal HRV in MSA patients is inherently complex and requires careful consideration of confounding factors, chief among them the extremely high prevalence of SDB. The presence of apneas, hypopneas, and stridor profoundly and directly alters autonomic function, superimposing its effects on those of the underlying neurodegeneration. Respiratory events, by causing intermittent hypoxia and sleep fragmentation through micro-arousals, trigger powerful and repeated activations of the sympathetic system. This translates into an increase in heart rate, blood pressure, and a marked alteration of HRV parameters, typically with a reduction in total variability and an increase in the LF/HF ratio. Stridor, by inducing increased inspiratory effort and abnormal respiratory work, can also have similar effects, but its effect has not been investigated directly.

1.3.4. Impact of CPAP Therapy on HRV in Sleep-Disordered Breathing

Obstructive sleep apnea alters cardiac autonomic function during sleep and wakefulness [103]. In patients with OSA during sleep, cardiovascular activity varies in synchrony with the apnea-hyperpnea respiratory cycle, with bradycardia and hypotension during apneas and tachycardia and hypertension following apnea termination [104]. This leads to a formal limitation of HRV application to sleep studies in OSA. Indeed, the analysis of HRV during sleep is limited by the presence of repetitive apneas, leg movements, or arousals, which artificially modify HRV analysis, introducing a "rhythmic" biological noise able to alter autonomic cardiovascular oscillations [105, 106]. In fact, severe OSA induced an important modification in breathing pattern, which could have per se been a relevant confounding factor able to impinge upon HRV rhythmical oscillations. For this reason, cardiovascular autonomic assessment in severe OSA patients can importantly be affected by non-neural oscillations related to the continuous episodes of apnea which could thereby alter HRV analysis; this factor must be taken into account when analyzing PSG data and interpreting the results [105].

Various studies have performed frequency domain analysis of OSA patients during the night, and although there is high variability of protocols and kind of patients, they demonstrate a global shift of the sympathovagal balance toward sympathetic predominance both in sleep and wake, as the increased LF power and LF/HF ratio in OSA patients are relatively consistent findings, and tend to correlate with the severity of the disease [107, 103, 108, 109]. However, some studies do not confirm these findings, perhaps due to the small sample size. [110] The arousal index strongly correlates with LF/HF ratio and VLF in patients with OSA [111, 109] and negatively correlates with HF components [112, 109]. Moreover, the arousal index was found to be a more powerful contributing factor than AHI for cardiac autonomic dysfunction in patients with OSA. [109]

Studies combining both spectral and symbolic analysis confirm sympathetic predominance in patients with moderate and severe forms of OSA (increase in LFnu and decrease in HFabs in moderate OSA as compared to normal subjects, and a monotonic increase in LF/HF with OSA severity) alongside an increase in the occurrence of 0V and a decrease in 2LV in patients with moderate and severe forms of OSA, as compared to normal controls. [113]

CPAP acts as a pneumatic splint, mechanically preventing airway collapse during sleep. This eliminates the primary triggers of autonomic dysfunction: intermittent hypoxia, hypercapnia, and sleep fragmentation. By eliminating these triggers, CPAP should effectively reduce the powerful sympathetic activation that characterizes SDB.

As to autonomic effects of CPAP, a pioneer study by Somers and colleagues showed that CPAP is able to acutely affect ANS, with a significant decrease of muscle sympathetic nerve activity during wake and sleep. [103] Interestingly, even one night of CPAP treatment was able to affect HRV with a reduction of sympathetic modulation and an improvement of baroreflex control. [114, 115] Previous studies have examined the effect of CPAP on sympathetic hyperactivity in OSA patients, and repeatedly demonstrated a significant reduction in LF during acute CPAP treatment. [116, 117, 118, 119] A recent metanalysis assessing the impact of any treatment of OSA on HRV [120] also confirmed a significant reduction of VLF across

different studies, while changes in LF, HF and LF/HF were not significant, probably due to the high heterogeneity of the included studies (i.e. assessing not only CPAP but also weight loss, positional therapy, etc).

With consistent use over months, CPAP could promote a recalibration of the ANS, with a reduction in sustained daytime sympathetic hyperactivity, an effect that may take 6 to 12 months of adherent use to become clearly evident. This is confirmed by long term observations, up to 3-9 months [121], [122] and even 2 years [116]. Moreover, reports suggest that long term CPAP (2 years treatment) is able to improve the coupling between parasympathetic modulation and delta wave sleep [123] suggesting a positive effect of this therapy on central and peripheral oscillations.

Another interesting marker of HRV is cardiorespiratory coupling (CRC)—the temporal alignment between heart period and respiration (e.g., quantified by K2)—which captures the integrity of brainstem autonomic–respiratory integration and complements conventional HRV by indexing coordinated timing rather than variability amplitude alone. In patients with OSA, CRC is characteristically reduced, consistent with arousal- and hypoxemia-driven fragmentation of heart–breathing timing, whereas CPAP therapy enhances CRC by stabilizing ventilation and reducing arousals and desaturation burden [124], [89]

The benefits of CPAP on HRV and cardiovascular risk are critically dependent on patient adherence, typically defined as ≥4 hours of use per night. A clear dose-response relationship exists, where more hours of nightly use correlate with greater clinical benefits, and the same is yet to be specifically demonstrated for HRV as well.

To our knowledge no study has specifically studied the impact on HRV of OSA and stridor, both isolate and in combination, in MSA patients, nor the effect of CPAP therapy used for the treatment of either stridor or OSA on HRV in these patients.

2. Aim of the study

Sleep disorders are a common non-motor manifestation in all a-synucleinopathies. Sleep-related breathing disorders are particularly frequent in MSA, and can manifest in a variety of ways, the most common being inspiratory stridor and obstructive sleep apnoea. All these disturbs have an clear impact on the quality of life and sleep of both patients and caregivers and may even influence survival [47], [57]. CPAP has gained a growing role in the past years in this setting and is currently considered the 1st line treatment of stridor [42]. The impact of CPAP therapy on autonomic dysfunction, as assessed by HRV, is yet to be defined.

Given this background, the present study was designed to

1) To comprehensively characterize HRV parameters (cardiorespiratory coupling and non-linear symbolic analysis) during different sleep stages (NREM and REM), obtained from polysomnographic recordings, in a large cohort of patients diagnosed with MSA.
2) To evaluate the impact of the different SDB on HRV parameters.
3) To investigate the conservation of natural evolution of HRV in the wake, NREM and REM.
4) To assess the impact of CPAP therapy on HRV.

3. Materials and methods

3.1. Study population

MSA patients who underwent a video-polysomnographic study (v-PSG) at the sleep unit of Pitié Salpêtrière Hospital, Paris, France, between June 2016 and November 2024 were retrospectively enrolled in a larger study on the impact of CPAP on PSG parameters in MSA patients, as previously published [74].

In a subset of patients, we performed the present exploratory study analysing HRV parameters in the different phases of the recordings (wake, NREM, REM).

Inclusion criteria were:

- diagnosis of either probable MSA or possible MSA according to the second Consensus Conference on the diagnosis of MSA at evaluation [27] *AND*
- extensive clinical documentation, with at least two neurological follow-up visits with a confirmed neurological diagnosis.

Exclusion criteria were:

1. other major neurologic or psychiatric diseases;
2. final diagnosis at last clinical evaluation non consistent with MSA;
3. missing or incomplete clinical documentation;
4. low quality of the PSG recording;
5. low quality of ECG trace;
6. absence of stable sinus rhythm on ECG, history of coronary artery disease, the presence of cardiac stimulators, onco-haematological conditions, ongoing acute clinical conditions.

In case of multiple PSG recordings available for the same patient, a priority to the first PSG demonstrating stridor was given. In case of sequential studies negative for stridor, the last available was chosen for the study.

The diagnosis of SDB was defined by the presence of either:

- stridor

- apnoea-hypopnea index (AHI) ≥ 15
- their combination

In accordance with the French law on clinical research, patients were informed that their clinical measurements (collected as part of routine care) could be subsequently anonymized and used for research purposes, if they formally did not object (non-objection procedure). Given the retrospective nature of the study, formal approval by an ethics committee and written consent are dispensed with. The sleep clinic received validation (MR003 #1999732) for this procedure. Data were collected, archived and analysed anonymously following the current European laws for data protection.

3.2. Clinical-demographic data set

For each enrolled patient, the following information were collected from clinical records contemporary to PSG:

1) clinic-demographic data: sex, age at evaluation and at onset of symptoms, disease duration, symptoms at onset, motor phenotype, Hoenh and Yahr (H&Y) stage, Global Disability Scale (GDS), weight, height, body mass index (BMI), disease duration at last visit and/or death.
2) non-motor comorbidities: hyposmia, constipation, urinary disturbs, supine hypertension, orthostatic hypotension, cardiovascular and respiratory comorbidities.
3) motor features at the time of PSG: scores of UMSARS scale [125] on speech, swallowing, falling, posture, body sway and gait collected prospectively. If not available, the scores were retrospectively estimated from medical charts when possible.
4) clinical data about sleep: Epworth sleepiness scale, history of stridor and RBD, previous diagnosis of OSA.
5) pharmacologic treatment: use of L-Dopa, L-Dopa daily dose, use of dopamine agonists, L-Dopa equivalent daily dosage (LEDD) calculated according to Tomlinson et al. [126], use of anti-hypertensive drugs, anti-hypotensive drugs, anti-depressive drugs, benzodiazepines, antipsychotics, melatonin.

3.3. Polysomnography dataset

All enrolled patients underwent an inpatient video-polysomnography of at least two consecutive nights. The PSG recording included: three electroencephalograms, two electro-oculograms, a surface electromyogram of the mentalis and left and right tibialis anterior muscles, 1-lead electrocardiogram, video and audio recordings, measure of nasal pressure and transcutaneous pulse oximetry, and assessment of thoracic and abdominal movements, body position. Sleep was monitored from lights off to lights on. The sleep stages, arousals, periodic leg movements, respiratory events, and chin EMG activities (after excluding snoring artefacts) were scored by sleep experts according to international criteria [127].

REM sleep without atonia was defined as an epoch with at least 50% of the duration with chin EMG amplitude greater than the minimum amplitude demonstrated in NREM sleep, as recommended [127] The percentage of REM sleep without atonia was the ratio between the number of epochs without atonia and the total number of epochs. We considered a cut-off of REM sleep without atonia greater than 18% of REM sleep time as abnormal [128]. Clinical RBD corresponded to a clinical history of dream enactment. Definite RBD met the following criteria: a history of clinical RBD, including repeated episodes of sleep related vocalization and/or complex motor behaviours; documented behaviours on vPSG during REM sleep or a clinical history of dream enactment; REM sleep without atonia greater than 18%; and no better explanation by another sleep disorder, mental disorder, medication, or substance use [129].

Video recordings were carefully examined to detect any abnormal movement during REM sleep, and the audio was evaluated for stridor. Stridor is an easily recognizable harsh and strained high-pitched sound, usually inspiratory, sometimes expiratory, or biphasic[130]. Sleep apnoea-hypopnea syndrome was defined as an AHI ≥15 per hour. Abnormal breathing events during sleep were classified as either *apnoea* (a complete cessation of airflow lasting ≥10 s) or *hypopnea* (requiring either >50% airflow reduction or a smaller airflow reduction with associated >3% oxygen desaturation or arousal). Predominant OSA was defined if ≥ 5 predominantly obstructive respiratory events (obstructive and mixed apnoeas, hypopneas, or

respiratory effort-related arousals) per hour of sleep occurred during a PSG, and if the total number of central apnoeas and/or central hypopneas was less than 50% of the total number of apnoeas and hypopneas [129]. Alternatively (≥ 5 respiratory events plus a total number of central apnoeas and/or central hypopneas ≥ 50% of the total number of apnoeas and hypopneas), CSA was defined.

Overall, the following parameters of PSG recording were collected: alpha rhythm during wake, sleep efficiency, total sleep time (TST) and sleep stages as the percentage of TST, latency to sleep onset, latency to REM sleep, number of cycles, sleep fragmentation with number of arousals per hour, periodic movements of sleep (PMS) per hour, AHI, number and type of apnoea/hypopnea events, presence of stridor during wake and sleep, presence of periodic respiration, arterial oxygen saturation (SaO2) during wake and sleep, desaturation index, total time with SaO2 < 90%.

ECG and respiratory traces were extracted from PSG recordings using a specialized software. The QRS complexes were identified, and parabolic interpolation was used to locate the apex of the R waves. All the ECG traces were linearly detrended and carefully checked to avoid any missing beats and any incorrect detection of QRS complexes. These traces were then categorized into wakefulness (Wake), NREM sleep (N1+N2+N3), and REM sleep. The analysis focused on the first two complete sleep cycles for each subject (NREM and REM sleep). Consecutive samples of 250–300 beats were automatically determined based on sleep phases (Wake, NREM, and REM), and HRV analysis (cardiorespiratory coupling and non-linear symbolic analysis).

3.4. Statistical analysis

Qualitative variables were summarized by frequency and percentage, quantitative variables by mean ± standard deviation if normally distributed, or by median (1st quartile; 3rd quartile) if not normally distributed according to the Kolmogorow-Smirnow test. Qualitative variables were compared by means of the Pearson's X2 test (or Fisher's exact test, if appropriate). Quantitative variables were compared

between groups using the Mann-Whitney U (if non-normally distributed) or the T-test (if Gaussian distributed).

Clinical-demographic characteristics and PSG characteristics of patients with and without indication to SDB were compared with univariate analysis.

To compare baseline cardiovascular parameters across the four study groups (noSDB, OSA, isolated stridor, and OSA+stridor) a one-way analysis of variance (ANOVA) was performed. Prior to the analysis, model assumptions were evaluated. The normality of the distribution within each group was tested using the Shapiro–Wilk test, and the homogeneity of variances across groups was assessed with Levene's test. No significant violations of these assumptions were observed, allowing for the application of the ANOVA. The analysis compared group means to determine whether statistically significant differences existed. In the event of a significant overall effect, post-hoc pairwise comparisons with correction for multiple testing (e.g., Tukey's test) were planned to identify which groups differed from one another.

To assess the evolution of HRV indices during the different phases a mixed design with "Group" (noSBD, OSA, isolated stridor, OSA+stridor) as the between-subject factor and "Phase" (Wake, NREM, REM) as the within-subject factor was used.

Primary inference used mixed ANOVA (subject as the repeated unit), assessing sphericity with Mauchly's test. Significant effects were followed by phase-wise one-way ANOVAs with pairwise comparisons between groups; significant Phase effects were followed by within-group, repeated-measures pairwise contrasts.

To evaluate the effect of CPAP during Night2, quantitative variables of the two nights were compared the Wilcoxon signed-rank tests (if non-normally distributed) or the paired T-test (if Gaussian distributed). To evaluate the differences between patients with and without CPAP, a two-way mixed-design ANOVA was utilized. This model included 'Group' (CPAP vs. noCPAP) as the between-subjects factor and 'Time' (Night 1 vs. Night 2) as the within-subjects (repeated measures) factor. The primary outcome of this analysis was the Group × Time interaction effect, which assesses whether the overnight change in parameters differed between the two groups. To confirm these findings with a more robust model, a Linear Mixed-

Effects Model (LMM) was also fitted, including fixed effects for group, time, and their interaction, with a random intercept specified for each subject.

Statistical analysis was performed by means of SPSS®, version 24.0 or upgrades.

P values < 0.05 were considered statistically significant.

4. Results

4.1. Clinical description of the cohort

A total of 24 patients diagnosed with MSA who underwent a v-PSG over two consecutive nights were included in the final analysis. The cohort was composed of 9 patients with MSA-P and 15 with MSA-C. The mean disease duration from symptom onset for the entire cohort was 4.9 ± 2.1 years.

The primary grouping variable was the presence of SDB, defined by the presence of either stridor or AHI ≥ 15, or both, stratifying patients into an SDB-negative group (noSDB, n=6) and an SDB-positive group (SDB+, n=18). Key demographic and clinical variables were compared between these two groups (see *Table 1*). There were no statistically significant differences in terms of mean age (63.8 ± 7.8 vs. 63.3 ± 8.1 years; p = 0.729), sex distribution (83.3% vs. 50.0% male; p = 0.151), or BMI (26.7 ± 5.8 vs. 25.8 ± 6.1 kg/m², respectively; p = 0.773).

Furthermore, the groups were also comparable for key clinical characteristics. The distribution of MSA phenotype did not differ significantly between the SDB- and SDB+ groups (p = 0.088), although the cerebellar phenotype was more frequent in the SBD+ group. Similarly, the mean disease duration was not significantly different between the two groups (5.5 ± 2.7 vs. 4.8 ± 2.4 years, respectively; p = 0.167). These results indicate that the two clinical groups were well-matched for all key baseline characteristics.

	Total	SDB-	SDB+	p value
Number of patients	24	6	18	–
Sex, male	58.3 % (14)	83.3% (5)	50.0% (9)	0.151
Age at PSG, years	62.9 ± 8.0	63.8 ± 7.8	63.3 ± 8.1	0.729
Age at disease onset, years	57.3 ± 8.8	58.3 ± 8.5	58.5 ± 8.3	0.933
Disease duration, years	5.6 ± 3.2	5.5 ± 2.7	4.8 ± 2.4	0.167
Motor phenotype				
MSA-P	37.5% (9)	66.7% (4)	27.8% (5)	0.088
MSA-C	62.5% (15)	33.3% (2)	72.2% (13)	
Diagnostic probability				
Probable	83.3% (20)	66.7% (4)	88.9% (16)	
Possible	16.7% (4)	33.3% (2)	11.1% (2)	0.206
Clinical scales				
H&Y	3 (3; 4)	3 (3; 4)	3 (3; 4)	0.527
GDS	3 (2; 4)	3 (2; 4)	2.5 (2; 4)	0.504
ESS	7 (3; 12)	5 (3; 10)	7.5 (4; 12)	0.122
Biometric indexes				
Weight	75.0 ± 21.7	79.7±20.0	73.3 ± 22.7	0.554
Height	168.8±10.7	172.7±6.7	167.3±11.7	0.307
BMI	26.0±5.9	26.7±5.8	25.8±6.1	0.773

Table 1. Baseline Demographic and Clinical Characteristics of the Study Cohort.

Data are presented as mean ± standard deviation for continuous variables (Age, BMI, Disease Duration) and as n (%) for categorical variables (Sex, MSA Phenotype). P-values compare characteristics between the SDB- and SDB+ groups and were derived from independent samples t-tests for continuous variables and Chi-squared tests for categorical variables.

Polysomnographic features of the Night1 were analyzed to characterize the presence and severity of SDB at baseline.

Regarding sleep architecture, the SDB+ group spent a significantly greater percentage of the Total Sleep Time in N1 sleep compared to the noSDB group ($p = 0.033$). Other parameters, including Total Sleep Time, Sleep Efficiency, latency to sleep and REM sleep and the percentage of time spent in N2, N3, and REM sleep, were also comparable between the two groups ($p > 0.05$ for all).

As expected, the SDB+ group exhibited a significantly higher AHI compared to the noSDB group (34.1 ± 24.2 vs. 1.9 ± 3.0 events/hour, respectively; $p < 0.001$), which was mainly due to hypopneas (p 0.003). The increased burden of respiratory events in the SDB+ group was also associated with more significant nocturnal hypoxemia, measured by the percentage of the night with oxygen saturation below 90% (T90) ($p = 0.002$). The desaturation index was also significantly higher in the SDB+ group ($p = 0.013$). The arousal index tended to be elevated in SDB+ patients (17.1 ± 8.6 vs. 9.4 ± 7.3 events/h), but this difference did not reach statistical significance ($p = 0.058$). Importantly, periodic leg movements (PLM) were significantly more frequent in the SDB+ group compared with SDB– (66.3 ± 64.7 vs. 18.7 ± 25.7 events/h; $p = 0.018$), suggesting an additional marker of sleep fragmentation in patients with SDB.

	Total	SDB-	SDB+	p
Total sleep time (min)	383.4 ± 88.3	367.9 ± 67.5	388.6 ± 95.4	0.630
Sleep efficiency (%)	73.6±13.4	78.0 ± 9.2	72.2±14.5	0.366
Latency to sleep onset	24.1±27.3	32.7±52.0	21.3±13.6	0.391
Latency to REM sleep	136.8±112.5	100.9±70.1	148.8±122.8	0.379
Sleep stages (% of TST)				
N1	5.6±6.2	2.2±1.5	7.2±7.0	0.033
N2	55.9±16.3	62.7±15.3	53.6±16.4	0.280
N3	24.5±0.7	23.1±11.2	25.0±10.7	0.770
REM sleep	14.6±7.9	12.0±6.9	15.5±8.3	0.343
Sleep fragmentation (events/h)				
Arousal index	15.2±8.8	9.4±7.3	17.1±8.6	0.058
AHI	26.0±25.3	1.9±3.0	34.1±24.2	**<0.001**
Central apneas	0 (0; 3.8)	0 (0; 1.5)	1 (0; 4.3)	0.535
Obstructive apneas	0 (0; 18)	0 (0; 2.5)	2.5 (0; 29.5)	0.518
Mixed apneas	0 (0; 2.8)	0 (0; 0.5)	0 (0; 4.3)	0.268
Hypopneas	77 (15.8; 169.3)	2.5 (0; 17.8)	131 (73.8; 191.8)	**0.003**
Desaturation index	26.5 (10.3; 50.2)	1.9 (0; 20.7)	33.1 (13.4; 66.0)	**0.013**
SpO2 <90% (% of TST)	0.8 (0; 4.6)	0 (0.2; 3.5)	22.2 (5.4; 77.4)	**0.003**
Mean SpO2 wake	94 (93; 95)	93.5 (92.8; 95.3)	94 (93; 95)	1.000
Mean SpO2 sleep	93 (92; 94)	92 (92; 95.3)	93 (91.2; 93)	0.056
Mean SpO2 N3	93 (92; 94)	92 (92; 95.3)	93 (92.3; 94)	0.747
Mean SpO2 REM	93 (91; 94)	92.5 (91.8; 96)	93 (91; 94)	0.770
Periodic leg movements	54.5±60.7	18.7±25.7	66.3±64.7	**0.018**

Table 2. Baseline sleep and respiratory characteristics.

Data are presented as mean ± standard deviation for normally distributed continuous variables and median (1^{st} quartile; 3^{rd} quartile) for not normally distributed variables. P-values compare characteristics between the SDB- and SDB+ groups and were derived from independent samples t-tests for normal variables and Mann-Whitney U for not normal variables.

4.2. Differences in HRV in patients with and without SDB

We first compared nocturnal cardiovascular parameters between noSDB and SDB+ patients during the Night1, to describe the autonomic state at baseline, as described in *Table2*.

This primary analysis revealed a profound impairment of cardiorespiratory regulation in the SDB+ group. The most significant disparities were found in the K2 index of cardiorespiratory coupling, which was markedly lower in the SDB+ cohort across all recorded states: wakefulness, NREM, and REM sleep ($p < 0.001$ for all). Similarly, 2LV% symbolic patterns, a marker of vagal modulation, were significantly reduced in the SDB group in all states (p 0.012, < 0.001 and 0,003 respectively). Conversely, a state-specific increase in sympathetic-related 0V% patterns was noted in the SDB+ group during REM sleep ($p = 0.005$).

No significant differences were observed for HR.

	SDB- n = 6	SDB+ n = 18	p
Wake			
HR	64,74 ± 5,25	70,05 ± 9,60	0,147
K2	0,86 ± 0,12	0,51 ± 0,26	**<0.001**
0V%	27,49 ± 12,11	39,22 ± 22,23	0,198
2UV%	18,48 ± 9,45	19,30 ± 9,62	0,857
2LV%	9,20 ± 4,54	4,60 ± 3,11	**0,012**
NREM sleep			
HR	61,70 ± 4,37	64,42 ± 9,14	0,449
K2	0,93 ± 0,07	0,56 ± 0,27	**<0.001**
0V%	16,42 ± 8,61	24,93 ± 18,88	0,218
2UV%	24,96 ± 11,23	26,48 ± 10,65	0,771
2LV%	24,96 ± 11,23	6,56 ± 4,91	**<0,001**
REM sleep			
HR	64,87 ± 5,59	67,11 ± 9,67	0,582
K2	0,86 ± 0,13	0,40 ± 0,25	**<0.001**
0V%	24,11 ± 8,18	50,56 ± 22,38	**0,005**
2UV%	22,77 ± 9,49	14,80 ± 9,33	0,086
2LV%	6,77 ± 3,52	2,23 ± 2,29	**0,003**

Table 3. **Comparison of nocturnal cardiovascular parameters between the control and the combined SDB cohort.**

Data are presented as mean ± standard deviation for the noSDB group and SDB+ group. The final column shows the p-value from the independent samples t-test. Significant p-values ($p < 0.05$) are highlighted in bold.

The next step was to further explore potential heterogeneity within the SDB+ cohort and assess the impact of specific SBD on HRV. Thus, a secondary analysis was performed by stratifying the patients into three distinct phenotypes: patients with OSA (AHI ≥ 15), with isolated stridor, and with both OSA and stridor (named "OSA+stridor").

The most consistent differences were observed in the K2 index of cardiorespiratory coupling, which was significantly different between groups in all recorded states. During wakefulness ($p = 0.026$), the SDB- group exhibited significantly higher coupling than both the OSA and OSA+stridor subgroups. This pattern was amplified during REM sleep ($p = 0.021$), where the SDB- group showed higher K2 values than all three SDB+ patient subgroups. During NREM sleep ($p = 0.012$), the SDB- group again surpassed the OSA and OSA+stridor subgroups; notably, a difference was also found within the SDB+ cohort, with the isolated stridor group showing significantly higher coupling than the OSA group.

Significant group differences were found for 2LV% symbolic patterns, predominantly during sleep. During NREM sleep ($p = 0.011$), the noSDB group had significantly higher 2LV% values than all three patient subgroups. Similarly, during REM sleep ($p = 0.021$), the noSDB group showed higher values than the isolated stridor and OSA+stridor subgroups, while OSA remained at an intermediate level overlapping with both. No significant differences emerged during wakefulness.

In contrast, no statistically significant inter-group differences were found in the mean HR nor 2UV% in any state. Similarly, the overall ANOVA 0V% was not significant. However, an exploratory pairwise t-test during REM sleep identified a significantly lower 0V% in the noSDB group compared to the isolated stridor group ($p = 0.013$).

	SDB- n = 6	OSA n = 5	Isolated stridor n =5	OSA+stridor n= 8	p
Wake					
HR	64,74 ± 5,25ª	71,36 ± 11,21ª	71,54 ± 3,60 ª	66,88 ± 10,59ª	0,697
K2	0,86 ± 0,12ª	0,34 ± 0,16^{b}	0,68 ± 0,27 ^{ab}	0,49 ± 0,26^{b}	**0,026**
0V%	30,24 ± 10,87ª	42,99 ± 25,12ª	50,55 ± 29,81 ª	32,02 ± 18,23 ª	0,569
2UV%	18,48 ± 9,45 ª	18,82 ± 12,91ª	15,11 ± 10,28 ª	21,58 ± 6,47 ª	0,811
2LV%	9,20 ± 4,54 ª	3,54 ± 3.01 ª	2,51 ± 2,16 ª	5,92 ± 3,25 ª	0,086
NREM sleep					
HR	61,70 ± 4,37 ab	64,48 ± 6,80 ª	72,26 ± 6,31^{a}	60,92 ± 9,67^{b}	0,098
K2	0,93 ± 0,07ª	0,31 ± 0,17 ^{b}	0,74 ± 0,21 ^{ac}	0,59 ± 0,27 ^{bc}	**0,012**
0V%	16,31 ± 9,80 ª	29,72 ± 26,49ª	36,44 ± 19,49 ª	19,05 ± 12,96ª	0,401
2UV%	24,96 ± 11,23ª	22,26 ± 13,23ª	20,42 ± 8,41 ª	30,28 ± 9,10 ª	0,596
2LV%	24,96 ± 11,23ª	9,08 ± 5,33 ^{b}	3,56 ± 2,87 ^{b}	7,96 ± 4,14 ^{b}	**0,011**
REM sleep					
HR	64,87 ± 5,59 ª	68,68 ± 11,61ª	72,67 ± 4,62 ª	63,42 ± 9,06 ª	0,443
K2	0,86 ± 0,13 ª	0,32 ± 0,14 b	0,43 ± 0,34 b	0,41 ± 0,28 b	**0,021**
0V%	26,76 ± 5,22 ª	43,35 ± 26,73ª	62,60 ± 22,01 ª	46,67 ± 18,29ª	0,158
2UV%	22,77 ± 9,49 ª	16,84 ± 12,08ª	9,01 ± 4,88 ª	16,68 ± 10,51ª	0,503
2LV%	6,19 ± 3,46 ª	3,75 ± 3,21 ^{ab}	1,13 ± 0,87 ^{b}	1,97 ± 1,30 ^{b}	**0,021**

Table 4: Comparison of nocturnal cardiovascular parameters across the control group and MSA patient subgroups.

Data are presented as mean ± standard deviation. The final column shows the p-value from the one-way Analysis of Variance (ANOVA). Within each row, means not sharing a common superscript letter (a, b, c) are significantly different from each other (post-hoc analysis, $p < 0.05$).

4.3. Phase-dependent evolution of HRV parameters

Across states, patients with MSA without sleep-breathing disorders mirrored the modulation that would be expected in a healthy population: 0V% was higher in Wake and REM and lowest in NREM, 2UV% rose from Wake to NREM then declined in REM, 2LV% peaked in NREM (low in Wake/REM), and K2 remained high with a slight NREM rise. Relative to this pattern, clinical groups diverged mainly on K2 and 2LV%.

For K2, between-group differences were significant in all phases (Wake $p = 0.026$; NREM $p = 0.012$; REM $p = 0.021$): in Wake, noSBD > OSA and > OSA+stridor (noSBD vs isolated stridor n.s.); in NREM, noSBD > OSA and > OSA+stridor, and isolated stridor > OSA; in REM, noSBD > all clinical groups (OSA, isolated stridor, OSA+stridor mutually n.s.).

For 2LV%, differences were robust in NREM and REM (NREM $p = 0.011$; REM $p = 0.021$; Wake trend $p = 0.086$): in NREM, noSBD > OSA, isolated stridor, and OSA+stridor; in REM, noSBD > isolated stridor and > OSA+stridor (noSBD vs OSA n.s.; clinical–clinical pairs n.s.).

By contrast, 0V% and 2UV% did not differ between groups within phase (0V%—Wake $p = 0.569$, NREM $p = 0.401$, REM $p = 0.158$; 2UV%—Wake $p = 0.811$, NREM $p = 0.596$, REM $p = 0.503$), despite descriptive REM changes in stridor (higher 0V%, lower 2UV%).

Overall, patients without SDB had a relatively preserved, state-dependent autonomic profile, whereas OSA and stridor, especially in REM, showed lower K2, greater rigidity (higher 0V%), and a blunted NREM 2LV% peak.

4.4. The impact of CPAP on HRV

A subsequent subgroup analysis assessed the impact of CPAP on HRV parameters. Specifically, the evolution of HRV parameters between the two nights in 10 patients who used the CPAP during Night2 and 5 patients of the SDB- group who did not have an indication to CPAP.

Within the CPAP group, widespread changes in autonomic modulation compared to baseline could be observed during the treatment night. A significant reduction in HR was observed during both NREM and REM sleep ($p = 0.02$). Analysis of symbolic dynamics revealed a profound decrease in 0V% across in wake and REM sleep ($p \leq 0.02$). Conversely, patterns reflecting vagal activity generally increased, with both 2UV and 2LV patterns rising significantly during wake and REM sleep. Furthermore, the K2 index showed a significant increase during wake and REM sleep (p 0.05 and 0.04).

Such changes were not present in the noSBD groups, as shown in Figures 4.1 – 4.3.

	Night1	Night2	p
Wake			
HR	69,93 ± 6,36	70,28 ± 9,10	0,92
K2	0,63 ± 0,23	0,83 ± 0,17	**0,05**
0V%	49,82 ± 24,35	27,52 ± 12,03	**0,02**
2UV%	15,18 ± 6,83	20,19 ± 8,11	**0,04**
2LV%	3,24 ± 2,75	6,38 ± 3,21	**0,01**
NREM sleep			
HR	67,99 ± 7,89	64,01 ± 7,72	**0,02**
K2	0,69 ± 0,26	0,71 ± 0,24	0,84
0V%	34,74 ± 20,90	27,18 ± 7,98	0,30
2UV%	21,19 ± 8,50	18,67 ± 7,53	0,40
2LV%	4,92 ± 3,20	6,72 ± 2,49	0,08
REM sleep			
HR	70,80 ± 9,45	65,16 ± 9,51	**0,02**
K2	0,49 ± 0,24	0,69 ± 0,23	**0,04**
0V%	58,91 ± 21,72	31,03 ± 8,61	**< 0,001**
2UV%	11,78 ± 8,65	17,71 ± 6,29	**0,03**
2LV%	1,65 ± 1,17	4,86 ± 3,11	**0,02**

Table 5: evolution of HRV parameters in the different recorded phases between Night1 and Night2 in patients treated with CPAP.

Data are presented as mean ± standard deviation for the Night1 (no CPAP) and Night2 (with CPAP). The final column shows the p-value from the independent samples t-test. Significant p-values ($p < 0.05$) are highlighted in bold.

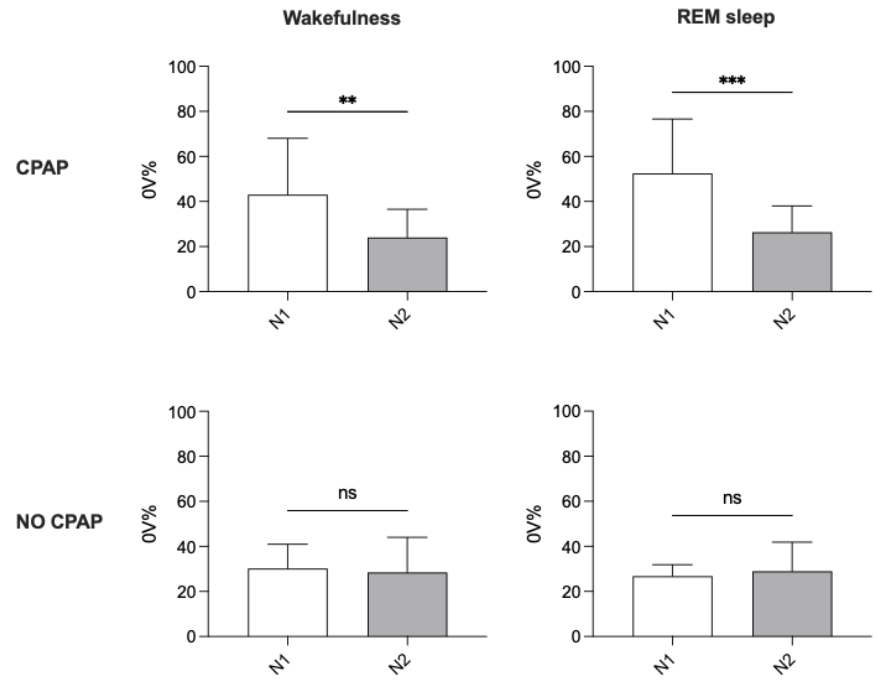

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Figure 4.1:. *Nocturnal Changes in 0V Symbolic Dynamics Patterns*

The bar charts illustrate the mean percentage (± standard deviation) of 0V patterns, a marker of sympathetic activity, during Night1 (N1) and N2 (Night2). Data are shown for the CPAP treatment group (top panels) and the NO CPAP group (bottom panels) during both wakefulness and REM sleep. Asterisks denote statistically significant differences between N1 and N2 within each group. **: $p < 0.01$; ***: $p < 0.001$; ns: not significant.

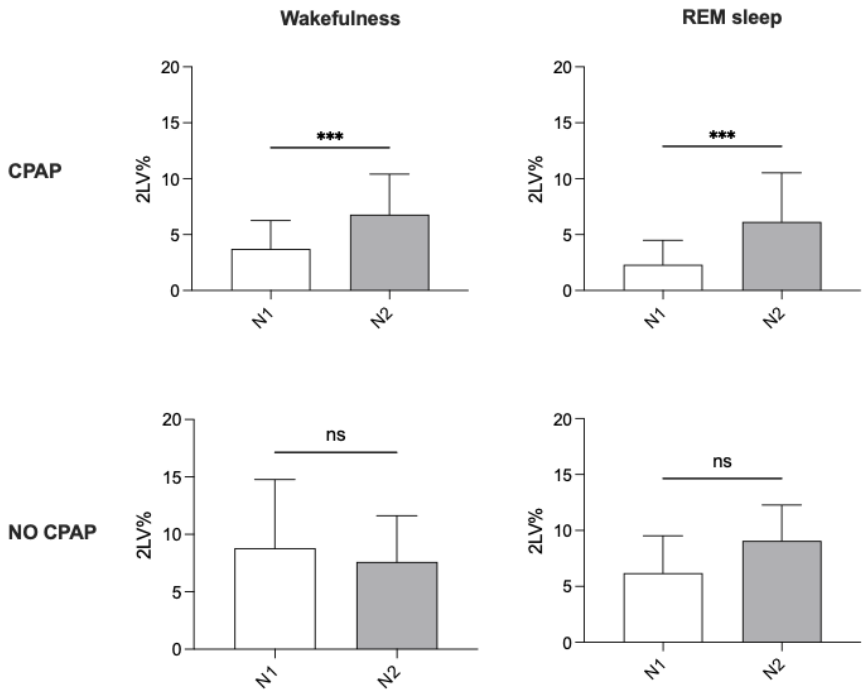

Figure 4.2: *Nocturnal Changes in 2LV Symbolic Dynamics Patterns.*

The bar charts illustrate the mean percentage (± standard deviation) of 2LV patterns, during Night1 (N1) and Nigh2 (N2). Data are shown for the CPAP treatment group (top panels) and the NO CPAP (SDB-) (bottom panels) during both wakefulness and REM sleep. Asterisks denote statistically significant differences between N1 and N2 within each group. ***: $p < 0.001$; ns: not significant.

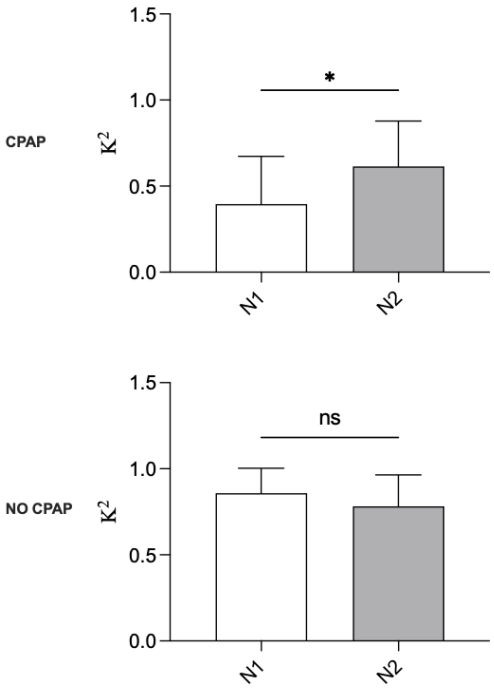

Figure 4.3: *Nocturnal Changes in Cardiorespiratory Coupling (K^2 Index).*

The chart illustrates the change in the K^2 index of cardiorespiratory coupling between the Night1 (N1) and Night2 (N2). Results are shown separately for the CPAP treatment group (top panel) and the noCPAP (bottom panel). Bars represent the mean value, and error bars represent the standard deviation. The asterisk (*) denotes a statistically significant difference ($p < 0.05$) between N2 and N2; ns: not significant

5. Discussion

MSA represents a unique model of centrally originated autonomic failure, given its early and severe involvement of brainstem autonomic networks [1, 131, 2]. Sleep offers a controlled physiological context to describe this failure, since autonomic set-points differ predictably between NREM and REM sleep, with vagal predominance in NREM and sympathetic lability in REM, and daytime behavioural confounders are minimized. However, in patients suffering from MSA SDB, including OSA and stridor, can perturb autonomic control via intermittent hypoxia, respiratory effort, and fragmentation [67 58]. These factors limit the interpretability of conventional frequency-domain HRV indexes during v-PSG, where respiratory "noise" can dominate neural oscillations [105, 106]. The present study therefore combined a coupling metric (K2) indexing heart–breathing entrainment with symbolic dynamics (0V, 2UV, 2LV), which capture non-linear properties of beat-to-beat variability robust to short segments and respiratory confounding [88, 87].

To the best of our knowledge, this is the first study assessing HRV parameters MSA patients from recordings of a video-PSG over two different nights and evaluating the impact of CPAP therapy during the second night on HRV in a subgroup of patients.

We were able to include in the first analysis a total of 24 patients, with a slightly higher prevalence of the cerebellar than the parkinsonian phenotype (15 vs 9). Apart from that, the general cohort showed demographical characteristics in line with large European epidemiological studies, with the two sexes almost equally represented and a mean age at disease onset of 57.3 ± 8.8. [5] More than 90% had a probable diagnosis according to Gilman criteria. [27]

In this general cohort, 13/24 patients had stridor, the majority of which presented also an AHI > 15 (8/13), coherently with what we previously reported on larger numbers [74]–suggesting the frequent coexistence of an apnoea syndrome, or a possible impact of stridor in generating hypopneas.

The homogeneity of demographical parameters suggests that no difference derives from sex or age differences between patients with and without SDB. Moreover,

disease duration was similar in the two subgroups, even though with a tendency of patients with SBD towards a shorter duration of the disease.

On the other hand, sleep characteristics over the first night of PSG were profoundly different in patients with SDB than those without. Higher AHI and number and of hypopneas could be observed in patients with SBD. The number of hypopneas was disproportionally high (median 131, interquartile range 73.8; 191.8), compared to apnoeas, suggesting that such respiratory events play an even relevant role in the increase of AHI in MSA patients than apnoeas, possibly due to the compresence of stridor. As previously reported in other MSA cohorts [60], an higher AHI is not associated with a greater BMI or differences in other biometric indexes. As expectable, mean oxygen saturation during sleep was lower in the cohort with SDB, with a longer time spent with SpO2 <90% and higher desaturation index. A higher frequency of PLMs was noted, probably linked to the higher number of respiratory events and the more fragmented sleep.

At baseline (Night 1), SDB+ patients showed a markedly impaired autonomic profile versus patients without SDB, with both K2 and 2LV% reduced in each state and 0V% selectively higher in REM. This observation could be confirmed when stratifying the patients depending on the specific presence of stridor/OSA/their combination, The clearest separation between groups emerged for cardiorespiratory coupling: K2 was consistently higher in patients with MSA without SDB and significantly reduced in all three groups of SDB patients. These results indicate that SDB is associated with a generalized degradation of heart–breathing coordination throughout the night, accentuated in REM. From a physiological perspective, repeated arousals, hypoxic dips, and load-related perturbations likely disrupt stable vagal gating of respiratory sinus arrhythmia and induce variable sinoatrial timing, impairing coherent entrainment [122] .

Symbolic dynamics further delineated the stage dependence of vagal organization. In patients without SBD, 2LV% exhibited the expected physiological NREM peak and low REM values, closely mirroring classical sleep-stage autonomic modulation [97], [98]. In SDB groups, that NREM 2LV% peak was consistently blunted, and 2LV% remained low in REM, with the lowest values in stridor phenotypes. These findings

suggest that SDB erodes the normal NREM vagal modulation, producing flatter and less organized dynamics even when respiratory events are not explicitly sampled. On the other hand, between-group effects for 0V% and 2UV% did not reach significance at the phase level. Nevertheless, their directional behavior aligned with physiology and respiratory burden: 0V% increased in REM in stridor, and 2UV% tended to fall in REM in stridor. The absence of robust phase-wise group effects for 0V% and 2UV% likely reflects limited power and high within-group heterogeneity; both indices are known to be sensitive to arousal timing and hypoxia microstructure, which vary markedly across SDB phenotypes and nights. Notably, mean HR did not differ between groups in any phase, highlighting that this measure is far less sensitive than non-linear variability and coupling metrics to state-dependent dysautonomia in MSA.

Laryngeal stridor is a distinctive feature of MSA, reflecting impaired vocal fold motion and brainstem dysfunction and associated with worse outcomes, including sudden nocturnal death in some series [42, 47, 57]. In our cohort, isolated stridor showed a particularly REM-vulnerable autonomic profile: K2 dropped most steeply in REM, 0V% rose, and both 2UV% and 2LV% were lowest among groups in REM. This pattern is physiologically coherent. REM combines intrinsic sympathetic surges and motor atonia with ventilatory instability, amplifying the respiratory load imposed by paradoxical adduction or abduction restriction of the larynx [132], [133]. The conjunction of central autonomic degeneration and peripheral airway dysfunction plausibly yields both weaker central timing (lower K2) and a collapse of vagal structuring (lower 2UV/2LV) during REM. In NREM, the finding that isolated stridor had higher K2 than pure OSA, while still below noSBD, suggests that the autonomic impact of stridor may be less dominated by repetitive obstructive events and more by sustained inspiratory load and reflexogenic stress, with different temporal signatures than typical OSA [122] .

As we previously showed on a larger number of patients [74], on the second night, CPAP improved respiratory indices. These gains were paralleled by a clear autonomic shift. In particular, heart rate decreased in NREM and REM and K2 increased in wake and NREM under CPAP, indicating stronger cardio–respiratory entrainment once obstructive events and stridor were suppressed. In parallel,

symbolic-dynamics metrics moved toward a more favourable balance, with lower 0V% and higher 2UV% and 2LV% across states, consistent with reduced sympathetic rigidity and at least partially restored vagal structuring, especially during NREM. Mechanistically, the stabilization of ventilation and the reduction in arousal-related perturbations likely permitted more regular respiratory gating of sinoatrial timing, thereby enhancing coupling; the larger NREM effect is coherent with that stage's vagal predominance, while REM remains more vulnerable to residual instability. Overall, the concordant rise in K2 and the improvement in symbolic markers under CPAP support a rapid autonomic recalibration toward more coherent, physiologically organized cardio–respiratory control. All these differences were not observed in the subgroup of patients who did not receive an indication to CPAP, ruling out a trivial Night1 / Night2 effect. These differential night effects support a causal link between alleviating SDB and restoring both coupling and vagal structure.

Our observations may have different clinical implications. First, the notion that mean heart rate alone is not informative for phenotyping dysautonomia during sleep in MSA gives relevance to non-linear variability and coupling, which offer much greater sensitivity to state-anchored disruption. In particular, K2 and 2LV% carry complementary information: at baseline, both distinguish patients with and without SBD in a stage-specific manner, and under CPAP they improve together with respiratory stabilization. Moreover, REM stage should receive focused attention in stridor, where the combination of high 0V%, low 2UV% and 2LV%, and low K2 marks a stage of increased vulnerability that may be clinically relevant to adverse nocturnal events. These observations could also expand CPAP indication to an even earlier phase of the disease, should be considered early in MSA patients with SDB to reduce sympathetic rigidity and restore vagal organization. Finally, coupling metrics under intervention should be interpreted alongside symbolic dynamics and respiratory context; in this cohort, the increase in K2 with CPAP aligns with improved oxygenation, fewer arousals, and more coherent vagal structure, supporting its value as a marker of therapeutic response, like previously shown in CPAP patients. [124]

Unfortunately, one of the main practical issues with CPAP therapy is its scarce tolerability and the long time needed for the patient to adapt to the machine. Long-term follow-up may also be unsatisfactory, particularly when the disease progresses. A follow-up study is currently ongoing to understand how CPAP was accepted in our cohort and whether autonomic effects shown on the second night were maintained in follow up registrations.

The study has different limitations to be acknowledged. First, its retrospective design inherently restricts the level of control over clinical, therapeutic, and technical variables, as all observations are derived from real-world clinical practice. The decision to start CPAP therapy in MSA patients is highly variable in different centres, but in the present study it was limited to the presence of stridor or AHI>15. A heterogeneity in CPAP settings may also be expected. Moreover, group sizes were modest after stratification into OSA, isolated stridor, and OSA+stridor, reducing power for some phase-wise contrasts, particularly for 0V% and 2UV%. Although we used stage-matched segments, residual contamination from arousals, periodic limb movements, or subclinical respiratory events cannot be fully excluded and may blur phase-wise contrasts. An additional conceptual limitation is related to K2, which, though highly informative, reflects both neural and mechanical components of cardiorespiratory coupling. Changes in respiratory pattern (e.g., upper airway resistance, respiratory effort, ventilatory instability) may therefore modulate K2 independently of autonomic outflow, complicating interpretation. Future studies incorporating event-locked analyses and multimodal signals (beat-to-beat blood pressure, baroreflex indices, respiratory effort, as well as acoustic features of stridor) could help disentangle these contributions.

Finally, this study focuses on the acute effect of a single night of CPAP. It remains unclear whether the autonomic improvements observed are sustained over time, whether they translate into measurable clinical benefits (e.g., improved sleep continuity, daytime symptoms, or long-term autonomic stability), and which MSA phenotypes may derive the greatest advantage from respiratory support.

6. Conclusions

In the present study, we assessed HRV parameters in a cohort of MSA patients with a video-PSG over two consecutive nights and evaluated the impact of CPAP therapy during the second night on HRV in a subgroup of patients.

Overall, our results suggest that MSA patients without sleep-breathing disorders retain a recognizable, stage-dependent autonomic profile, whereas the presence of SBD is associated with lower cardiorespiratory coupling across states, blunting of NREM vagal structuring, and REM-accentuated rigidity, most prominently in patients with stridor.

The autonomic signature shifted in a favorable direction under CPAP, indicating a rapid recalibration toward more coherent cardiorespiratory entrainment and restored vagal organization once respiratory load and arousals are mitigated.

Prospective protocols are needed to establish the long-term effect and clinical relevance of the autonomic improvements observed and to clarify which patients may benefit the most. Future longitudinal studies should enroll stratified MSA phenotypes (isolated stridor, OSA, OSA+stridor), use stage-specific endpoints that capture REM vulnerability and the NREM vagal peak, and link changes in cardiorespiratory coupling and symbolic dynamics to patient-centred outcomes, including sleep consolidation, daytime autonomic function, and survival.

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